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RECOMBINANT DNA REGULATION ACT, 1977

HEARING

BEFORE THE

SUBCOMMITTEE ON HEALTH AND SCIENTIFIC RESEARCH

OF THE

COMMITTEE ON HUMAN RESOURCES UNITED STATES SENATE

NINETY-FIFTH CONGRESS

FIRST SESSION

ON

S. 1217

TO REGULATE ACTIVITIES INVOLVING RECOMBINANT DEOXYRIBONUCLEIC ACID

AND RELATED BILLS

APRIL 6, 1977



Printed for the use of the Committee on Human Resources

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RECOMBINANT DNA REGULATION ACT, 1977

WEDNESDAY, APRIL 6, 1977

U.S. SENATE,
SUBCOMMITTEE ON HEALTH AND SCIENTIFIC RESEARCH
OF THE COMMITTEE ON HUMAN RESOURCES,
Washington, D.C.

The subcommittee met, pursuant to notice, at 9:39 a.m., in room 6202, Dirksen Senate Office Building, Senator Edward M. Kennedy (chairman of the subcommittee) presiding.

Present: Senators Kennedy, Pell, and Chafee.

Senator KENNEDY. The subcommittee will come to order.

Today the Senate Health and Scientific Research Subcommittee holds a legislative hearing of truly historic importance. We are considering legislation to give the Federal Government the right to regulate a specific area of biomedical research—research with recombinant DNA.

The freedom of scientific inquiry is cherished in this country. Nonetheless, many scientists are themselves advocating that recombinant DNA research be regulated by the Federal Government. Scientists and lay persons alike have concluded that this research has the potential to significantly alter our society—for good or ill. The extent of that potential alteration is not known today—and cannot be known until more research is done.

It may be that the risks are currently being grossly exaggerated. It may be that the benefits are overstated. But it may also be that the reverse is true. Because we do not know and cannot know at the present time, I believe we must err on the side of caution. I believe it is this feeling that has produced the unprecedented agreements that some form of legislation is necessary.

Whatever form the final legislation takes, it must build upon the unique process that has promoted the consensus which makes this regulation possible.

For the first time, a technical area of biomedical research has been the subject of widespread in-depth public discussions. Scientists and lay persons have been talking to each other in all parts of the country. They have gained mutual respect.

Scientists have learned that ordinary citizens can understand technical matters and make responsible decisions. Lay people have learned that the scientists can and will share information and that their proposals for self-regulation were not designed to slip something over on the public—but were responsible efforts at minimizing potential dangers.

To my mind, this unprecedented dialog between scientists' and citizens' minds must be built into the regulatory process. Because the

risks, if there are any, will involve scientists and lay persons alike, decisions as to how to contain them must be made cooperatively.

I believe a national commission, with a majority of nonscientists, would be the best vehicle to regulate this research. I would be interested in our witnesses' comments on this proposal.

In addition, I would like their reaction to the following propositions:

1. All recombinant DNA research in this country should be subject to the same regulations.

2. The regulatory process ought to be flexible and reflect new knowledge of risks and benefits as it becomes available.

3. The ethical, legal and moral implications of this work should be given careful scrutiny.

4. Local communities should retain the right to be stricter than the Federal regulations—including the right to prohibit recombinant DNA research in their own communities.

The decision to have the Federal Government intervene and regulate a specific area of scientific inquiry is not to be taken lightly. It establishes a precedent whose consequences are not yet clear. It should make us all somewhat uneasy.

What is reassuring, however, is that the impetus for this step has come from the ground up. It is not a case of Federal imposition of unwanted regulation on an uninformed and unsuspecting public. It is, rather, an example of the regulatory process at its best.

With the Government being responsive to the wishes of the people—scientists and lay persons alike. As such, there is great promise that this legislation will be accepted once enacted, and that this legislative precedent will be responsibly used in the future.

[Bills and introductory remarks of Senator Kennedy relating to the DNA legislation follow:]

[From the Congressional Record—Senate, Apr. 1, 1977]

By Mr. KENNEDY:

S. 1217. A bill to regulate activities involving recombinant deoxyribonucleic acid, the Committee on Human Resources.

Mr. KENNEDY. Mr. President, today I am pleased to introduce the administration's legislation regarding the urgent necessity to regulate and control recombinant DNA research. This legislation grows out of the recommendation of a broadly based interagency committee, which was chaired by the Director of the National Institutes of Health, Donald Frederickson. The need for such legislation is also recognized by the scientific community both here and abroad.

Mr. President, as chairman of the Senate Subcommittee on Health and Scientific Research, I have scheduled hearings on this and related legislation next Wednesday, April 6, 1977. These hearings are specifically designed to elucidate the relevant issues regarding this kind of genetic research so that it will be possible to promptly report a comprehensive bill from the committee to the Senate.

Recombinant DNA research has captured the attention and raised the anxiety of the Nation more than any other area of biomedical research in history. The potential to purposefully transform the nature of living organisms, for good or ill, is both an exhilarating and frightening prospect. The concern is not over current benefits or current risks—but over uncertain future benefits and uncertain future risks.

The questions of potential benefits and hazards of this research extend beyond the wall of the laboratory and the realm of freedom of scientific inquiry. These questions involve every citizen, scientist and layman alike. The assessment of risk and the judgment of how to balance risk against benefit of recombinant DNA research are responsibilities that clearly rest in the public domain. And it

follows that these issues must be decided through public processes. The vigorous discussion and debate amongst both the scientific community and the general public over this issue is very beneficial to the policymaking process. However, as the dialog unfolds throughout the Nation, I believe that there is a growing consensus throughout the country that regulatory legislation is necessary.

The legislation that I am introducing today presents the administration's views on regulating recombinant DNA research. I believe that the approach taken by the administration leaves several unresolved issues. First, I think that there is need for an alternative definition of recombinant DNA research to that given in the administration's proposal. I suggest that we define recombinant DNA research as any research, study, investigation, experiment, or act in which molecules or segments of molecules of deoxyribonucleic acid—DNA—that are not known to be otherwise capable of being propagated in a particular species of living cell are rendered capable of propogation in that species by joining them by any method outside of living cells to another DNA molecule or segment of DNA molecules. I would like the witnesses at the hearing to comment on this definition.

Second, I strongly feel that the public has a right to participate in the decisions concerning this area of research. I would like the witnesses at the hearing to consider whether a commission within the Department of Health, Education and Welfare should be established and be composed of a majority of individuals who are not involved in biomedical research.

Third, I have concern about the scope and nature of the standards to be promulgated under the administration's bill. Why should the thrust of the standards be limited to the production or possession of recombinant DNA? Why not include recombinant DNA research and include standards for the responsibilities and qualifications of those who both own research facilities and those who actually do the research.

In this regard, I take exception with the apparent limitation under the administration's approach. Should not the failure to comply with the standards be a ground for revoking a license. Is it not the main purpose of the licensure to assure that the standards are met?

I believe tha there must be a legislative standard under which the decision to grant a license is made. Therefore, I would like those who will be before my subcommittee next week to consider if a license should only be granted when the Government is satisfied that the research to be authorized by a license will be conducted in a manner as to assure the health and welfare of the persons to engage in the research, the environment, and the population of the surrounding community.

Finally, I would like to raise the following questions for consideration at the hearing:

First. Should there not be a comprehensive, thoughtful study which will consider and evaluate the ethical, societal, and legal implications of recombinant DNA research? I think that a group such as a subcommittee of the National Commission for the Protection of Human Subjects could be formed with the charge to study these long-range implications and make periodic reports to the Nation.

Second. Does the public not have a right to know who is conducting this type of research, where it is being conducted, and under what conditions?

Third. How much funding will this entire effort require and how many positions?

Fourth. Should there be a limit on how many facilities are to exist at the highest level of physical containment?

Fifth. Why should this research be exempt from environmental impact requirements?

Sxth. What information should be required to be submitted to the Government and what information should be disclosed to the public?

These are some of the concerns I have and I encourage the witnesses at the hearing next Tuesday to comment on the administration's proposal, the issues I have raised and to express their own concerns.

I ask unanimous consent that the bill be printed at this point in the Record.

There being no objection, the bill was ordered to be printed in the Record, as follows:

S. 1217

Be it enacted by the Senate and House of Representatives of the United States of America in Congress assembled, That this Act may be cited as the "Recombinant DNA Regulation Act".

FINDINGS

SEC. 2. The Congress finds that—

(1) The work with recombinant DNA will improve the understanding of basic biological processes and offers many potential benefits,

(2) there exists, however, a possible risk that microorganisms containing recombinant DNA may cause disease or alter the environment,

(3) microorganisms containing recombinant DNA could spread throughout the United States and to other countries, adversely affecting human health, the environment, industry, and agriculture,

(4) the only effective way to minimize the risk to health, the environment, industry, and agriculture is by regulating activities involving recombinant DNA, whether or not these activities are in interstate commerce, and therefore,

(5) all activities involving recombinant DNA either affect or are in interstate commerce.

GENERAL REQUIREMENTS

SEC. 3. (a) Except as provided in subsection (b), no person may possess or engage in the production of recombinant DNA unless—

(1) the production or possession complies with the standards promulgated under section 4,

(2) the production or possession occurs in a facility licensed under section 5, or the possession occurs while transporting recombinant DNA from one such facility to another and

(3) the production or possession occurs as part of a project that has been registered under section 6.

(b) the Secretary may exempt from some or all of the requirements of subsection (a) any category of activities he finds poses no significant risk to health or the environment (1) because of the nature of those activities, or (2) because the category is adequately regulated under other Federal law.

STANDARDS

SEC. 4. (a) For purposes of protecting the health and safety of individuals who work with recombinant DNA, the health and safety of the public at large, and the integrity of the environment, the Secretary—

(1) shall, no later than 90 days after the enactment of this Act, promulgate (without regard to section 102(2)(C) of the National Environmental Policy Act of 1969 or 6 U.S.C. 553) as interim standards applying to the production or possession or recombinant DNA the Recombinant DNA Research Guidelines (41 Fed. Reg. 27901 (1976)), with such modifications as he finds are needed,

(2) shall, no later than 180 days after the promulgation of interim standards under clause (1), initiate procedures to promulgate final standards applying to the production or possession of recombinant DNA,

(3) shall, no later than one year after the promulgation of interim standards under clause (1), promulgate final standards applying to the production or possession of recombinant DNA, and

(4) may, from time to time, promulgate (A) new standards applying to the production or possession of recombinant DNA, and (B) amendments to standards promulgated under this section.

(b)(1) Any person adversely affected by an action of the Secretary under this section may obtain review of the action in the United States Court of Appeals for the District of Columbia. The petition for review must be filed within sixty days of the action. Review shall conform to chapter 7 of title 5 of the United States Code.

(2) An action with respect to which review could have been obtained under paragraph (1) shall not be subject to judicial review in any other proceeding.

LICENSING OF FACILITIES

SEC. 5 (a) The Secretary may issue or renew a license for a facility to permit the production or possession (or certain categories of production or possession) of recombinant DNA at that facility only if (1) the facility submits an application therefor containing or accompanied by such information concerning recombinant DNA activities at that facility as the Secretary may prescribe, (2) the facility agrees and the Secretary determines that such production or possession

(including the transportation of recombinant DNA to or from that facility) will comply with the standards promulgated under section 4 and such ancillary conditions as he may prescribe, and (3) the facility agrees and the Secretary determines that such production or possession will occur only as part of a project registered under section 6.

(b) The Secretary may require payment of a fee from a facility for the issuance or renewal of a license under this section to cover all or part of the costs of administering this Act in respect to that facility.

(c) A license issued or renewed by the Secretary under this section is valid for the period prescribed by the Secretary, not to exceed three years.

(d) The Secretary may permit an appropriate State or local agency or a licensing or accrediting body to issue and renew licenses for facilities to permit the production or possession (or certain categories of production or possession) of recombinant DNA at those facilities if and for so long as the Secretary determines that the agency or body—

(1) requires a facility to comply with the standards promulgated under section 4 and such ancillary conditions as the Secretary may prescribe,

(2) requires a facility to permit the production or possession of recombinant DNA only as part of a project registered under section 6, and

(3) has the capacity to and does make provisions for assuring that the requirements of clauses (1) and (2) continue to be met.

(e) The Secretary may revoke, suspend, or limit a license issued under this section if he finds, after notice and opportunity for a hearing to the facility, that the facility—

(1) has misrepresented any material fact in obtaining the license,

(2) has engaged or attempted to engage or represented itself as entitled to perform any activities involving recombinant DNA not authorized by its license,

(3) has failed to comply with any of the terms or conditions of the license,

(4) has failed to comply with a request of the Secretary for any information or materials the Secretary finds necessary to determine the facility's continued eligibility for its license or to evaluate the possible effects on health or the environment of activities involving recombinant DNA.

(5) has failed to comply with a request of the Secretary to inspect any portion of the facility, its operations, or its records, which are related to activities involving recombinant DNA, or

(6) has violated or aided and abetted in the violation of any requirement established under this Act.

REGISTRATION

SEC. 6. The Secretary shall register any project involving recombinant DNA if the request for registration is accompanied by such information as the Secretary may prescribe concerning recombinant DNA activities which are part of that project.

INSPECTIONS

SEC. 7. An individual designated as an inspector by the Secretary, upon presenting appropriate credentials to the owner, operator, or agent (if any of these be present) in charge of a facility at which the inspector has reasonable grounds to believe that recombinant DNA is present or is being produced may enter that facility at reasonable times, and inspect, at reasonable times and in a reasonable manner, the facility and all things at (or being transported to or from) that facility which he has reasonable grounds to believe are involved with recombinant DNA and obtain appropriate samples of such things. When an inspector has completed such an inspection he shall, before he leaves the facility, inform the owner, operators, or agent in charge of the facility of any conditions or practices which in the inspector's judgment constitute a violation of any of the requirements of this Act. The inspector shall also prepare a written report of his findings and send it to the owner, operator, or agent in charge of the facility within a reasonable time.

RECORDS AND SAMPLES

SEC. 8. Each facility at which recombinant DNA is produced or located shall keep and make available to the Secretary such records (including medical records of personnel) and samples involving recombinant DNA at (or being transported to or from) that facility as the Secretary may prescribe.

REPORTS

SEC. 9 Each facility at which recombinant DNA is produced or located shall submit to the Secretary such reports concerning recombinant DNA at (or being transported to or from) that facility as the Secretary may prescribe.

EFFECT ON STATE AND LOCAL REQUIREMENTS

SEC. 10. (a) Except as provided in subsection (b), no State or political subdivision of a State may establish or continue in effect with respect to recombinant DNA activities any requirement which is different from, or in addition to, any requirement applicable under this Act to such activities.

(b) Upon application of a State or political subdivision on a State, the Secretary shall exempt from subsection (a) a requirement of that State or political subdivision applicable to recombinant DNA activities if he determines that the requirement is, and will be be administered so as to be, as stringent as, or more stringent than, a requirement under this Act. The Secretary may not withdraw any such exemption for so long as he finds that such requirement remains unchanged and continues to be so administered.

EMPLOYEE PROTECTION

SEC. 11. (a) No employer may discharge any employee or otherwise discriminate against any employee with respect to the employee's compensation, terms, conditions, or privileges of employment because the employee (or person acting pursuant to a request of the employee) has—

(1) commenced, cause to be commenced, or is about to commence or cause to be commenced a proceeding under this Act,

(2) testified or is about to testify in any such proceeding, or

(3) assisted or participated or is about to assist or participate in any manner in such a proceeding or in any other action to carry out the purposes of this Act.

(b) (1) Any employee who believes that the employee has been discharged or otherwise discriminated against by any person in violation of subsection (a) of this section may, within 30 days after such alleged violation occurs, file (or have any person file on the employee's behalf) a complaint with the Secretary of Labor (hereinafter in this section referred to as the "Secretary") alleging such discharge or discrimination. Upon receipt of such a complaint, the Secretary shall notify the person named in the complaint of the filing of the complaint.

(2) (A) Upon receipt of a complaint filed under paragraph (1), the Secretary shall conduct an investigation of the violation alleged in the complaint. Within 30 days of the receipt of such complaint, the Secretary shall complete such investigation and shall notify in writing the complainant (and any person acting on behalf of the complainant) and the person alleged to have committed such violation of the results of the investigation conducted pursuant to this paragraph. Within ninety days of the receipt of such complaint the Secretary shall, unless the proceeding on the complaint is terminated by the Secretary on the basis of a settlement entered into by the Secretary and the person alleged to have committed such violation, issue an order either providing the relief prescribed by subparagraph (B) or denying the complaint. An order of the Secretary shall be made on the record after notice and opportunity for agency hearing. The Secretary may not enter into a settlement terminating a proceeding on a complaint without the participation and consent of the complainant.

(B) If in response to a complaint filed under paragraph (1) the Secretary determines that a violation of subsection (a) of this section has occurred, the Secretary shall order (i) the person who committed such violation to take affirmative action to abate the violation, (ii) such person to reinstate the complainant to the complainant's former position together with the compensation (including back pay), terms, conditions, and privileegs of the complainant's employment, (iii) compensatory damages, and (iv) where appropriate, exemplary damages. If such an order is issued, the Secretary at the request of the complainant, shall assist against the person against whom the order is issued a sum equal to the aggregate amount of all costs and expenses (including attorney's fees) reasonably incurred, as determined by the Secretary, by the complainant for, or in connection with, the bringing of the complaint upon which the order was issued.

(c) (1) Any employee or employer adversely affected or aggrieved by an order issued under subsection (b) may obtain review of the order in the United

States Court of Appeals for the circuit in which the violation, with respect to which the order was issued, allegedly occurred. The petition for review must be filed within sixty days from the issuance of the Secretary's order. Review shall conform to chapter 7 of title 5 of the United States Code.

(2) An order of the Secretary, with respect to which review could have been obtained under paragraph (1), shall not be subject to judicial review in any criminal or other civil proceeding.

(a) Whenever a person has failed to comply with an order issued under subsection (b)(2), the Secretary shall file a civil action in the United States district court for the district in which the violation was found to occur to enforce such order. In actions brought under this subsection, the district courts shall have jurisdiction to grant all appropriate relief and compenstory and exemplary damages. Civil actions brought under this subsection shall be heard and decided expeditiously.

(e) Subsection (a) of this section shall not apply with respect to any employee who, acting without direction from the empolyee's employer (or any agent of the employer), deliberately causes a violation of any requirement of this Act.

CONSULTATION

SEC. 12. In administering this Act, the Secretary shall consult with the Secretaries of Agriculture, Commerce, Defense, Labor, and Transportation, the Administrators of the Environmental Protection Agency and Veterans' Affairs, the Director of the National Science Foundation, other appropriate officials, and such interagency committees and other advisory bodies as the Secretary may establish, concerning the promulgation of standards and of amendments to standards, the avoidance of duplicative requirements, and such other matters which may be of mutual interest.

ENFORCEMENT

SEC. 13. (a) Upon petition by the Secretary, the district court of the United States may restrain violations of this Act.

(b)(1) Any person who violates a provision of this Act (other than in section 11) may be assessed a civil penalty by the Secretary of not more than $5,000 for each violation.

(2) No civil penalty shall be assessed unless the person charged shall have been given notice and opportunity for a hearing on such charge. In determining the amount of the penalty the Secretary shall consider the appropriateness of such penalty to the gravity of the violation.

(3) In case of inability to collect such civil penalty or failure of any person to pay all, or such portion of such civil penalty as the Secretary may determine, the Secretary shall refer the matter to the Attorney General, who shall recover such amount by action in the district court of the United States for the district in which that person resides or has his principal place of business.

(c) Any person who knowingly or willfully violates any provision of this Act (other than in section 11) shall be subject, upon conviction, to a fine of not more than $5,000, or to imprisonment for not more than one year (and not more than ten years for a related series of violations), or both.

(d) Each day a violation of the Act continues shall constitute a separate violation for purposes of this section.

EMERGENCY PROCEDURE FOR HAZARDOUS RECOMBINANT DNA

SEC. 14. The Secretary may commence a civil action, by process of libel or otherwise, in an appropriate district court of the United States for the seizure or destruction of hazardous recombinant DNA or for other appropriate relief to prevent its production, movement, or spread.

ADMINISTRATIVE RESTRAINT OR SEIZURE

SEC. 15. If during an inspection under section 7 an inspector finds material he has reasonable grounds to believe is hazardous recombinant DNA, he may order the material not to be moved or may seize the material. Within twenty days after such action by an inspector the Secretary must commence a civil action under section 14 with respect to the inspector's action, unless the owner of the material has agreed otherwise.

TRAINING

SEC. 16. The Secretary may conduct and support training in the safe handling of recombinant DNA.

REPRESENTATION BY ATTORNEY GENERAL

SEC. 17. The Attorney General shall appear and represent the Secretary or the Secretary of Labor at any civil or criminal action initiated under this Act.

DEFINITIONS

SEC. 18. For purposes of this Act—

(1) "Secretary" (except as used in section 11) means the Secretary of Health, Education, and Welfare.

(2) "DNA" means deoxyribonucleic acid.

(3) "recombinant DNA" means DNA that consists of different segments of DNA which has been joined together in a cell-free system and that has the capacity to infect and replicate in some host cell either autonomously or as an integrated part of the host's genome.

(4) "hazardous recombinant DNA" means recombinant DNA which either—

(A) poses a significant risk to health or the environment, or

(B) (i) (I) is neither located at a facility licensed under section 5 nor being transported from one such facility to another, or

(II) is likely to be moved or to spread from such a facility or transportation to a location not at such a facility or part of such transportation, and

(ii) is not known not to pose a significant risk to health or the environment,

(5) "person" means any individual, partnership, corporation, association or any Federal, State, or local governmental entity,

(6) "district court of the United States" includes the District Court of Guam, the District Court of the Virgin Islands, the highest court of American Samoa, and a similar or equivalent court in any other United States territory or possession or in the Trust Territory of the Pacific Islands, and

(7) in relation to transportation to or from a facility, a suitable facility in a foreign country shall be treated as a facility licensed under section 5.

GEOGRAPHIC APPLICABILITY OF ACT

SEC. 19. This Act applies to the United States, its territories and possessions, and the Trust Territory of the Pacific Islands.

RELATIONSHIP TO OTHER FEDERAL LAWS

SEC. 20. (a) This Act shall not affect the authority of any Federal agency to regulate under any other Act activities involving recombinant DNA.

(b) In exercising any authority under this Act, the Secretary, or any person acting on his behalf or pursuant to the provisions of this Act, shall not, for purposes of section 4(b)(1) of the Occupational Safety and Health Act of 1970, be deemed to be exercising statutory authority to prescribe or enforce standards or regulations affecting occupational safety and health.

(c) (1) Upon request by the Secretary, each Federal agency is authorized—

(A) to make its services, personnel, and facilities available (with or without reimbursement) to the Secretary to assist the Secretary in the administration of this Act, and

(B) to furnish to the Secretary such information, data, estimates, and statistics, and to allow the Secretary access to all information in its possession, as the Secretary may reasonably determine to be necessary for the administration of this Act.

(2) Upon request by any Federal agency, the Secretary is authorized—

(A) to make the services, personnel, and facilities of the Department of Health, Education, and Welfare available (with or without reimbursement) to that agency to assist the agency in the administration of any Act with respect to activities involving recombinant DNA, and

(B) to furnish to that agency such information, data, estimates, and statistics, and to allow that agency access to all information in the Secretary's possession, as the agency may reasonably determine to be necessary for the administration of any Act with respect to activities involving recombinant DNA.

SEPARABILITY

SEC. 21. If any provision of this Act is held invalid by reason of being inconsistent with the Constitution, all provisions of this Act which are separable from that invalid provision shall remain in effect.

EFFECTIVE DATE AND EXPIRATION DATE OF ACT

SEC. 22. (a)(1) This Act is effective upon enactment, except that sections 3(a), 10(a), and 18(4)(B) are effective 180 days after enactment.

(2) Upon promulgation of standards under section 4, no person may possess or engage in the production of recombinant DNA unless the production or possession complies with those standards.

(b) This Act shall expire five years after its enactment except with respect to records made within that period.

95TH CONGRESS
1ST SESSION

S. 1217

IN THE SENATE OF THE UNITED STATES

APRIL 1 (legislative day, FEBRUARY 21), 1977

Mr. KENNEDY introduced the following bill; which was read twice and referred to the Committee on Human Resources

A BILL

To regulate activities involving recombinant deoxyribonucleic acid.

Be it enacted by the Senate and House of Representatives of the United States of America in Congress assembled, That this Act may be cited as the "Recombinant DNA Regulation Act".

FINDINGS

SEC. 2. The Congress finds that—

(1) work with recombinant DNA will improve the understanding of basic biological processes and offers many potential benefits,

(2) there exists, however, a possible risk that micro-

organisms containing recombinant DNA may cause disease or alter the environment,

(3) micro-organisms containing recombinant DNA could spread throughout the United States and to other countries, adversely affecting human health, the environment, industry, and agriculture,

(4) the only effective way to minimize the risk to health, the environment, industry, and agriculture is by regulating activities involving recombinant DNA, whether or not those activities are in interstate commerce, and therefore

(5) all activities involving recombinant DNA either affect or are in interstate commerce.

GENERAL REQUIREMENTS

SEC. 3. (a) Except as provided in subsection (b), no person may possess or engage in the production of recombinant DNA unless—

(1) the production or possession complies with the standards promulgated under section 4,

(2) the production or possession occurs in a facility licensed under section 5, or the possession occurs while transporting recombinant DNA from one such facility to another, and

(3) the production or possession occurs as part of a project that has been registered under section 6.

(b) The Secretary may exempt from some or all of the requirements of subsection (a) any category of activities he finds poses no significant risk to health or the environment (1) because of the nature of those activities, or (2) because the category is adequately regulated under other Federal law.

STANDARDS

SEC. 4. (a) For purposes of protecting the health and safety of individuals who work with recombinant DNA, the health and safety of the public at large, and the integrity of the environment, the Secretary—

(1) shall, no later than ninety days after the enactment of this Act, promulgate (without regard to section 102(2)(C) of the National Environmental Policy Act of 1969 or 5 U.S.C. 553) as interim standards applying to the production or possession of recombinant DNA the Recombinant DNA Research Guidelines (41 Federal Register 27901 (1976)), with such modifications as he finds are needed,

(2) shall, no later than one hundred and eighty days after the promulgation of interim standards under clause (1), initiate procedures to promulgate final standards applying to the production or possession of recombinant DNA,

(3) shall, no later than one year after the promulga-

tion of interim standards under clause (1), promulgate final standards applying to the production or possession of recombinant DNA, and

(4) may, from time to time, promulgate (A) new standards applying to the production or possession of recombinant DNA, and (B) amendments to standards promulgated under this section.

(b) (1) Any person adversely affected by an action of the Secretary under this section may obtain review of the action in the United States Court of Appeals for the District of Columbia. The petition for review must be filed within sixty days of the action. Review shall conform to chapter 7 of title 5 of the United States Code.

(2) An action with respect to which review could have been obtained under paragraph (1) shall not be subject to judicial review in any other proceeding.

LICENSING OF FACILITIES

SEC. 5. (a) The Secretary may issue or renew a license for a facility to permit the production or possession (or certain categories of production or possession) of recombinant DNA at that facility only if (1) the facility submits an application therefor containing or accompanied by such information concerning recombinant DNA activities at that facility as the Secretary may prescribe, (2) the facility agrees and the Secretary determines that such production or

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possession (including the transportation of recombinant DNA to or from that facility) will comply with the standards promulgated under section 4 and such ancillary conditions as he may prescribe, and (3) the facility agrees and the Secretary determines that such production or possession will occur only as part of a project registered under section 6.

(b) The Secretary may require payment of a fee from a facility for the issuance or renewal of a license under this section to cover all or part of the costs of administering this Act in respect to that facility.

(c) A license issued or renewed by the Secretary under this section is valid for the period prescribed by the Secretary, not to exceed three years.

(d) The Secretary may permit an appropriate State or local agency or a licensing or accrediting body to issue and renew licenses for facilities to permit the production or possession (or certain categories of production or possession) of recombinant DNA at those facilities if and for so long as the Secretary determines that the agency or body—

(1) requires a facility to comply with the standards promulgated under section 4 and such ancillary conditions as the Secretary may prescribe,

(2) requires a facility to permit the production or possession of recombinant DNA only as part of a project registered under section 6, and

(3) has the capacity to and does make provision for assuring that the requirements of clauses (1) and (2) continue to be met.

(e) The Secretary may revoke, suspend, or limit a license issued under this section if he finds, after notice and opportunity for a hearing to the facility, that the facility—

(1) has misrepresented any material fact in obtaining the license,

(2) has engaged or attempted to engage or represented itself as entitled to perform any activities involving recombinant DNA not authorized by its license,

(3) has failed to comply with any of the terms or conditions of the license,

(4) has failed to comply with a request of the Secretary for any information or materials the Secretary finds necessary to determine the facility's continued eligibility for its license or to evaluate the possible effects on health or the environment of activities involving recombinant DNA,

(5) has failed to comply with a request of the Secretary to inspect any portion of the facility, its operations, or its records, which are related to activities involving recombinant DNA, or

(6) has violated or aided and abetted in the violation of any requirement established under this Act.

REGISTRATION

SEC. 6. The Secretary shall register any project involving recombinant DNA if the request for registration is accompanied by such information as the Secretary may prescribe concerning recombinant DNA activities which are part of that project.

INSPECTIONS

SEC. 7. An individual designated as an inspector by the Secretary, upon presenting appropriate credentials to the owner, operator, or agent (if any of these be present) in charge of a facility at which the inspector has reasonable grounds to believe that recombinant DNA is present or is being produced may enter that facility at reasonable times, and inspect, at reasonable times and in a reasonable manner, the facility and all things at (or being transported to or from) that facility which he has reasonable grounds to believe are involved with recombinant DNA and obtain appropriate samples of such things. When an inspector has completed such an inspection he shall, before he leaves the facility, inform the owner, operator, or agent in charge of the facility of any conditions or practices which in the inspector's judgment constitute a violation of any of the requirements of this Act. The inspector shall also prepare a written report of his findings and send it to the owner, operator, or agent in charge of the facility within a reasonable time.

RECORDS AND SAMPLES

SEC. 8. Each facility at which recombinant DNA is produced or located shall keep and make available to the Secretary such records (including medical records of personnel) and samples involving recombinant DNA at (or being transported to or from) that facility as the Secretary may prescribe.

REPORTS

SEC. 9. Each facility at which recombinant DNA is produced or located shall submit to the Secretary such reports concerning recombinant DNA at (or being transported to or from) that facility as the Secretary may prescribe.

EFFECT ON STATE AND LOCAL REQUIREMENTS

SEC. 10. (a) Except as provided in subsection (b), no State or political subdivision of a State may establish or continue in effect with respect to recombinant DNA activities any requirement which is different from, or in addition to, any requirement applicable under this Act to such activities.

(b) Upon application of a State or political subdivision of a State, the Secretary shall exempt from subsection (a) a requirement of that State or political subdivision applicable to recombinant DNA activities if he determines that the requirment is, and will be administered so as to be, as stringent as, or more stringent than, a requirement under

this Act. The Secretary may not withdraw any such exemption for so long as he finds that such requirement remains unchanged and continues to be so administered.

EMPLOYEE PROTECTION

SEC. 11. (a) No employer may discharge any employee or otherwise discriminate against any employee with respect to the employee's compensation, terms, conditions, or privileges of employment because the employee (or any person acting pursuant to a request of the employee) has—

(1) commenced, caused to be commenced, or is about to commence or cause to be commenced a proceeding under this Act,

(2) testified or is about to testify in any such proceeding, or

(3) assisted or participated or is about to assist or participate in any manner in such a proceeding or in any other action to carry out the purposes of this Act.

(b) (1) Any employee who believes that the employee has been discharged or otherwise discriminated against by any person in violation of subsection (a) of this section may, within thirty days after such alleged violation occurs, file (or have any person file on the employee's behalf) a complaint with the Secretary of Labor (hereinafter in this section referred to as the "Secretary") alleging such discharge or

discrimination. Upon receipt of such a complaint, the Secretary shall notify the person named in the complaint of the filing of the complaint.

(2) (A) Upon receipt of a complaint filed under paragraph (1), the Secretary shall conduct an investigation of the violation alleged in the complaint. Within thirty days of the receipt of such complaint, the Secretary shall complete such investigation and shall notify in writing the complainant (and any person acting on behalf of the complainant) and the person alleged to have committed such violation of the results of the investigation conducted pursuant to this paragraph. Within ninety days of the receipt of such complaint the Secretary shall, unless the proceeding on the complaint is terminated by the Secretary on the basis of a settlement entered into by the Secretary and the person alleged to have committed such violation, issue an order either providing the relief prescribed by subparagraph (B) or denying the complaint. An order of the Secretary shall be made on the record after notice and opportunity for agency hearing. The Secretary may not enter into a settlement terminating a proceeding on a complaint without the participation and consent of the complainant.

(B) If in response to a complaint filed under paragraph (1) the Secretary determines that a violation of subsection (a) of this section has occurred, the Secretary shall order

(i) the person who committed such violation to take affirmative action to abate the violation, (ii) such person to reinstate the complainant to the complainant's former position together with the compensation (including back pay), terms, conditions, and privileges of the complainant's employment, (iii) compensatory damages, and (iv) where appropriate, exemplary damages. If such an order is issued, the Secretary, at the request of the complainant, shall assess against the person against whom the order is issued a sum equal to the aggregate amount of all costs and expenses (including attorney's fees) reasonably incurred, as determined by the Secretary, by the complainant for, or in connection with, the bringing of the complaint upon which the order was issued.

(c) (1) Any employee or employer adversely affected or aggrieved by an order issued under subsection (b) may obtain review of the order in the United States Court of Appeals for the circuit in which the violation, with respect to which the order was issued, allegedly occurred. The petition for review must be filed within sixty days from the issuance of the Secretary's order. Review shall conform to chapter 7 of title 5 of the United States Code.

(2) An order of the Secretary, with respect to which review could have been obtained under paragraph (1), shall not be subject to judicial review in any criminal or other civil proceeding.

(d) Whenever a person has failed to comply with an order issued under subsection (b) (2), the Secretary shall file a civil action in the United Statse district court for the district in which the violation was found to occur to enforce such order. In actions brought under this subsection, the district courts shall have jurisdiction to grant all appropriate relief, including injunctive relief and compensatory and exemplary damages. Civil actions brought under this subsection shall be heard and decided expeditiously.

(e) Subsection (a) of this section shall not apply with respect to any employee who, acting without direction from the employee's employer (or any agent of the employer), deliberately causes a violation of any requirement of this Act.

CONSULTATION

SEC. 12. In administering this Act, the Secretary shall consult with the Secretaries of Agriculture, Commerce, Defense, Labor, and Transportation, the Administrators of the Environmental Protection Agency and Veterans' Affairs, the Director of the National Science Foundation, other appropriate officials, and such interagency committees and other advisory bodies as the Secretary may establish, concerning the promulgation of standards and of amendments to standards, the avoidance of duplicative requirements, and such other matters which may be of mutual interest.

ENFORCEMENT

SEC. 13. (a) Upon petition by the Secretary, the district courts of the United States may restrain violations of this Act.

(b) (1) Any person who violates a provision of this Act (other than in section 11) may be assessed a civil penalty by the Secretary of not more than $5,000 for each violation.

(2) No civil penalty shall be assessed unless the person charged shall have been given notice and opportunity for a hearing on such charge. In determining the amount of the penalty the Secretary shall consider the appropriateness of such penalty to the gravity of the violation.

(3) In case of inability to collect such civil penalty or failure of any person to pay all, or such portion of such civil penalty as the Secretary may determine, the Secretary shall refer the matter to the Attorney General, who shall recover such amount by action in the district court of the United States for the district in which that person resides or has his principal place of business.

(c) Any person who knowingly or willfully violates any provision of this Act (other than in section 11) shall be subject, upon conviction, to a fine of not more than $5,000, or to imprisonment for not more than one year (and not

more than ten years for a related series of violations), or both.

(d) Each day a violation of this Act continues shall constitute a separate violation for purposes of this section.

EMERGENCY PROCEDURE FOR HAZARDOUS RECOMBINANT DNA

SEC. 14. The Secretary may commence a civil action, by process of libel or otherwise, in an appropriate district court of the United States for the seizure or destruction of hazardous recombinant DNA or for other appropriate relief to prevent its production, movement, or spread.

ADMINISTRATIVE RESTRAINT OR SEIZURE

SEC. 15. If during an inspection under section 7 an inspector finds material he has reasonable grounds to believe is hazardous recombinant DNA, he may order the material not to be moved or may seize the material. Within twenty days after such action by an inspector the Secretary must commence a civil action under section 14 with respect to the inspector's action, unless the owner of the material has agreed otherwise.

TRAINING

SEC. 16. The Secretary may conduct and support training in the safe handling of recombinant DNA.

REPRESENTATION BY ATTORNEY GENERAL

SEC. 17. The Attorney General shall appear and represent the Secretary or the Secretary of Labor at any civil or criminal action initiated under this Act.

DEFINITIONS

SEC. 18. For purposes of this Act—

(1) "Secretary" (except as used in section 11) means the Secretary of Health, Education, and Welfare,

(2) "DNA" means deoxyribonucleic acid,

(3) "recombinant DNA" means DNA that consists of different segments of DNA which have been joined together in a cell-free system and that has the capacity to infect and replicate in some host cell either autonomously or as an integrated part of the host's genome,

(4) "hazardous recombinant DNA" means recombinant DNA which either—

(A) poses a significant risk to health or the environment, or

(B) (i) (I) is neither located at a facility licensed under section 5 nor being transported from one such facility to another, or

(II) is likely to be moved or to spread from

such a facility or transportation to a location not at such a facility or part of such transportation, and

(ii) is not known not to pose a significant risk to health or the environment,

(5) "person" means any individual, partnership, corporation, association, or any Federal, State, or local government entity,

(6) "district court of the United States" includes the District Court of Guam, the District Court of the Virgin Islands, the highest court of American Samoa, and a similar or equivalent court in any other United States territory or possession or in the Trust Territory of the Pacific Islands, and

(7) in relation to transportation to or from a facility, a suitable facility in a foreign country shall be treated as a facility licensed under section 5.

GEOGRAPHIC APPLICABILITY OF ACT

SEC. 19. This Act applies to the United States, its territories and possessions, and the Trust Territory of the Pacific Islands.

RELATIONSHIP TO OTHER FEDERAL LAWS

SEC. 20. (a) This Act shall not affect the authority of any Federal agency to regulate under any other Act activities involving recombinant DNA.

(b) In exercising any authority under this Act, the Sec-

retary, or any person acting on his behalf or pursuant to the provisions of this Act, shall not, for purposes of section 4 (b) (1) of the Occupational Safety and Health Act of 1970, be deemed to be exercising statutory authority to prescribe or enforce standards or regulations affecting occupational safety and health.

(c) (1) Upon request by the Secretary, each Federal agency is authorized—

(A) to make its services, personnel, and facilities available (with or without reimbursement) to the Secretary to assist the Secretary in the administration of this Act, and

(B) to furnish to the Secretary such information, data, estimates, and statistics, and to allow the Secretary access to all information in its possession, as the Secretary may reasonably determine to be necessary for the administration of this Act.

(2) Upon request by any Federal agency, the Secretary is authorized—

(A) to make the services, personnel, and facilities of the Department of Health, Education, and Welfare available (with or without reimbursement) to that agency to assist the agency in the administration of any Act with respect to activities involving recombinant DNA, and

(B) to furnish to that agency such information, data, estimates, and statistics, and to allow that agency access to all information in the Secretary's possession, as the agency may reasonably determine to be necessary for the administration of any Act with respect to activities involving recombinant DNA.

SEPARABILITY

SEC. 21. If any provision of this Act is held invalid by reason of being inconsistent with the Constitution, all provisions of this Act which are separable from that invalid provision shall remain in effect.

EFFECTIVE DATE AND EXPIRATION DATE OF ACT

SEC. 22. (a) (1) This Act is effective upon enactment, except that sections 3(a), 10(a), and 18(4)(B) are effective one hundred and eighty days after enactment.

(2) Upon promulgation of standards under section 4, no person may possess or engage in the production of recombinant DNA unless the production or possession complies with those standards.

(b) This Act shall expire five years after its enactment except with respect to records made within that period.

95TH CONGRESS
1ST SESSION

S. 621

IN THE SENATE OF THE UNITED STATES

FEBRUARY 4 (legislative day, FEBRUARY 1), 1977

Mr. BUMPERS introduced the following bill; which was read twice and referred to the Committee on Labor and Public Welfare

A BILL

To provide for guidelines and strict liability in the development of research related to recombinant DNA.

Be it enacted by the Senate and House of Representatives of the United States of America in Congress assembled, That this Act may be cited as the "DNA Research Act of 1977".

FINDINGS

SEC. 2. The Congress finds that—

(1) research related to recombinant DNA is of exceptional importance, with many potential benefits, but also with major uncertainties regarding its possible effects on human beings and other organisms;

(2) research involving recombinant DNA may involve a high risk not only to those pursuing such research but to the population in general;

(3) it is essential in the public interest that the health and welfare of the Nation be protected by requiring that all research conducted with regard to recombinant DNA comply with strict standards of performance and safety; and

(4) all research with regard to recombinant DNA is either in interstate commerce or substantially affects such commerce and regulations by the Secretary of Health, Education, and Welfare as contemplated by this Act are necessary and proper to prevent and eliminate burdens on interstate commerce, to regulate such commerce effectively, and to protect the health and welfare of the Nation.

DEFINITIONS

SEC. 3. As used in this Act the term—

(1) "recombinant DNA" means molecules that consist of different segments of deoxyribonucleic acid which have been joined together in cell-free systems to infect and replicate in some host cell, either autonomously or on an integrated part of the host's genome;

(2) "Secretary" means the Secretary of Health, Education, and Welfare;

(3) "commerce" means—

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(A) any activity which affects commerce between any State or territory and any place outside thereof, and

(B) commerce within the District of Columbia or within any territory.

ESTABLISHMENT OF GUIDELINES

SEC. 4. The Secretary shall promulgate guidelines for research involving recombinant DNA within ninety days after the date of enactment of this Act.

APPLICATION OF GUIDELINES

SEC. 5. The guidelines promulgated under section 4 shall apply to all research involving recombinant DNA that is in or affects commerce, or that is carried on in any area subject to the jurisdiction of the United States.

PATENT RESTRICTIONS

SEC. 6. Notwithstanding any other law, no patent shall be granted on any procedure or organism which results from research on recombinant DNA unless all applicable guidelines have been strictly adhered to, and full and complete disclosure has been made with regard to such process or organism.

CIVIL LIABILITY

SEC. 7. Persons carrying out research involving recombinant DNA shall be strictly liable, without regard to fault, for all injury to persons or property caused by such research.

PROHIBITED ACTS

SEC. 8. No person shall solicit or accept, directly or indirectly, any specimen for research which shall involve recombinant DNA or conduct such research unless there is in effect a license for such research issued by the Secretary.

LICENSES

SEC. 9. (a) The Secretary is authorized to issue licenses for research involving recombinant DNA.

(b) A license issued by the Secretary under this section shall be valid for a period of one year, or such shorter period as the Secretary may establish for any research project and may be renewed in such manner as the Secretary may prescribe.

(c) The Secretary may require payment of reasonable fees for the issuance and renewal of licenses but the amount of such fee shall not exceed an amount necessary to defray the reasonable costs of reviewing and passing upon applications for licenses, and of enforcing guidelines.

(d) Licenses may contain such terms and conditions as the Secretary finds are necessary and appropriate to carry out the purposes of this Act.

APPLICATION REQUIREMENT

SEC. 10. A license shall not be issued by the Secretary unless—

(1) the application therefor contains or is accom-

panied by such information as the Secretary finds necessary; and

(2) the Secretary determines that such facility will be operated in accordance with the guidelines promulgated under section 4.

REVOCATION, SUSPENSION, OR LIMITATION

SEC. 11. A license issued by the Secretary may be revoked, suspended, or limited if the Secretary finds, after reasonable notice and opportunity for a hearing to the licensee, that such licensee—

(1) has been guilty of misrepresentation in obtaining a license;

(2) has engaged or attempted to engage or represented himself as entitled to perform any research or procedure or category of procedures not authorized by the license;

(3) has failed to comply with guidelines with respect to research facilities or personnel prescribed by the Secretary pursuant to this Act;

(4) has failed to comply with reasonable requests of the Secretary for any information or materials the Secretary deems necessary to determine continued eligibility for its license or continued compliance with the Secretary's guidelines;

(5) has refused a request from the Secretary or any

Federal officer or employee designated by the Secretary for permission to inspect the research facility and its operations and pertinent records at any reasonable time; or

(6) has violated or aided and abetted any violation of any provision of this section or of any guideline promulgated hereunder.

UNREASONABLE HAZARDS TO PUBLIC HEALTH

SEC. 12. Whenever the Secretary has reason to believe that a continuation of any activity by a research facility licensed under this section would constitute a significant hazard to the public health, the Attorney General may, at the Secretary's request, bring suit in the name of the United States in the district court for the district in which such facility is situated to enjoin continuation of such activity and upon a proper showing, a temporary injunction or restraining order against continuation of such activity pending issuance of a final order may be granted without bond.

INSPECTION AUTHORITY

SEC. 13. (a) In order to enforce this Act, officers, employees, or agents designated by the Secretary, upon presenting appropriate credentials to the owner, operator, or agent in charge of a facility engaged in research involving recombinant DNA, are authorized—

(1) to enter, at reasonable times, any such research facility; and

(2) to inspect, at reasonable times and in a reasonable manner, such research facility and all equipment, materials, containers, records, files, papers, processes, controls, facilities, and other things therein, in order to determine whether such research facility is being operated in compliance with this Act, the guidelines issued hereunder, and the terms of any license that may have been issued with respect to such research facility.

(b) Upon completion of any such inspection and prior to leaving the premises, the officer, employee, or agent making the inspection shall give to the owner, operator, or agent in charge a report summarizing any conditions or practices observed by him by which, in his judgment, indicate a violation of this Act or any guideline or license issued hereunder. He shall also prepare a written report of his findings and send it to such owner, operator, or agent within a reasonable time.

VIOLATIONS AND PENALTIES

SEC. 14. (a) Any person who willfully violates any provision of this Act or any guideline promulgated hereunder shall be guilty of a misdemeanor and shall, upon conviction therefor, be subject to imprisonment for not more than one

year, or a fine of not more than $10,000, or both. For purposes of computing such fine, each day of a continuing violation shall be deemed a separate violation. In addition, the court may order that the person or entity convicted be ineligible for Federal funds, in whole or in part, for a period of up to five years.

(b) No research facility employer shall discharge any employee or otherwise discriminate against any employee with respect to his compensation, terms, conditions, or privileges of employment because the employee has—

(A) commenced, caused to be commenced, or is about to commence or cause to be commenced a proceeding under this Act;

(B) testified or is about to testify in any such proceeding; or

(C) assisted or participated in or is about to assist or participate in such a proceeding or in any other action to carry out the purposes of this Act.

95TH CONGRESS
1ST SESSION

S. 945

IN THE SENATE OF THE UNITED STATES

MARCH 8 (legislative day, FEBRUARY 21), 1977

Mr. METZENBAUM introduced the following bill; which was read twice and referred to the Committee on Human Resources

A BILL

To regulate the conduct and development of research related to recombinant DNA, to establish a national commission to study recombinant DNA research and technology, and to provide citizens and governmental remedies.

Be it enacted by the Senate and House of Representatives of the United States of America in Congress assembled, That this Act may be cited as the "Recombinant DNA Standards Act of 1977".

TITLE I—REGULATIONS GOVERNING THE CONDUCT AND DEVELOPMENT OF RECOMBINANT DNA RESEARCH

PURPOSE

SEC. 2. The recent acceleration of research involving recombinant DNA has provided far-reaching scientific advancements. While these scientific advancements promise many far-reaching benefits, they also raise serious questions regarding hazards to human beings and other organisms. It is the interest of Congress to protect the health and welfare of the Nation by regulating the conduct and development of recombinant DNA research.

FINDINGS

SEC. 3. The Congress finds that:

(1) Research related to recombinant DNA raises serious questions regarding potential benefits and hazards to human beings and other organisms.

(2) Research involving recombinant DNA may involve a high risk not only to those pursuing such research but to the population in general.

(3) It is essential in the public interest that the health and welfare of the Nation be protected by requiring that the conduct and development of all research involving recombinant DNA comply with minimum Federal standards of safety and performance.

(4) It is essential in the public interest that moral,

environmental and scientific issues involved in recombinant DNA research be thoroughly studied and that findings and recommendations be made to the Congress and the President.

(5) It is premature to establish a finalized policy with respect to the advantages of continued recombinant DNA research. Therefore, it is necessary to give the minimum safety procedures established by the National Institutes of Health the force of law over all recombinant DNA research.

DEFINITIONS

SEC. 4. As used in this Act the term—

(a) "recombinant DNA" means molecules that consist of different segments of deoxyribonucleic acid which have been joined together in cell-free systems to infect and replicate in some host cell, either autonomously or as an integrated part of the host's genome;

(b) "Secretary" means the Secretary of Health, Education, and Welfare;

(c) "person" means any individual, corporation, governmental entity or group of individuals with similar interests.

PROMULGATION OF STANDARDS FOR RESEARCH RELATING TO RECOMBINANT DNA

SEC. 5. The Secretary shall, within ninety days after the date of enactment of this Act, promulgate regulations applicable to research relating to recombinant DNA which are

at least equivalent to those standards promulgated in regulations of the Secretary on July 7, 1976 (41 Fed. Reg. 131).

APPLICATION OF REGULATIONS

SEC. 6. The regulations promulgated under section 5 shall apply to all research involving recombinant DNA that is in or affects commerce, or that is carried on in any area subject to the jurisdiction of the United States.

INSPECTION AUTHORITY

SEC. 7. (a) In order to enforce this Act, officers, employees, or agents designated by the Secretary, upon presenting appropriate credentials to the owner, operator, or agent in charge of a facility engaged in research involving recombinant DNA are authorized—

(1) to enter, at reasonable times, any such research facility; and

(2) to inspect, at reasonable times and in a reasonable manner, such research facility and all equipment, materials, containers, records, files, papers, processes, controls, facilities, and other things therein, in order to determine whether such research facility is being operated in compliance with this Act, the regulations issued hereunder.

(b) Upon completion of any such inspection, the officer, employee, or agent making the inspection shall deliver or mail to the owner, operator, or agent in charge a written

report summarizing any conditions or practices observed by him or her which, in his or her judgment, indicate a violation of this Act or of any regulation.

INJUNCTIVE AUTHORITY

SEC. 8. (a) Whenever the Secretary has reason to believe that a continuation of any activity by a facility conducting research involving recombinant DNA would constitute a significant hazard to the public health, the Attorney General shall, at the Secretary's request, bring suit in the name of the United States in the district court for the district in which such facility or person is situated or in any district in which such person is doing business, to enjoin continuation of such activity and upon a proper showing, a temporary injunction or restraining order against continuation of such activity pending issuance of a final order may be granted without bond.

(b) Whenever any person has reason to believe that a continuation of any activity by a facility conducting research involving recombinant DNA constitutes a significant hazard to the public health, that person may bring suit in the United States district court in which such facility is situated to enjoin continuation of such activity and upon a proper showing, a temporary injunction or restraining order against continuation of such activity pending issuance of a final order may be granted without bond.

ATTORNEYS' FEES; FEES AND COSTS OF EXPERTS; LITIGATION EXPENSES

SEC. 10. (a) For the purpose of this section, the term "person" means any person defined by section 4(c) of this title and includes a class of individuals and any individual member of such class.

(b) Notwithstanding any other provision of law, any party or party intervenor in a civil action shall be entitled to recover reasonable attorneys' fees, fees and costs of experts, and other reasonable costs of litigation, including taxable costs, from the defendant if—

(1) the court affords such person the relief sought in substantial measure; or

(2) the court determines that such action served an important public purpose.

(c) Reasonable attorneys' fees, fees and costs of experts, and other costs of litigation awarded under this section shall be based upon prevailing market rates for the kind and quality of the services furnished.

VIOLATIONS AND PENALTIES

SEC. 11. (a) Any person who violates any provision of this Act or any regulation promulgated hereunder shall be guilty of a misdemeanor and shall, upon conviction therefor, be subject to imprisonment for not more than one year, or a fine of not more than $10,000, or both.

(b) Any person who willfully violates any provision of

this Act or any regulation promulgated hereunder shall be guilty of a felony and shall, upon conviction therefor, be subject to imprisonment for not less than one year, or a fine of not less than $10,000, or both.

(c) No research facility employer shall discharge any employee or otherwise discriminate against any employee with respect to his or her compensation, terms, conditions, or privileges of employment because the employee has—

(1) commenced, caused to be commenced, or is about to commence or cause to be commenced, a proceeding under this Act;

(2) testified or is about to testify in any such proceeding; or

(3) assisted or participated in or is about to assist or participate in such a proceeding or in any other action to carry out the purposes of this Act.

TITLE II—STUDY OF RECOMBINANT DNA RESEARCH AND TECHNOLOGY

PART A—NATIONAL COMMISSION FOR THE STUDY OF RECOMBINANT DNA RESEARCH AND TECHNOLOGY

ESTABLISHMENT OF COMMISSION

SEC. 201. (a) There is established a Commission to be known as the National Commission for the Study of Recombinant DNA Research and Technology (hereinafter in this title referred to as the "Commission").

(b) (1) The Commission shall be composed of thirteen members appointed by the Secretary of Health, Education, and Welfare. The Secretary shall select members of the Commission from individuals distinguished in the fields of medicine, law, ethics, theology, the biological, physical, and environmental sciences, philosophy, humanities, health administration, government, and public affairs; but six (and not more than six) of the members of the Commission shall be individuals who are or who have been engaged in recombinant DNA research and shall reflect diverse opinion on safety and appropriateness. In appointing members of the Commission, the Secretary shall give consideration to recommendations from the National Academy of Sciences and other appropriate entities. Members of the Commission shall be appointed for the life of the Commission. The Secretary shall appoint the members of the Commission within sixty days of the date of the enactment of this Act.

(2) (A) Except as provided in subparagraph (B), members of the Commission shall each be entitled to receive the daily equivalent of the annual rate of the basic pay in effect for grade GS–18 of the General Schedule for each day (including traveltime) during which they are engaged in the actual performance of the duties of the Commission.

(B) Members of the Commission who are full-time offi-

cers or employees of the United States shall receive no additional pay on account of their service on the Commission.

(C) While away from their homes or regular places of business in the performance of duties of the Commission, members of the Commission shall be allowed travel expenses, including per diem in lieu of subsistence, in the same manner as persons employed intermittently in the Government service are allowed expenses under section 5703 (b) of title 5 of the United States Code.

(c) The Chairman of the Commission shall be selected by the members of the Commission from among their number.

(d) (1) The Commission may appoint and fix the pay of such staff personnel as it deems desirable. Such personnel shall be appointed subject to the provisions of title 5, United States Code, governing appointments in the competitive service, and shall be paid in accordance with the provisions of chapter 51 of subchapter III of chapter 53 of such title relating to classification and General Schedule pay rates.

(2) The Commission may procure temporary and intermittent services to the same extent as is authorized by section 3109 (b) of title 5 of the United States Code, but at rates for individuals not to exceed the daily equivalent of the annual rate of basic pay in effect for grade GS–18 of the General Schedule.

COMMISSION DUTIES

SEC. 202. (a) The Commission shall carry out the following:

(1) (A) The Commission shall (i) conduct a study as to the appropriateness of continuing recombinant DNA research; (ii) conduct a comprehensive investigation and study to identify the basic ethical and scientific principles which should underlie the conduct of DNA research; (iii) develop guidelines which should be followed in such research to assure that it is conducted in accordance with such principles; and (iv) make recommendations to the Secretary (I) for such administrative action as may be appropriate to apply such guidelines to recombinant DNA research conducted or supported under programs administered by the Secretary, and (II) concerning any other matter pertaining to recombinant DNA research and technology.

(B) In carrying out subparagraph (A), the Commission shall consider at least the following:

(i) The protection of researchers and the general public from the dangers of recombinant DNA research.

(ii) The role of assessment of risk-benefit criteria in the determination of the appropriateness of research involving recombinant DNA.

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(iii) Appropriate guidelines for the conduct of recombinant DNA research.

(iv) Mechanisms for evaluating and monitoring the performance of review mechanisms established by the National Institutes of Health and National Cancer Institute and appropriate enforcement mechanisms for carrying out their decisions.

(v) Determine the status and extent of recombinant DNA research conducted in foreign countries and consult with such individuals as they deem appropriate.

SPECIAL STUDY

SEC. 203. The Commission shall undertake a comprehensive study of the ethical, social, and legal implications of advances in recombinant DNA research and technology. Such study shall include—

(1) an analysis and evaluation of scientific and technological advances in past, present, and projected recombinant DNA research and services;

(2) an analysis and evaluation of the implications of such advances, both for individuals and for society;

(3) an analysis and evaluation of laws and moral and ethical principles governing the use of technology in the medical practice and other fields;

(4) an analysis and evaluation of public under-

standing of and attitudes toward such implications and laws and principles; and

(5) an analysis and evaluation of implications for public policy of such findings as are made by the Commission with respect to advances in recombinant DNA research and technology and public attitudes toward such advances.

ADMINISTRATIVE PROVISIONS

SEC. 204. (a) The Commission may for the purpose of carrying out its duties under sections 202 and 203 hold such hearings, sit and act at such times and places, take such testimony, and receive such evidence as the Commission deems advisable.

(b) The Commission may secure directly from any department or agency of the United States information necessary to enable it to carry out its duties. Upon the request of the Chairman of the Commission, the head of such department or agency shall furnish such information to the Commission.

(c) The Commission shall not disclose any information reported to or otherwise obtained by it in carrying out its duties which contains or relates to a trade secret or other matter referred to in section 1905 of title 18 of the United States Code.

(d) Except as provided in subsection (b) of section

202, the Commission shall complete its duties under sections 202 and 203 not later than the expiration of the twenty-four-month period beginning on the first day of the first month that follows the date on which all the members of the Commission have taken office. The Commission shall make periodic reports to the President, the Congress, and the Secretary respecting its activities under sections 202 and 203 and shall, not later than ninety days after the expiration of such twenty-four-month period, make a final report to the President, the Congress, and the Secretary respecting such activities and including its recommendations for administrative action and legislation.

(e) The Commission shall cease to exist thirty days following the submission of its final report pursuant to subsection (d).

DUTIES OF THE SECRETARY

SEC. 205. Within sixty days of the receipt of any recommendation made by the Commission under section 202, the Secretary shall publish it in the Federal Register and provide opportunity for interested persons to submit written data, views, and arguments with respect to such recommendation. The Secretary shall consider the Commission's recommendation and relevant matter submitted with respect to it and, within one hundred and eighty days of the date of its publication in the Federal Register, the Secretary shall (1) deter-

mine whether the administrative action proposed by such recommendation is appropriate to assure the safety and appropriateness of recombinant DNA research conducted or supported under programs administered by him, and (2) if he determines that such action is not so appropriate, publish in the Federal Register such determination together with an adequate statement of the reasons for his determination. If the Secretary determines that administrative action recommended by the Commission should be undertaken by him, he shall undertake such action as expeditiously as is feasible.

Senator KENNEDY. I am delighted to have as our opening witnesses two of our colleagues: Senator Bumpers and Senator Metzenbaum.

We will start off with Senator Bumpers, who has introduced legislation of his own as has Senator Metzenbaum.

Senator Bumpers has been extremely interested in health issues and has been a great help to this committee on questions of immunization and other health programs.

STATEMENT OF HON. DALE BUMPERS, A U.S. SENATOR FROM THE STATE OF ARKANSAS

Senator BUMPERS. Thank you very much.

I especially appreciate your opening remarks, and I hope my testimony addresses most of the items that you have mentioned.

I think that the issue of recombinant DNA research is one of the most, if not the most, important issues that Congress is going to face. The advances in biological research that made this powerful new research technique possible are clearly some of the most important of our lifetimes, and it is incumbent on scientists, politicians, Governmental officials, and public citizens alike, to be responsible in the way we deal with both the research and the application of the knowledge gained through it.

There are two issues that I think can be separated in our considerations. The first is public safety, which is clearly the one requiring immediate attention. The scientists involved in this research deserve our profound gratitude for recognizing the potential dangers of their experiments, and then for making public their concerns and developing procedures to insure uniform safety standards.

Those efforts within the scientific community were unique in the history of the science. They point out not only the responsible attitudes of the researchers, but also the responsibility we have as representatives of the public.

The second issue we face is the way we apply the advances in technology and the knowledge gained during the research efforts. The questions that arise here are not specifically restricted to recombinant DNA research, but come rather from the broader field sometimes called genetic engineering.

Nevertheless, the power of the recombination technique is such that the possibilities of manipulating human characteristics, of cloning, and the like are increased substantially. I think this broader question must be addressed, but not necessarily or properly in the legislation we are considering.

My primary concern today is with the question of public safety, and that is what prompted my introduction of S. 621.

I raised three issues in that bill that seem critical to me—first, the nature and extent of guidelines and licensing for all research on recombinant DNA molecules; second, procedures for issuance of patents; and, third, liability in case of accident.

Each of these must be considered carefully in any legislation, because we must be certain that the public health and safety are preserved without unnecessarily restricting the development of science or the legitimate advances that could come from this particular research technique.

It is absolutely essential that legislation provide a clear margin of safety in setting regulations and certifying laboratories in which the research may be carried out.

There seems to be general agreement among the legislative proposals that there ought to be some form of licensing for laboratories, researchers, and individual experiments.

I feel that it is definitely advisable to license researchers and laboratories so that strict standards will apply whenever such research is contemplated. Individual research projects should also be licensed or registered in a way that makes them open to public scrutiny.

There have been several suggestions that special research centers be designated as national labs where all the most dangerous experiments would be conducted.

I would support legislation that provides for a very limited number of these national labs because this would limit proliferation of at least the most dangerous research for a period during which more knowledge about the hazards could be developed.

It would be inappropriate for legislation to be inflexible with respect to specific guidelines. New knowledge gained from experiments designed to define the hazards in the research will undoubtedly lead to modifications in any guidelines.

I do not want to talk today about specific safety problems or the current NIH guidelines. However, I would like to suggest that a central issue that deserves the attention of everyone is the use of *E. coli*—perhaps the most widespread bacterium in the world—as the major organism on which gene splicing is carried out.

It is true that there are efforts under way to develop strains of *E. coli* that are unable to live anywhere other than specific laboratory facilities. Nevertheless, it seems foolhardy to me to take any chance that a dangerous strain of *E. coli* could be produced, because the consequences could be enormous.

The next issue I would like to mention is the advisability of patents on the products or techniques developed during recombinant DNA research. Frankly, I am not convinced that there should be any patents given—especially on "new" organisms or plasmids—until we have had more time to evaluate the long-term implications of this technology.

If there are patents to be issued, then I feel strongly that full compliance with Federal guidelines should be mandatory. This should include prior disclosure of the research plans so that independent, technically competent people can evaluate both the advisability of the plans and the adequacy of safety precautions to be used.

S. 621 clearly makes that point, although I recognize that under current patent law, public disclosure of research, in advance, precludes the issuance of patents. Nevertheless, I think that prior review of research proposals is so important that we may have to sacrifice—if that is the right word—some commercial development in this field for a few years.

A final issue of some controversy is liability. S. 621 provides for strict liability, without regard to fault, for injury caused by research involving recombinant DNA. This section was not intended as a penalty or as a back door way to preclude the research by removing the possibility of insurance for research facilities, as some have claimed,

but was intended to serve rather as an inducement to comply with strict guidelines.

It might conceivably even encourage researchers or institutions to carry out procedures that are even more conservative on the side of safety. I would certainly consider an amendment that removed the strict liability provision for those researchers complying with NIH guidelines.

Throughout the deliberations related to both public safety and the long-term implications of the research, there must be public involvement at all levels. There has been some criticism of the current NIH guidelines for the lack of nonscientist involvement in their drafting, and that criticism may be well founded, despite the fact that it is probably a result of historical developments, and not the result of ill intent on the part of anyone concerned. I do not agree with such criticism.

The guidelines, or revisions, must be done with input from people and groups supporting as many different perspectives and backgrounds as possible.

The citizens of Cambridge have shown that intelligent laymen cannot only understand the complexity of the problems involved with this research, but can contribute reasonable and prudent suggestions for new safety procedures.

I congratulate the citizens of Cambridge, and particularly the review panel, for their efforts, and I am especially pleased that several of their representatives will be testifying here today.

The decisions we make as a society on this issue could easily determine the course of all future human generations, and those decisions therefore must be made by the people in the broadest sense of the word.

Finally, I would like to say a few words about the world of science in general.

Freedom of inquiry has been cherished by researchers in most of the world for several centuries, and certainly in this country during the explosion of scientific knowledge that occurred in the last century.

I would be the last person to suggest interfering unnecessarily with this freedom, and the legislation I have introduced was not conceived with that in mind. But I do feel that this particular issue raises completely new questions for those of us responsible for public welfare.

The human species has been evolving for probably more than 3 million years, and during that entire period we have learned about nature and developed new technologies—starting fire and the wheel. Thus far, there have been no fatal mistakes, but there is surely no assurance that the next bit of knowledge or the newest technological development will be harmless. Each day is a new one for the world, and it is increasingly clear that if we do not use reason in the development of human knowledge, the consequences could be tragic. We may all regret the way we have used advances in research on nuclear physics.

The lesson we must take from our previous experience is not that knowledge is bad, but that indiscriminate developments based on some knowledge can be very mistaken. It is our highest responsibility as representatives of the people of this country and of the world. For that matter, to evaluate the way recombinant DNA research is conducted, and to insure, as best we can, that the research is safe and that applications based on the technology are appropriate.

Mr. Chairman, I applaud your efforts here today, and I extend my thanks to you and the subcommittee for taking an interest in the DNA issue long before most Americans had even heard of it.

My concern is not that any particular bill passes unchanged, but rather that all the important issues are carefully considered and satisfactorily resolved.

Senator KENNEDY. I want to thank you, Senator Bumpers, for a very helpful statement. You identified some of the matters of great importance which we are going to have to wrestle with.

One is in the question of who and what should be licensed. Whether it should only be the facility or should the owners, operators and researchers be licensed as authorized by the license? In the administration's bill, it is targeted to facilities but not to identifiable persons.

I think your bill raises the important issue of compensation for injuries caused by this research. You propose a standard of strict liability. A question has been raised by many universities concerning the strict liability approach which you have outlined. They question whether it will virtually prohibit universities from being involved in this research.

Can a university conduct this research if it is subject to this kind of liability provision?

Would they risk conducting this work? If some accident did occur, under strict liability, the university might close down.

No private university would be able to do the research under a strict liability mechanism. That may or may not be so. But that is what the universities have said.

Senator BUMPERS. I understand they cannot buy insurance today, they cannot buy insurance right now.

Senator KENNEDY. How do you deal effectively with that? I do not know.

I am hopeful we will hear suggestions from universities as we move along in dealing with this legislation. It is an important question.

I agree with the thrust of what you are saying. But I think it raises another problem and could effectively halt university research in this area.

Senator BUMPERS. Mr. Chairman, I recognize the harshness of that proposal. Quite frankly, I am not hung up on it.

What I am really hung up on is that everybody, whether they are using Federal funds or not, comply with NIH guidelines. Once that is done, the strict liability part of it can go out.

Senator KENNEDY. One thing which we must focus on is the nature and structure of the regulatory body to implement this legislation. We must also emphasize the role of public in the decisionmaking of this body. I hope that Senator Metzenbaum would perhaps comment on this.

What we do not want is this responsibility fractionated between various agencies. We have already seen this problem in the preparation of the Administration's bill. Many agencies with different views and interests had bickered to have their point of view win out. I think we ought to have one agency responsibile for all recombinant DNA research. This includes careful monitoring, regulation and oversight of the research as it continues, so that we get a better understanding of

potential risks as well as the benefits from it. As good as interagency task forces are, I do not believe they should be the mechanism for this.

A second point concerning the structure of the regulatory commission which I think is terribly important is the composition of the decisionmaking body and the need for a majority of lay persons. We have seen this work well with the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research. It has functioned extraordinarily well in very complex and difficult issues. I think we ought to insure that there is a substantial number of lay persons on this recombinant DNA commission. I have suggested a majority. The implications from this research are as complex and mind boggling as some of the difficult issues handled so well by the Protection of Human Subjects Commission.

It is very, very important that the public be involved as decisionmakers on whatever regulatory agency is created for recombinant DNA as well as in studying the moral and ethical questions which are really in the long run the basic ones.

Senator BUMPERS. I wish we could have addressed those in a rational timely manner before this research ever got started. That is where the real flaw is. I think the gene is out of the bottle now. We have to cope with it as best we can.

Senator KENNEDY. Particularly in the area of not only research, but applicability of the research. I think there are obviously two major concerns.

One is the safety of the research; the other is its application and use.

Then I think you raised the question as to who ought to be guiding the application and use. If you consider the risks and benefits of the applications in terms of the public and society, it is of the magnitude of the breakthrough of the atom. In that situation the public, in terms of the Government, has the control of use and application in part because of national security. Who is going to establish and control the use and application of recombinant DNA research?

It is going to be the public?

What standards should be used?

Would it be big brother Government making these decisions with all the implications that this would have?

These are enormously tough, difficult, and complex problems that reach the essence of our civilization and society. You have raised some important concerns here.

Senator BUMPERS. Unfortunately, it is the sort of issue that puts most people to sleep.

Senator KENNEDY. Or wake them up. It ought to wake them up.

Thank you very much.

Senator BUMPERS. Thank you very much, Mr. Chairman.

Senator KENNEDY. We want to keep you informed and we hope your staff will work with us. We welcome that.

We are going to need the best judgment of thoughtful Members of the Senate as well as the public. We welcome your comments.

Senator BUMPERS. Thank you very much.

Senator KENNEDY. Senator Metzenbaum, this last discussion leads right into some of your cautious comments about the way we ought to proceed. I have had a chance to review your testimony. It is helpful and raises many of these same questions.

From personal experience, I know of your profound interest in health and health matters generally, and the stress you put on the delivery aspects of health as well as on these aspects in terms of research.

So I know you have given a lot of thought to these problems and questions, and we welcome your comments today.

STATEMENT OF HON. HOWARD METZENBAUM, A U.S. SENATOR FROM THE STATE OF OHIO

Senator METZENBAUM. I appreciate that, Senator Kennedy. I must say I am particularly pleased that it is you who has taken the leadership in connection with this subject. It was your leadership in developing the whole Commission on the Protection of Human Subjects.

This is in a different phase of that same kind of concern really in my opinion, and as Senator Bumpers so appropriately states, puts people to sleep, but at some point in our lives very well could awaken them with a kind of bombshell effect.

I think that is what is so frightening about this entire subject, the combination of the need to have recombinant DNA research and, at the same time, concern ourselves about the possible negative impacts it could have upon all of humanity.

I am pleased to be here today to testify in support of S. 945, a bill I introduced to establish a national commission to study whether continued DNA research is in the national interest, and if it is, to identify the basic policies which should underlie recombinant DNA research.

It would be easy to come forward with a piece of legislation to abolish or to terminate any such research. But that would obviously be totally inappropriate. Yet, the manner in which we are presently proceeding, and that is with having only a segment of the entire research being regulated by the NIH, and about 75 percent of it, or some such figure, being totally unregulated, creates an equally great problem.

Senator KENNEDY. You are absolutely right, Senator.

As you are probably aware, when initial guidelines came out, they only applied to NIH and they did not apply to other agencies. However, with reluctance the other agencies have agreed to apply the guidelines. The pharmaceutical industry in our earlier hearings, indicated they were going to conform to the NIH guidelines.

But you are absolutely right in terms of the need for broad applicability of the guidelines. I think it is a point well worth stressing.

Senator METZENBAUM. I think there are really two major thrusts of the legislation which I speak to here today.

One is the need to establish the National Commission to inquire into the whole area of recombinant DNA research, and even to the question of whether or not it should go forward at all.

If it does go forward, under what kinds of guidelines?

But, in the interim, to extend to the balance of the research that is being done in this country, the standards that have already been established by the NIH for research under their aegis.

This bill would extend these guidelines on recombinant DNA research to all persons or companies engaged in this area of study until such time as the Commission makes its recommendation.

I want to say, as I move forward, that Senator Bumpers presents legislation which has some extremely good points in it that are not covered in S. 945. I would hope as we move forward, as the committee moves forward on this subject, that out of the administration bill, which I think has some major gaps in it, such as the whole question of a National Commission, and such as the question of doing something immediately as pertains to NIH guidelines, but I think putting together these bills might be extremely helpful in a very meaningful program in this area.

As you well know, within the past decade alone, enzymes have been discovered that are capable of breaking DNA strands at specific sites and restructuring the broken fragments into new combinations, making possible the introduction of foreign genes into viruses and plasmids. These, in turn, can be used to insert foreign genes into bacteria and cells of different life forms. This technique holds great promise—new methods to produce insulin and interferon—cures for Tay-Sachs disease and sickle cell anemia—and vaccines for influenza and hepatitis.

It also holds great risks—new pathogenic viruses—alteration of the hereditary makeup of man—and the creation of man—and the creation of highly resistant plants which could wipe out other vegetation and animal life in its path.

The seriousness of this problem is reflected by the fact that, in 1974, the scientific community—without any governmental intervention—imposed a voluntary moratorium on virtually all recombinant DNA research until safety guidelines could be developed.

While these associated benefits and risks are well known to those in the field, the vast majority of the public remains totally in the dark.

Recombinant DNA research is a term probably not known by 95 percent of the American people, but it touches upon the very basics of life and its implications may have a profound impact on our future.

Despite the gravity of the nature of this research, the public has been left out of the decisionmaking process.

For example, both the scientific community and the NIH have directed their attentions toward making recombinant DNA safe without first asking whether man should be involved in the creation of new life forms?

I believe that American citizens have the right to participate in this decisionmaking process. For too long, these decisions have been reserved to scientists who perform the research, to private industries, and to government agencies.

The commission I have proposed will be staffed by members of the medical, scientific, legal, ethical, theological, environmental, and governmental community, and will take on the basic questions which recombinant DNA research raises. Is continued DNA research in the national interest?

What are the basic policies which should govern it?

What safeguards are necessary to protect citizens from the hazards of DNA research?

Such a commission would report back to the President and the Congress no later than 2 years after its formation with its recommendations and guidelines.

Let me point out to the chairman, in answer to a couple of your questions, three of your questions.

You asked the question, should there be a National Commission? My bill would answer that with an unequivocal, yes.

Your second question is, should the commission have the majority of nonscientists?

My answer to that in the legislation is an unequivocal yes. Thirteen member commission with a 7-to-6 ratio.

But it would be something else. Of the scientists who would be on the commission, three of them would be for the continuation of research, and three of them would have to be those who had publicly indicated their opposition to the continuation of the research.

And your question No. 3, should it study the moral and ethical questions involved?

I certainly answer that also with an unequivocal, yes.

Mr. Chairman, this commission is vitally necessary, but there is also a need to insure that the public is protected while the commission is making its study.

To this end, S. 945 would extend existing NIH safety guidelines to include all individuals or private companies now engaged or about to be engaged in recombinant DNA research.

Presently, the NIH guidelines apply only to research supported by Federal funds. The guidelines establish four standards of physical containment from P–1, the standard microbiological containment, to P–4, which involves such sophisticated techniques as monolithic walls, floors and ceilings, airlocks, and protective clothing and showering requirements.

In addition, the guidelines prescribe acceptable hosts and vector to be used in experiments and prohibit certain kinds of experiments, among which are the following (1) cloning of recombinant DNA with certain pathogenic organisms; (2) the use of certain oncogenic viruses; (4) the recombination of genes from patent toxins; and (4) the transfer of drug resistant traits.

These guidelines are at best a compromise between proponents and opponents of recombinant DNA research. It may turn out that far stricter regulations are necessary.

On the other hand, experience with DNA research may indicate that the NIH guidelines are adequate.

While I am not in a position today, Mr. Chairman, to say with absolute certainty which of these conclusions will be drawn, I do know that, in the last 5 years, mounting controversy has arisen in the scientific, medical and ethical communities over the potential benefits and liabilities of DNA research.

Despite this substantial controversy, nonfederally funded recombinant DNA research continues unchecked. Take the case of pharmaceutical companies.

At least five major pharmaceutical companies are engaged or about to be engaged in recombinant DNA research holding the same potential risk for disaster as that being conducted in the federally-funded research community. Yet, this research is totally unregulated by the NIH guidelines. Further, a recent check with public officials in three cities where pharmaceutical companies were engaged in recombinant DNA research disclosed that none of the public officials, or the NIH, had any knowledge that this kind of research was being conducted.

In testimony before the House Subcommittee on Health and Envi-

ronment, Dr. John Finklea, Director of the National Institute for Occupational Safety and Health, stated:

> We have found that private industries, including some of the corporations, conducting or planning recombinant DNA research, have not always voluntarily and quickly dealt with other occupational safety and health problems. For example, one corporation (DuPont) when contacted, assured NIOSH that medical records for recombinant DNA research workers would be made available and therefore no central registry of such workers was necessary. The corporation at the same time took legal action disputing our authority for access to employment and medical records necessary to evaluate health risks at a plant with an apparent increase in cancer among the workers.

It seems, Mr. Chairman, that the high degree of uncertainty surrounding this area necessitates that all recombinant DNA—including that conducted by private industries—be subject to NIH guidelines.

Mr. Chairman, there have been other proposals which address the question of recombinant DNA research. I would only urge upon the committee that we not accept the assumption that there ought to be a continuation of research in this area, but that there be a means employed by commission, if you please, as suggested by my legislation, but a commission from outside, including laymen and professionals, not an interagency commission or committee, which I believe would not bring that degree of objectivity to the subject that is needed in this area.

It has also been suggested that perhaps the Commission which you so ably brought into being, the Commission for the Protection of Human Subjects, might encompass this within its responsibilities. That at first blush would appear to be an excellent suggestion, except I am not at all certain that that Commission has those people with that kind of expertise in this field, and I do not know what your own thinking on the subject is.

Senator KENNEDY. We would like the moral and ethical questions to be studied by a panel of the human subjects and we would have a separate commission that would be made up of scientists and lay persons to be the principal mechanism for regulation of the research facilities.

We are all wrestling around with similar kinds of things.

Your statements and comments are very close in terms of the concerns, the stress on ethical and moral questions, and the independence of the decisionmaking process. You have got real harmony in terms of your approach.

One thing that we have tried to make sure is that in the fashioning of any kind of guidelines such as the NIH guidelines it is important to keep alive the regulatory process so that as we get additional kinds of information on the potential risks or potential dangers that we can modify or adjust the guidelines to accommodate the new knowledge. This is one of the concerns that I have about just sort of locking those guidelines in in terms of cement, and why I would hope that the whole regulatory process would be kind of a living process.

It would be a good deal different from other regulatory processes. But we are talking about a subject matter which is a good deal different.

Senator METZENBAUM. I very much agree with the approach of the chairman. Certainly the leadership you have given this country in the whole area of health needs, as well as so many areas, particularly

in the health needs field, makes me gratified that this subject is in your jurisdiction.

I would only close with one parting remark, and that is I think this matter of recombinant DNA research is moving forward so rapidly that I do believe we need to approach it with a sense of urgency and to move forward as rapidly as we can. And you have my commitment that if in any way I can be helpful in that process, I would like to do so, regardless of what the legislation, the particular legislation is.

Thank you, Mr. Chairman.

Senator KENNEDY. Thank you very much, Senator Metzenbaum, and particularly for raising and stressing these questions in terms of the moral and ethical considerations—other testimony we will have this morning does not even mention them—I think they are the key.

The importance of stressing the layperson's involvement in this whole decisionmaking process, the caution that you suggest, I mean these are all important lessons. I know you have given a good deal of thought to it.

We will consider your recommendations very seriously. I want to thank you very much.

As I say, we invite your people to stay in close touch with us. We will need all the help we can get.

Senator METZENBAUM. Thank you very much.

[The prepared statement of Senator Metzenbaum follows:]

TESTIMONY BY

Senator Howard M. Metzenbaum

on

S. 945:

Recombinant DNA Standards Act of 1977

Before the

Subcommittee on Health and Scientific Research

Committee on Human Resources

United States Senate

April 6, 1977

Mr. Chairman and members of the Subcommittee on Health and Scientific Research:

I am pleased to have the opportunity to testify in support of S. 945 -- a bill I introduced to establish a national commission to study whether continued DNA research is in the national interest, and if it is, to identify the basic policies which should underlie recombinant DNA research. S. 945 would also extend existing NIH guidelines on recombinant DNA research to all persons or companies engaged in this area of study until such time as the commission makes its recommendation.

I commend the distinguished chairman, of this subcommittee, Senator Kennedy, for moving ahead on this important topic. The need to provide comprehensive guidelines governing recombinant DNA research is only now coming to light.

Within the past decade alone, enzymes have been discovered that are capable of breaking DNA strands at specific sites and restructuring the broken fragments into new combinations, making possible the introduction of foreign genes into viruses and plasmids. These, in turn, can be used to insert foreign genes into bacteria and cells of different life forms. This technique holds great promise -- new methods to produce insuline and interferon -- cures for tay-sachs disease and sickle cell anemia -- and vaccines for influenza and hepatitis. It also holds great risks -- new pathogenic viruses -- alteration of the hereditary makeup of man -- and the creation of highly resistant plants which could wipe out other vegetation and animal life in its path. The seriousness of this problem is reflected by the fact that in 1974, the scientific community -- without any governmental intervention -- imposed a voluntary moratorium on virtually all recombinant DNA research until safety guidelines could be developed.

While these associated benefits and risks are well known to those in the field, the vast majority of the public remains totally in the dark.

Recombinant DNA research touches upon the very basics of life and its implications may have a profound impact on our

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future. Despite the gravity of the nature of this research, the public has been left out of the decision-making process. For example, both the scientific community and the NIH have directed their attentions toward making recombinant DNA safe without first asking whether man should be involved in the creation of new life forms?

I believe that American citizens have the right to participate in this decision-making process. For too long these decisions have been reserved to scientists who perform the research, to private industries, and to government agencies.

To accomplish this, I have proposed a national commission for the study or recombinant DNA research and technology. This proposal is based on a fundamental premise: some decisions are so broad in their impact that they are not solely scientific decisions but social decisions.

Two examples where citizens have effectively participated in this kind of broad decision making are:

1) The National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (Ryan Commission) which was initiated by Senator Kennedy. This Commission has brought about increased public understanding of, and support for, necessary human experimentation.

2) The joint Mental Health and Mental Retardation Advisory and Review Commission, created by the Ohio 111th General Assembly to involve the public in the ongoing review and development of patient care and research and training programs in the Ohio Department of Mental Retardation.

Each of these special boards or commissions has demonstrated the value of broad citizen participation in difficult public policy areas. S. 945 is one further step in this valuable and necessary participation.

The commission I have proposed will be staffed by members of the medical, scientific, legal, ethical, theological, environmental, and governmental community, and will take on the basic questions which recombinant DNA research

raises -- Is continued DNA research in the national interest? -- What are the basic policies which should govern it? -- What safeguards are necessary to protect citizens from the hazards of DNA research? Such a commission would report back to the President and the Congress no later than two years after its formation with its recommendations and guidelines.

Mr. Chairman, this commission is vitally necessary, but there is also a need to insure that the public is protected while the commission is making its study. To this end, S. 945 would extend existing NIH safety guidelines to include all individuals or private companies now engaged or about to be engaged in recombinant DNA research. Presently, the NIH guidelines apply only to research supported by Federal funds. The guidelines establish four standards of physical containment from P-1, the standard microbiological containment, to P-4, which involves such sophisticated techniques as monolithic walls, floors and ceilings, airlocks, and protective clothing and showering requirements. In addition, the guidelines prescribe acceptable hosts and vector to be used in experiments and prohibit certain kinds of experiments, among which are the following: 1) cloning of recombinant DNA with certain pathogenic organisms; 2) the use of certain oncogenic viruses; 3) the recombination of genes from potent toxins; and 4) the transfer of drug resistant traits.

These guidelines are at best a compromise between proponents and opponents of recombinant DNA research. It may turn out that far stricter regulations are necessary. On the other hand, experience with DNA research may indicate that the NIH guidelines are adequate. While I am not in a position today, Mr. Chairman, to say with absolute certainty which of these conclusions will be drawn, I do know that in the last five years mounting controversy has arisen in the scientific, medical, and ethical communities over the potential benefits and liabilities of DNA research. Despite this substantial controversy, non-Federally funded recombinant DNA research continues unchecked. Take the case of pharmaceutical companies:

> At least five major pharmaceutical companies are engaged or about to be engaged in recombinant DNA research holding the same potential risk for disaster as that being conducted in the Federally-funded research community. Yet, this research is totally

unregulated by the NIH guidelines. Further, a recent check with public officials in three cities where pharmaceutical companies were engaged in recombinant DNA research disclosed that none of the public officials, or the NIH, had any knowledge that this kind of research was being conducted.

In testimony before the House Subcommitte on Health and Environment Dr. John Finklea, Director of the National Institute for Occupational Safety and Health stated:

"We have found that private industries, including some of the corporations conducting or planning recombinant DNA research, have not always voluntarily and quickly dealt with other occupational safety and health problems. For example, one corporation (DuPont) when contacted assured NIOSH that medical records for recombinant DNA research workers would be made available and therefore no central registry of such workers was necessary. The corporation at the same time took legal action disputing our authority for access to employment and medical records necessary to evaluate health risks at a plant with an apparent increase in cancer among the workers."

It seems, Mr. Chairman, that the high degree of uncertainty surrounding this area necessitates that all recombinant DNA -- including that conducted by private industries -- be subject to NIH guidelines.

Mr. Chairman, there have been other proposals which address the question of recombinant DNA research. The administration bill, recently introduced, suffers from one basic defect -- it assumes the necessity and propriety of this research. If we enact laws to control the research and production of recombinant DNA in the detail proposed -- getting into the minutiae of licensing and the number of facilities -- we have assumed that the research should be continued. I believe that such a conclusion without a complete study by the commission I have outlined would be premature.

It has also been suggested that perhaps the Ryan Commission should be the vehicle to examine the complex issues. This Commission was designed and staffed to examine human, not laboratory research. Not one of the commissioners, nor any of its professional staff members, are experienced in

recombinant DNA research. The unique and highly complex nature of DNA research requires that the composition of any commission studing this area reflect those knowledgeable in DNA research.

DNA research may have serious implications on our lives. I believe S. 945 addresses this question with the critical sense of urgency necessary to protect the public interest.

Senator KENNEDY. Our next witness is a good personal friend of mine, the Governor of the Commonwealth of Massachusetts.

There has been considerable controversy in Massachusetts concerning recombinant DNA.

We will hear later from witnesses from Cambridge, Mass., who dealt with this controversy in a very meaningful and productive way.

Governor, we are happy to have you here this morning commenting on these important issues.

Governor Byrne notified us last evening that because of flooding in New Jersey he would be unable to attend the hearing. The Governor has asked that his statement be included in the record, which will be made a part of the record.

First of all, Governor, bring us news from Massachusetts.

Is Nantucket and Martha's Vineyard still part of our Commonwealth?

Governor DUKAKIS. Yes, Mr. Chairman, and so is Fort Devens. I am delighted to say all is well in the Commonwealth this morning.

Senator KENNEDY. We are delighted you are here this morning. We hope that in your comments that one of the areas we will be interested in is if there is legislation for national standards what your attitude is on the flexibility that we ought to give to the States in perhaps establishing stricter guidelines and perhaps what roles we ought to permit in terms of communities, their involvement.

I think Massachusetts has had a very unique experience and a very successful one. I think there is a very great interest in it. I think what happened up there may very well set the pattern for the rest of the country in this important area of the health policy research.

We are looking forward to your testimony.

STATEMENT OF HON. MICHAEL S. DUKAKIS, GOVERNOR, COMMONWEALTH OF MASSACHUSETTS

Governor DUKAKIS. Thank you very much, Mr. Chairman. Thank you for inviting me.

This committee, as you so well know, is taking the first step in tackling an issue which has been bitterly dividing the scientific community and certainly baffles the rest of us.

The much broader constituency which is making the decisions of the scientists is equally divided. There is probably only one thing that those who are familiar with research on recombinant DNA will agree, and that is the precedent-shattering significance of these discoveries.

Genetic manipulation to create new forms of life places biologists at a threshold similar to that which physicists reached when they first split the atom. It is, by all accounts, a scientific breakthrough of such magnitude that no one is sure exactly where it will lead.

This very uncertainty about the potential nature of the results of the recombinant DNA research is central to the controversy.

The claims and charges by opposing sides in the debate around the issue only underscore the speculative nature of the entire question. Those who want the research to proceed quickly insist the result of this research may revolutionize agriculture, simplify pollution control, lead to cures for cancer and other diseases, and permit factory production of unlimited amounts of human insulin.

Their critics voice concern about worldwide epidemics that cannot be controlled with available antibiotics, of cancer viruses spreading through human or animal populations, or of ecological disasters so far unimagined because the causative organism has not yet been created.

But, as I understand the debate from the point of view of a non scientist and a layman, who I hasten to add has no claim to expertise, Mr. Chairman. As a matter of fact, if I had not gotten a D minus in physics at Swarthmore College, I probably would not be here in a political role. I might be here in some other role.

No one knows where this research will lead, while everyone agrees, in vastly different ways, that it will probably affect all of our lives.

Present realities indicate that this research will continue. Laboratories around the world are proceeding with varying degrees of control, and I think it is fair to say that the genie is out of the bottle.

What remains to be done here is to establish the precautions that must be taken to minimize the potential dangers.

The detailed guidelines published last June by the National Institutes of Health applied only to those institutions receiving research grants from the NIH. The legislation now before this committee includes binding regulations based on those guidelines.

I want to associate myself with the remarks you made just a minute ago which means this has to be living and evolving regulatory process and not one that is fixed in cement for all time or for the foreseeable future.

And those regulations apply to all recombinant DNA research performed in this country—private as well as public. The legislation also estabishes a mechanism by which the controls can be enforced. Both the extension and enforcement provisions of this legislation are necessary and perhaps already overdue. I endorse both provisions completely.

The decisions you make here will have worldwide repercussions. Publication of the NIH guidelines last year resulted in increased concern and controls in other countries where similar research is being performed.

Senator KENNEDY. I am glad you mention this, Governor. It is the first time it has been mentioned during the course of the day. But there is recombinant DNA research taking place around the world.

We are going to ask the Secretary, who is appearing after you, about what efforts we are making in terms of the whole international health area and to address this issue with the World Health Organization in early May.

It is a very, very profound question. That is really why that what happens here is so important—other countries can obviously make their own judgment—but clearly the United States is in the lead in terms of research, and hopefully we can be the leader in terms of coming up with rational and thoughtful solutions.

Governor DUKAKIS. I would agree, Mr. Chairman.

I do think it gives us an opportunity to undertake some leadership in the international field which is positive and affirmative and helpful and desirable. And that is a role we have not always played in the past.

So it does give us a real opportunity to do that.

Considering all of these things, I feel strongly that the proposed legislation you report out of this committee must establish Federal standards of control based on the NIH guidelines.

I do want to address myself, if I can, for just a minute or two, Mr. Chairman, to the question of preemption.

I think it would be a great mistake for this legislation, either directly or by implication, to deny to States or to local communities the power to involve themselves in this issue, or to go further if they so desire.

I am relieved that the legislation, as now written, does not do so, and seems to include a sensible approach to the question of State and local initiatives in this area.

For if this legislation were to preempt any possible involvement or regulation or supervision at the State or local level, it seems to me it would set a very dangerous and unfortunate precedent by, in effect, telling the citizens of the area closest to the potential danger that this part of their lives is beyond their control and they can do nothing and must stand back and let someone in Washington do it for them.

I believe that the Congress and the administration can trust in the ability of the general public and the elected representatives and citizens at the State and local level to act responsibly and rationally to concerns that directly affect them all. In fact, if this or similar legislation were to remove such authority, then it would also very quickly restrict public debate and cut off a very legitimate public interest in what public and private research institutes are doing.

You mentioned, Mr. Chairman, the example of Cambridge, Mass. Obviously, that is one of the reasons I am here.

Cambridge was the first city in the Nation to insist on playing a role in determining whether or not the controversial experiments should be done. It was not easy, and it was a little messy, and there was a lot of sound and fury.

As you know, some very long and warm debate during the course of a long and warm summer in that city.

The city council of that city called for the establishment of an Advisory Committee, Cambridge Experimentation Review Board, which is made up of nonscientists who knew little or nothing about DNA before they were appointed to the board.

The members of the review board were keenly aware throughout the deliberations of their role in setting a precedent for citizens' participation in science policymaking.

The review board's report, released January 5, recommended that the research be allowed to continue under the NIH restrictions, with the extra precautions that a specially weakened strain of bacteria be used for any "moderate risk" experiments.

In addition, they urged that a Cambridge Biohazards Committee should be set up to oversee regulatory and monitoring procedures carried out by the university biohazard committees.

And, finally, the committee recommended passage of a city ordinance to give these recommendations the force of law.

I understand Cambridge City Councillor Dave Clem will testify at these hearings. I think I saw Dan Hayes, who is chairman of that board, and did a superb job of chairing and guiding it to its conclusion.

I think they will agree with me that the measured approach which the city of Cambridge took may prove to be a model of effective community review of recombination research and perhaps, as time goes on, other types of scientific research as well.

In short, it seems to me that what we need is Federal legislation to set some broad national standards which will be adhered to wherever this research is being done, but we also need reasonable control at the State or local level, and the Cambridge model suggests that we can have it.

Draft language in the bill seems to me to be a nice accommodation between Federal interests, on the one hand, and the interest, at least in some cases, of States and cities who want to either go further or involve themselves in some broader way in this particular issue.

It does seem to me without any way ignoring the potential real benefits of this research, and there are many, Mr. Chairman, that this is an issue where we really cannot err on the side of caution.

You are particularly familiar with the fact that in the past 20 years we have been assaulted with man-made environmental hazards, ranging from DDT to PCB's and PBB's to vinyl chloride, kepones and freon, which I am sure a lot of us understand, but certainly give us grave concern.

Let me simply conclude, Mr. Chairman, by saying that when faced with potential threat which may possibly be wider ranging and more pervasive than any of those things, I believe it would be folly for the Federal Government to say, in effect to those of us at the State and local level, we will protect you this far but, under no circumstances, will we allow you to protect yourselves further.

Thank you very much, Mr. Chairman.

I would be happy to respond as best I can to any questions.

I do think that people from Cambridge who spent so much time and effort on this are the folks that really can explain in greater detail to you and to everyone here exactly what happened in that community and why we think that the ultimate response was responsible and constructive and probably set a standard for the Nation in that particular community which will help guide the entire country in precisely what we do.

Senator KENNEDY. Thank you very much, Governor. It is very helpful testimony, and particularly in this area, the relationship between the Federal Government and the States and the local communities.

As I mentioned earlier, there has been a relationship which has had unique circumstances in our own Commonwealth and worked out very satisfactorily.

I think, because of it, it will have an important impact in terms of phrasing that particular aspect of the legislation that deals between Federal and States.

At the present time, do you have any legislation that is before the State now?

Has anyone introduced or fashioned any legislation?

Governor DUKAKIS. I do not know whether someone has something kicking around in the hopper. There might have been a brief attempt to do that. I have not, as Governor, filed any such legislation at this point, and obviously we have been working closely with you on these efforts down here.

Senator KENNEDY. It would be difficult until you know the shape of our approach here. You can do it temporarily, obviously, but it would seem to me this would be the process where we would hope to be able to act before May 15.

We obviously look forward to working with you.

Governor DUKAKIS. I know the State of California has had actual legislation in its legislature, and you are probably familiar with it. We have not gone that far.

Here again, I think it is another example of how there is not exclusive wisdom at the State level either. This was a case where one particular community, long before anybody in our statehouse was really sensitive or aware, was dealing with the problem, and, in effect, Cambridge was teaching us some things about how to deal with the problem as well as those at the Federal level.

Again I think it is a good example of how, so long as we are all cooperating together, some initiative and some room for development of those initiatives at the local level as well as the State level, and it is very, very important in what happens here.

Senator KENNEDY. Let me ask you, Do you think that we ought to permit a local community to prohibit research in a given facility if they make that decision?

Governor DUKAKIS. I would not have any problem with that basic proposition.

Senator KENNEDY. I do not either.

Governor DUKAKIS. Two reasons.

To begin with, I doubt very much if that decision, if it were to be taken at the local level, would seriously cripple the DNA research. It is very easy to move next doór or down the street. Some people have expressed concern at that kind of decision taken by particular community would trigger all kinds of alarms and concerns in other communities.

As I often say to my deputies these days, democracy is held, but it is the only system we have. And occasionally those kinds of things happen from community to community.

But I do not see that type of decision, if it were to be made, would be in any way significant in a national sense in terms of choking off research elsewhere.

Personally, I doubt very much that any community would make that decision. I have got a lot of faith and confidence in the judgment of our people, and Cambridge, Mass., who are probably more sensitive to the human condition per square foot than any other community I know, and proceeded as it did, responsibly and constructively, and I honestly do not think that any community would make that decision, but if they chose to do so, I would not propose, either by State or Federal legislation, to bar that kind of judgment.

It seems to me that is one they ought to be able to make.

Senator KENNEDY. We did not get into the discussion with you on the important kinds of moral and ethical questions that are going to be raised by such research. I know these are matters that you are concerned and interested about, these enormously important kinds of issues and questions.

I think we will value very highly your recommendation on this issue affecting States and Federal Government. I think it is very clear and convincing as far as I am concerned, and we are hopeful that whatever legislation we get out will incorporate your recommendations.

Governor DUKAKIS. Thank you very much.

[The prepared statements of Governors Byrne and Dukakis follow:]

Recombinant DNA Research

Senate Human Resources Committee Subcommittee on Health

Testimony of the Honorable Brendan T. Byrne
Governor, State of New Jersey

I come before this committee today with the conviction that federal legislation is required to deal with the problems and valid concerns to which recombinant DNA research has given rise. I appreciate this opportunity to share with you the reasons I feel this type of national legislation is essential. I would also like to discuss with you the underlying principles upon which such legislation should be structured, and suggest to you certain provisions which I believe should be included in this legislation.

The arguments in support of having legislation enacted at the national level seem overwhelming. States simply have not the expertise and resources to deal with these issues and exercise proper oversight. And even if the individual states were to attempt to develop the expertise and were in fact successful in doing so, the inevitable result would be a wasteful duplication of effort and expense not merely in the development of standards but also in the enforcement of those standards by inspectors who would need to be highly trained to inspect perhaps only one or two research laboratories within a given state. Moreover, if the states must go their separate ways in this field, there will inevitably be developed varying standards in different states. Private firms with laboratories in more than one state will face the prospect of having to meet different standards in different labs; individual private firms and even educational institutions may be tempted to shop for jurisdictions where greater leeway is given in carrying on recombinant DNA research. And citizens in some states may suspect the safety standards are too lax in their states in contrast to others, while some researchers may believe that the restrictions imposed on them may be too stringent in contrast to other states.

Gentlemen, I hope that you have little doubt that the resources of the federal government must be brought to bear to insure as best we can that biological research does not threaten the well being of our citizens.

But I do not wish to be misconstrued. I believe that we must permit recombinant DNA research to continue. I am sure that the benefits have been revealed to you by other witnesses and in your own study of this issue. Aside from those experiments which are so dangerous that they must be strictly forbidden and for which it has been determined that no physical containment is sufficient, recombinant DNA research and the benefits offered thereby must be pursued.

The difficulty in drawing the line is substantial. As Governor of New Jersey, circumstances afford me a special insight into this problem. The Governor's residence in New Jersey is Morven, which is located not in Trenton but in nearby Princeton. In the last few months, the citizens of Princeton and the scientists at Princeton University have been grappling with the problem of what safeguards to place on the recombinant DNA research being carried on at the

University. It was the scientists themselves who suggested a temporary moratorium on this research until proper safeguards could be established and, in large measure, it was the scientists who brought the problem to the attention of the citizens. After extensive review, the University Research Board delivered to the President of Princeton University its "Recommendations for the Conduct of Research with Biohazardous Materials." This report is currently under review. In town meetings and from recent newspaper articles, it is clear that the scientists at Princeton are anxious to continue their research but recognize the valid concerns of the Princeton townspeople; on the other hand, Princeton citizens recognize the need for continuing this type of research, but, nonetheless express their concern that safety factors be adequate.

We have given some consideration to developing State legislation in this area; however, for the reasons I have already noted, we now urge federal legislation which will effectively protect the citizens of New Jersey and other states where research involving DNA recombination is or will be occurring. The legislation you develop must convince the citizens of all 50 states that when this type of research is being carried on, and especially when it is being carried on in their immediate geographic area, they are not assuming any risk from the performance of that activity. In a matter such as this, it is not merely the reality but also the appearance of safety which is important. But where the appearance of safety could only be increased through complete cessation of experimentation, citizens must realize the benefits which recombinant DNA research may bring in the future.

The restrictions imposed on scientific researchers must be reasonable. We should recall that, in great measure, it was the scientists who brought the dangers of recombinant DNA research to our attention. We must not impose restrictions and requirements that will tempt researchers to avoid compliance with the law; we should keep in mind that while we can make earnest and able efforts to police the standards we establish, to a great degree, we must rely on the scientists to carefully control the scope of the experiments they carry on in their individual labs. We should impose restrictions which are appropriate, which means, in part that those restrictions should not be needlessly burdensome. With these thoughts in mind, I would offer certain suggestions for legislation in this area. I would note, in passing, that I rely on the finding of the Interim Report of the Federal Interagency Committee on Recombinant DNA Research that existing legislation is not sufficient and that new legislation is needed to impose all of the requirements and to cover all of the research on DNA research which should be covered.

The basic element of any legislation must be the adoption of the National Institute of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules or of standards as or more stringent than these guidelines; these standards should apply to all research and production involving recombinant DNA, whether such research or production is governmentally or privately funded. The secretary of HEW should have the authority to modify these standards, and in addition, to adopt new standards. An important issue before you is whether a moratorium should be imposed on further recombinant DNA experimentation and production until appropriate standards are developed and promulgated by the Secretary. I believe you should study this issue very carefully. At this point, and pending

further review of this difficult issue, it seems to me that the NIH guidelines should be statutorily imposed immediately. If a researcher or producer is capable of meeting those standards, he should be permitted to continue with the understanding that the Secretary may, within a certain period of time, impose new and perhaps more stringent standards. It would seem unfair to require a researcher who is performing under clearly safe conditions to perhaps lose the fruits of his research efforts because of an absolute moratorium; however, if activity is being carried on under conditions which would violate NIH guidelines and which thus pose a threat to the safety of people, such activity must either be immediately conformed to meet NIH standards or else cease. Consideration should also be given to whether, during this initial period following the effective date of the statute to be enacted, local standards which are more stringent than NIH guidelines should prevail.

All facilities at which the more dangerous forms of recombinant DNA research are carried on should be licensed. The legislation should set forth criteria for determining which forms of research should lead to the requirement that the facility be licensed and the purposes for which a facility may be licensed. At least in the initial legislation in this area, there should not be a limit set on the number of such facilities which may be licensed; the Secretary can insure that safety standards are sufficiently stringent for all such facilities. As I will note later in my remarks, a Commission should be established to deal with recombinant DNA research; among its duties, this Commission should evaluate the need to curb any dangerous proliferation of licensed facilities. It may be anticipated that the cost of building facilities to meet the standards to perform the most dangerous types of research will serve to minimize the number of such facilities.

All of the more dangerous projects should be registered in a national registry. The Secretary of HEW should have the authority to determine which classes of projects do not require registration. Among its purposes, the registration procedure should serve to insure that the researcher and all workers involved in the project have been properly trained to perform their work safely and that they are aware of the nature of their work and any potential health risk. The registration requirements should be structured to protect the proprietary rights of the researchers. Any provision which would permit institutions, firms, or individuals to have their projects exempt from registration and disclosure because of national security reasons should be critically reviewed. The burden of justifying such exemptions should rest with the applicant.

Each public or private institution at which recombinant DNA research is being carried on should have a biohazardous research committee to review programs being carried on at that institution; federal funding should perhaps be provided for the operation of these committees.

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Federal inspectors should be specifically trained to inspect facilities at which recombinant DNA research is being carried on to ensure compliance with standards and with other statutory requirements. If state inspectors are to be employed, they should be trained at federal educational centers or at federally approved facilities. Federal funding to maintain these inspectors should be provided. It should be made clear that in order to carry out appropriate inspections, these inspectors have right of entry into premises where recombinant DNA research or production is being performed. Employees who come forward with information or evidence to assist in the enforcement of this legislation should be appropriately protected from being fired or other forms of reprisal. Appropriate civil and criminal penalties should be available for failure to comply with the statutory requirements, and such penalties should be especially severe for any individual who carries on a project which is absolutely prohibited because of the extreme risk involved. The Secretary should also be able to seek injunctive relief to halt a project which is inherently dangerous or is being carried on in a dangerous manner.

The legislation should establish a commission on recombinant DNA research. This commission should study the broader long term issues involved in this type of research. The commissions should also evaluate this initial legislation and, at some designated point in the future, perhaps in two years, make recommendations for revision.

Finally, the federal legislation should provide that it pre-empts any state regulation in the field of recombinant DNA research except that, where local community or where State standards are as or more stringent than the federal standards, the Secretary should have the authority to permit the local standards to apply.

The legislation regarding recombinant DNA research should also make clear that, while other federal agencies may regulate this type of research under appropriate statutory authority, the standards and requirements imposed under this legislation will prevail if conflicts arise between these standards and requirements and those promulgated by another federal agency.

Because of the substantial and justified concern which has arisen over the risks involved in recombinant DNA research, Congress should act quickly to abate this concern while permitting this valuable research to proceed in a manner which assures the public safety.

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FROM THE OFFICE OF GOVERNOR DUKAKIS

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FOR RELEASE 10:00 A.M. WEDNESDAY,
APRIL 6, 1977

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TESTIMONY OF GOVERNOR MICHAEL S. DUKAKIS OF MASSACHUSETTS
BEFORE THE U. S. SENATE SUBCOMMITTEE
ON HEALTH AND SCIENTIFIC RESEARCH
WASHINGTON, D. C.
WEDNESDAY, APRIL 6, 1977 - 10:00 A.M.

Mr. Chairman, Committee Members, Ladies and Gentlemen,

This Committee is taking the first steps in tackling an issue which bitterly divides the scientific community. The much broader constituency which is making the decisions of the scientists is equally divided. There is probably only one thing that those who are familiar with research on recombinant DNA will agree and that is the precedent-shattering significance of these discoveries.

Genetic manipulation to create new forms of life places biologists at a threshold similar to that which physicists reached when they first split the atom. It is, by all accounts, a scientific breakthrough of such magnitude that no one is sure exactly where it will lead. This very uncertainty about the potential nature of the results of recombinant DNA research is central to the controversy.

The claims and charges by opposing sides in the debate around the issue only underscore the speculative nature of the entire question. Those who want the research to proceed quickly insist the result of this research may revolutionize agriculture, simplify pollution control, lead to cures for cancer and other diseases, and permit factory production of unlimited amounts of human insulin. Their critics voice concern about worldwide epidemics that cannot be controlled with available antibiotics, of cancer viruses spreading through human or animal populations, or of ecological disasters so far unimagined because the causative organism has not yet been created.

But as I understand the debate from the point of view of a non-scientist, a layman with no claim to expertise in the field, all of this is only speculation. No one knows where this research will lead, while everyone agrees, in vastly different ways, that it will probably affect all of our lives.

Present realities indicate that this research will continue. Laboratories around the world are proceeding with varying degrees of control and I think it is fair to say that the genie is out of the bottle.

What remains to be done here is to establish the precautions that must be taken to minimize the potential dangers.

The detailed guidelines published last June by the National Institutes of Health applied only to those institutions receiving research grants from the NIH. The legislation now before this committee includes binding regulations based on those guidelines. And those regulations apply to all recombinant DNA research performed in this country -- private as well as public. The legislation also establishes a mechanism by which the controls can be enforced. Both the extension and enforcement provisions of this legislation are necessary and perhaps already overdue. I endorse both provisions completely.

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The decisions you make here will have world-wide repercussions. Publication of the NIH guidelines last year resulted in increased concern and controls in other countries where similar research is being performed. It is undoubtedly fair to assume that the decisions you make in turning these guidelines into enforceable regulations will also be carefully watched and acted on elsewhere.

Considering that, I feel strongly that the proposed legislation you report out of this Committee must establish federal standards of control based on the NIH guidelines. These controls should not pre-empt state and local regulations regarding recombinant DNA research. I am relieved that the legislation as it is now written does not do so. For such a pre-emption would, I believe, set a dangerous and unfortunate precedent by telling the citizens of the areas closest to the potential danger that this part of their lives is beyond their control, that they can do nothing and must stand back.

I believe that you must trust in the ability of the general public and elected representatives on the state and local level to act responsibly and rationally to concerns that directly affect them all. Legislation removing their authority would also very likely restrict public debate and cut off legitimate public interest in what the private and public research institutes are doing.

When the scientists conducting the research are so divided, that should set off a clear warning signal that the situation needs to be watched carefully. These experiments are likely to break into uncharted territory so quickly that I believe it would be prudent and wise to allow the closest authorities to have a say in the standards the experiments must meet.

The example set recently in Cambridge, Massachusetts, is a useful one. Cambridge was the first city to insist on playing a role in determining whether or not the controversial experiments should be done. The city council called for the establishment of an advisory committee, the Cambridge Experimentation Review Board, which was made up of non-scientists who knew little or nothing about DNA before they were appointed to the board.

The members of the review board were keenly aware throughout their deliberations of their role in setting a precedent for citizens'participation in science policy making.

The review board's report, released January 5, recommended that the research be allowed to continue under the NIH restrictions, with the extra precautions that a specially weakened strain of bacteria (labeled EK 2 in the NIH guidelines) be used for any "moderate risk" (P03) experiments. In addition, they urged that a Cambridge Biohazards Committee should be set up to oversee regulatory and monitoring procedures carried out by the university biohazard committees. And finally the committee recommended passage of a city ordinance to give these recommendations the force of law.

I understand that Cambridge City Councillor David Clem will also testify at these hearings. I think he will agree that this measured approach taken by the CERB may prove to be a model of effective community review or recombination research and perhaps other types of scientific research as well.

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In closing I want to re-emphasize my belief in the importance of allowing state and local governments to set more stringent controls than the federal standards. Elimination of control by local authorities would in all likelihood mean that research institutions will be less closely watched by the people most likely to be affected by their carelessness.

We need the federal legislation to set a minimum standard. I think we also need reasonable control at the state or local level and the Cambridge model suggests that we can have it.

This is an issue where we really cannot err on the side of caution. In the last twenty years we have been assaulted with man-made environmental hazards ranging from DDT to PCBs and PBBs to vinyl chloride, kepones and Freon.

When faced with a potential threat which may possibly be wider-ranging and more pervasive than any of these it would be folly for the federal government to say "We will protect you this far. You may not protect yourself further."

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Senator KENNEDY. Our next witness will be Secretary Califano.

Secretary Califano has to leave by 11 o'clock. You will be out of here by 11, but we are going to have to recess for about 4 minutes.

[Short recess.]

Senator KENNEDY. We will come to order.

We are delighted to have the Secretary. He is accompanied by Dr. Fredrickson, Director of NIH, and Ken Levine, who is Deputy Assistant Secretary for Legislation.

Now, I have had a chance to go through the testimony, Mr. Secretary. I do not know how you would like to proceed.

I have got some questions, and I know Dr. Fredrickson will be able to remain afterward.

I think we can move along quickly, but if you would like to give a brief comment, and then we will develop some of these thoughts.

STATEMENT OF HON. JOSEPH A. CALIFANO, JR., SECRETARY, DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE, ACCOMPANIED BY DR. FREDRICKSON, DIRECTOR, NIH, AND KEN LEVINE, DEPUTY ASSISTANT FOR LEGISLATION

Secretary CALIFANO. Mr. Chairman, I might just say briefly that we will submit the statement for the record.

Dr. Fredrickson is with me. He has spent many, many months, as you know, on these problems.

I am conscious that every journey into the unknown, which is what recombinant DNA is, is filled with both excitement and promise and risks of danger and imbued with many moral and ethical problems.

I do not see any point in dealing in detail with legislation which we deeply appreciate the chairman introducing, which is the administration's proposal in this area.

I might make a couple of comments touched on by Governor Dukakis and answer questions about our contact in the foreign field.

As far as preemption is concerned, the legislation, as it now stands, provides that the Federal standards will go into effect, but where the Secretary determines that State standards are stricter and are being implemented or will be implemented properly, that he must permit those State standards to take effect.

I think it is important that both elements of that proposition be present before those standards, State standards, do go in effect. Not only that they be stricter, but it be clear that they will be implemented.

As far as the moral and ethical issues are concerned about which you raised a question, Mr. Chairman, my own view of those is such that I have discussed with Dr. Fredrickson and told him that I would like to have the existing Committee for the Protection of Human Subjects begin to look at these questions now. I see no reason to wait on that. I intend to ask them to start on this as soon as they can because these are serious questions.

They are obviously questions of great concern to a great many Americans.

As far as creating additional commissions or committees are concerned, as some have suggested in connection with this legislation, I am in the process of reviewing the health and related commissions of HEW. We have 320 committees on health, a large number of them

established by the Congress. They have a membership of several thousand people; 1,000 individuals in membership change every year, and I really think we do not need additional commissions and committees in the health area or pretty much in any other area over there.

I would prefer to use existing mechanisms for these purposes.

With respect to your question, Mr. Chairman, as to the extent to which HEW or NIH or the Executive is aware of and concerned about international activities, I know you are concerned about that. I can only report to you that we are obviously—well, we are deeply involved with the World Health Organization, with the International Council of Scientific Unions, which Council gets us into the world of Iron Curtain countries as well as free world countries, with the European Scientific Foundation, with the European Molecular Biology Organization.

The NIH guidelines which would become interim guidelines under this legislation have been distributed throughout the world. They are now in effect among other countries, France, Germany, Switzerland, and the Netherlands.

Dr. Fredrickson and our people are very conscious of the problems and the international aspects of recombinant DNA.

I think that is it, Mr. Chairman. We appreciate your interest in this subject and the help of your staff over the past few weeks since the NIH interagency report was made public in helping us work on the legislation..

Senator KENNEDY. Thank you very much.

[The prepared statement of Mr. Califano follows:]

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DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

STATEMENT

OF

JOSEPH A. CALIFANO, JR.

SECRETARY OF HEALTH, EDUCATION, AND WELFARE

BEFORE THE

SUBCOMMITTEE ON HEALTH AND SCIENTIFIC RESEARCH

COMMITTEE ON HUMAN RESOURCES

UNITED STATES SENATE

WEDNESDAY, APRIL 6, 1977

MR. CHAIRMAN AND MEMBERS OF THE SUBCOMMITTEE:

I am pleased to be here to discuss Federal policies on recombinant DNA research. Specifically, I will outline the main provisions of an Administration bill which I believe will best serve the public interest in this complex and controversial area.

It is clear that, with recombinant DNA, a new scientific tool of extraordinary potential has become available to mankind. By enabling scientists to examine basic life processes in new ways, recombinant DNA research offers the promise that we may better understand and possibly improve our treatment of human disease.

But while the new research techniques hold the promise of significant scientific advances, they also pose risks

of uncertain magnitude. For example, recombinant DNA in a microorganism may alter it in unpredictable, and possibly undesirable ways. Should the altered microorganism escape from a laboratory, it might cause disease or modify the environment.

Under these circumstances, the basic public policy issue is to determine a way in which we can secure the benefits of this new form of research, while at the same time safeguard adequately against the risks created by this type of scientific inquiry. Moreover, we must make this decision in light of the basic principle in our society that scientists should be allowed to pursue knowledge and truth without government interference.

At the present time, it is extremely difficult to assess accurately the nature of the hazards posed by recombinant DNA research. More precise assessment of the risks must await further inquiry. Until this additional information on the nature of the risks posed becomes available, the Administration believes that the best course is to allow recombinant DNA research to go forward but only under strict conditions to insure safety.

It is this broad goal which the Administration's proposed legislation seeks to achieve.

I understand that legislation in this area constitutes unusual government involvement in the workings of basic science, but the potential risks inherent in present recombinant DNA techniques justify such a measure at the present time. Indeed, legislation is necessary not merely to safeguard the public but also to assure that basic research in this potentially fruitful area is allowed to continue.

Let me review briefly where we are today with respect to this issue, and how we got there.

The National Institutes of Health have engaged in extensive scientific and public review of the new technology. On June 23, 1976, it released guidelines that established strict conditions for the conduct of NIH-supported research on recombinant DNA molecules. The NIH Guidelines, addressing the potential risks of the research by a double system of physical and biological containment, were published in the Federal Register for public comment and were widely distributed nationally and internationally. Changes in the Guidelines

will be considered as public comments are received and new scientific information emerges.

Other efforts have been made to assure public participation in this process. For example, the NIH has undertaken an environmental impact assessment of research allowed by the Guidelines. A draft statement was filed and published in the Federal Register in September 1976 for public review and comment. These comments are currently being evaluated.

Thus, the NIH has sought to facilitate public scrutiny of its activities and to ensure that the research is proceeding under strict safety conditions with full public understanding and support.

Last fall a Federal Interagency Committee on Recombinant DNA Research was created to determine the applicability of the NIH Guidelines to research throughout the public and private sectors and to recommend whether legislative or executive action was necessary to assure compliance with these Guidelines. The committee consists of approximately 25 representatives of Departments

and agencies that conduct or support this research or that have potential regulatory authority over it. After extensive review of current Federal statutes, the Committee concluded that no single existing authority can adequately regulate recombinant DNA research and that new legislation is therefore required.

After lengthy deliberations on March 10 and 14, 1977, the Committee agreed on a set of elements for proposed legislation. Their recommendations were forwarded to me on March 15. This report was released to the general public the next day. Subsequently, legislation has been developed embodying the Interagency Committee's recommendations.

We are grateful for your support of this legislation, Mr. Chairman, as well as for the support of other members of the Committee.

This legislation proposes to regulate basic laboratory research to a most unusual degree. While such regulation runs counter to my own commitment to the principle that intellectual inquiry can thrive only when free of constraint, I am persuaded that, with respect to the use of

recombinant DNA techniques, caution and restraint is required. Many distinguished scientists have called for a similar approach. They have agreed upon certain prohibitions and guidelines to govern their research for the present. I believe that within the scientific community there is a widely held apprehension, shared by the public, that not only Government-sponsored research, but all such use of these recombinant DNA techniques, must take place under uniform minimum standards.

There is no reasonable alternative to regulation under law. Only continued research, proceeding under strict safeguards, will tell us whether these restrictions must continue in force or whether they can be relaxed at some time in the future.

I would like to address briefly certain aspects of the Administration bill which I believe are essential for effective national regulation. As the Interagency Committee recommended, this bill provides that the Department of Health, Education and Welfare have responsibility for regulation and that the NIH Guidelines be promulgated as initial interim standards.

The scope of regulation in the bill is directed to the use and production of recombinant DNA molecules. The Interagency Committee recommended this broad language because of the difficulty in determining the border between research, pilot production, and commercial application. The language thus covers all use of recombinant DNA techniques. The language also assures however that no other agency with existing authority over aspects of this problem is precluded from continuing to exercise that authority in this area.

It is therefore both desirable and necessary that the Department exercise its regulatory authority in close consultation with other agencies. These include those regulatory agencies that may be better equipped for regulation of certain activities, or empowered to deal with the protection of the environment and occupational safety. We must also assure that standard-setting is carried out in close cooperation and coordination with all the other Federal reserach agencies. We will develop effective interagency mechanisms to bring this about. We will also assure appropriate public participation in these processes.

We plan, for example, to establish a permanent interagency advisory committee to propose or review revisions of the NIH Guidelines. Through such a committee, or through other

arrangements in addition to or in place of it, we will be working closely with other Federal agencies to assure the prompt revision of the Guidelines to accommodate developments in recombinant DNA research. Such cooperation is necessary regarding the use of recombinant DNA for agricultural purposes. At present the Guidelines prohibit the deliberate release into the environment of any organism containing a recombinant DNA molecule. Promising research is now going forward, however, on the use of recombinant DNA molecules for important agricultural applications. If this research proves successful, we would promulgate, after consultation with the Department of Agriculture, a change in the Guidelines to allow the release of safe and effective products of agricultural benefit.

Concern over potential hazards on using recombinant DNA techniques has already evoked some legislative and executive action at State and local levels. I believe this has been due, at least in part, to lack of generally applicable Federal standards. The Administration bill nevertheless provides that the Secretary must permit States or local governments to impose more stringent standards in their jurisdiction if he determines that the standards as adopted and implemented are, indeed, more stringent.

This Administration bill, I believe, is directly responsive to the perceived need for national standards and regulations to govern research and commercial activities in this new scientific area. It generally reflects the consensus of the Federal Interagency Committee, which took into account other bills introduced in Congress for regulation of this research, various reports, and recommendations at the State and local level for the regulation of recombinant DNA research. In addition, the Committee also solicited the views of other interested parties, such as agricultural scientists, biomedical scientists, environmentalists, labor unions, and private industry.

In closing, I should emphasize that the Administration regards this new regulatory activity as a high priority and it is our intention to fund the activity within the resources requested for NIH by the President in his 1978 budget.

The Department would be pleased to work with your Subcommittee, Mr. Chairman, on this necessary legislation which will allow scientific progress to be made and at the same time assure protection of the public health and environment.

Senator KENNEDY. I think, Mr. Secretary, it would be useful to the extent that you might be able to, just to review for a moment or two what your own assessment of the benefit and the risks are in terms of this DNA research.

Dr. Fredrickson might comment a little later as well. But I think the magnitude and the importance is such that the chief spokesperson for the country on health matters should really spell out for us what you think the dimensions of it are and the importance and the significance. I think that is terribly important so we understand a sense of urgency and the importance you place on it and the administration places on it in dealing with this issue.

Secretary CALIFANO. Mr. Chairman, we view recombinant DNA as a new scientific tool of extraordinary potential. We also recognize that it is in many ways moving us into the unknown.

With the caveats in that area, I might review some of the benefits and risks that we see and that our scientific people see.

The one obvious and hoped for and expected benefit is a substantial increase of basic biological processes. The DNA recombinant methodology provides simple and inexpensive way to prepare large quantities of genetic material that can be used to study the structure and functions of genes.

A second broad category includes practical applications for medicine, for agriculture, and for industry. These applications will only be realized if the reproduction of the recombined form DNA and host cell is followed by expression of genetic information contained in DNA.

Potential benefits here include the synthesis of medically important substances, such as insulin, hormones, clotting factors, and antibodies.

The Agriculture Department, I might say, as Secretary Bergland believes, there is strong potential here for agriculture, and I think the committee might want to get a statement directly from that Department at least for its record.

There are industrial possibilities with DNA, including the possibility of using genetically modified micro-organisms in the energy area, to neutralize pollutants such as oilspills.

As far as the dangers are concerned, Mr. Chairman, they are hard to predict. But the insertion of DNA of one species into a cell of a different species could be neutral, advantageous, or deleterious to either the recipient or other forms of life which come into contact with the recipient.

We have 3 years, more than 3 years of DNA experimentation. As yet, there is no known instance in which a hazardous agent has been created by recombinant DNA technology.

However, that, by no means, means it is not possible for such a thing to be created.

The most serious furtherest out possibility is a kind of Andromeda strain possibility that people conjure up. We see no evidence of that. But there is always the danger of something like that, the perceived danger of something like that. That is one of the reasons why we feel there has to be such strict guidelines in the conduct of recombinant DNA research.

I do not know if Dr. Fredrickson wants to comment.

Senator KENNEDY. Dr. Fredrickson, while we are on this subject, which I think is a very basic one, you might want to add to that.

Dr. FREDRICKSON. I think the Secretary has covered in the main the major aspects of both benefits and risks, Mr. Chairman.

Clearly, there are already some benefits that have been realized in the preparation of genetic material. It is also clear we have no clear estimate of the probabilities of certain of the benefits which have been projected. They could be very great, they could be very limited.

I think that we must today admit that the potential hazards are entirely speculative beyond the question of what we know about the risks of potential hosts or vectors that are being proposed to be used in this kind of experimentation.

The risks may indeed prove to be very much less than has been proposed. But since we do not know, I think we have to proceed with the prudence that is represented by the guidelines and this legislation.

Senator KENNEDY. One of the features that I think is important, and I am interested in your reactions. With the guidelines established, you have sort of an interim period, and then there would be revised guidelines established. After you establish them, they will be virtually fixed subject to the Administrative Procedures Act, which can be cumbersome and can be rather lengthy.

I am just wondering about your own thoughts in permitting a greater flexibility in monitoring and updating, and in the very careful kind of oversight which is terribly important?

Secretary CALIFANO. Mr. Chairman, the reason we feel interim guidelines should go in effect immediately, because we do not think there should be any gap, and we think all recombinant DNA activity should be covered promptly, and those guidelines now cover all Federal activity. I mentioned they covered recombinant DNA activity in several foreign countries.

The question of how to go about changing those guidelines, once they go into effect in our decision to use traditional APA procedures is because there is so much interest in this area. There obviously will be a large number of not only institutions, both academic and commercial, but a large number of citizens, environmental groups and others, who will want to be heard fairly, and who we will want to hear from fairly in the course of changing those guidelines.

We thought those procedures were the best to do it. We can expedite them to the greatest extent possible. There are ways if there should be a dramatic turn one way or the other, we can move more promptly than procedures would provide.

Senator KENNEDY. One of the things that I think is terribly important is that as good as interagency cooperation is and the task forces are, on an issue as important as this, I am not personally satisfied with the mechanisms that could be set up. I am just wondering in your bill, I just mention these are technical provisions, and I am more interested in a general comment. However, the bill allows exemptions from the requirements if other Federal agencies have regulatory authority, and then the bill also has language which requires consultation among agencies. Yet, the bill does not establish a single approach.

I am just wondering what your attitude is about getting a single approach on an issue this important, within a single agency that is going to be making decisions, that is going to stop the constant badgering between various kinds of agencies, and that will act with more authority and responsibility and also, hopefully, enlightenment, which

is so important in this issue. It is not going to go back into a lot of bickering between various agencies, so I am interested in your philosophy approach on it.

We have seen, as you well know—you have got three different Government regulations for HMO's, whether under medicaid or planning legislation of other kinds, and if we do not tie this up in terms of trying to deal with this issue in ways that make some sense, I think we fail in a very important area.

I want you to address how you think it can best be done.

Secretary CALIFANO. Mr. Chairman, I share that kind of concern as a general proposition.

As far as the interdepartmental problems are concerned, I think the legislation should and is intended to clearly give the authority and the power to HEW. It should be in one place. There is no question about that in the final decisionmaking.

As far as what happens within the Department itself, our present intentions are to have NIH with appropriate consultation, but have NIH recommend the revisions in the guidelines, review and clarify the technical issues, and handle, if you will, the standard setting, the philosophy, the scientific input, and then turn over to CDC the functions of licensing facilities and maintaining the registry and operating and promulgating the standards.

Both organizations would obviously have to be involved in training workers and in working with those outside the Federal Establishment as well as inside that are involved in recombinant DNA research.

We, in the course of developing this legislation, warded off a great number of attempts within the executive branch to fragment authority or provide a whole host of exceptions that we thought were not warranted, certainly at this point.

Senator KENNEDY. The problem, as I see it, just having NIH make the recommendations is whether you are going to have the kind of components which I think is so essential in an area of research as important as this that is going to have the impact in the terms of the public having that degree of input.

I am for the protection of human subjects panel considering ethical and moral kinds of questions, but I think the other kinds of issues in terms of scientific research, there ought to be a lay impact in terms of reviewing even the technical kind of information. And I do not think the way it is set up in terms of NIH, with their recommendations coming on, that you are insuring that kind of input on it.

Secretary CALIFANO. Senator, I do not in any way disagree with the need for that kind of input. I think we need it. I think we get it through the APA procedures in which there will be, I am sure, a great deal of lay participation.

My concern, as I said earlier, is that——

Senator KENNEDY. Do you know something new?

As chairman of the Administrative Practices Committee for 10 years, the role of the public in those procedures—if I said it was pretty near nonexistent, it would be stretching a point on it.

We will have to wrestle as to how the procedures ought to be established. I am not interested today in trying to tie all the knots up, but just getting more of an approach on this.

I do think you need centralized authority that it is not going to divide this thing up, and you see in terms of the Department of Agriculture what they are interested in, and it has to be centralized authority that can move on this.

I think outside the very broad kinds of ethical and moral kinds of questions, you have to have, even in terms of decisionmaking process from scientific point of view, a lay impact. How we are going to deal with this, I think, is important.

Secretary CALIFANO. I do not doubt that. I would rather use to the extent possible the existing mechanisms like that Commission on the Protection of Human Subjects.

My point earlier, we literally have 321 health committees in NIH, a thousand members change every year, and I think we are reaching a point where nobody knows who is advising whom on what.

Senator KENNEDY. That is fine. I think there is a question that you are going to have to deal with those. We are open to recommendations.

But there is a significant difference between advisory committee on community mental health and the kind of panel that we are talking about here. It is an entirely different kind of concept.

We have to understand that in terms of both its power and its types of decisions.

As you pointed out, the magnitude of this particular kind of problem is mind boggling.

Let me ask, because I know you have to go, and I have taken a lot of time, and we will submit other questions for the record, but you talked about not only the scientific research, but the applicability of this DNA research in your testimony, the use of it.

Secretary CALIFANO. Yes, Mr. Chairman.

Senator KENNEDY. That is an extremely important kind of question about the fact that it is going to be the Government, the governmental agency is going to be, or who within the Government is going to be making these kinds of decisions when they see the research going down certain kinds of roads, and who is going to be making the decisions in terms of the implications and the modifications and all the other factors on it?

Who is going to be making those kinds of decisions on it?

What is the public accountability?

Where does the public input come in in terms of that?

Is that going to be people waiting under the Administrative Procedures Act because certain kinds of groups out at NIH has made a decision to do "Y"?

I think those are tough kinds of questions. We are not going to resolve them here today.

I know you have thought about them. I am interested to hear your reaction.

Secretary CALIFANO. Mr. Chairman, any time the Government intrudes into the area of scientific inquiry, which is essentially a search for knowledge and truth and better understanding of God's world, we are—I am troubled. But I think in this area there is such a great degree of unknown, that there is a necessity for some Government intrusion representing hopefully the interests of the public, and overseeing in its executive actions by the Congress branch of the Government and this committee and others.

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When we move to the arena of applying in either commercial or noncommercial ways and attempting to get the benefits and uses of research and new discoveries of knowledge, there is a tradition in this country of tremendous intrusion.

I am not arguing that it has been effective, as effective as it should be, but the FDA is deeply involved in the activities of pharmaceutical companies who purvey new drugs.

We have increasing environmental areas of EPA in connection with chemicals, with the Toxic Substances Act, and we are deeply involved in the use of many of those substances.

I guess I have less concern about Government involvement in the application in practical terms in this area than I do with the very special kind of involvement that is involved here actually in the search for knowledge, if you will.

Senator KENNEDY. I will just give you basically a philosophical comment with regard to it.

There are those within the scientific community that believe that really the scientists ought to be able to make the decisions in terms of development of the technology, and the public ought to be involved only in terms of application of these factors.

It is my view that with regard to a matter as important as this that the public ought to be involved on the ground level in terms of the whole kind of process of scientific development, as well as in the application.

I think it is imperative that as we move into this area, and that we are going to build the kind of institutional framework. Since this is so incredibly significant and important, unlike really any of the other kinds of advisory groups, et cetera, we are going to insist on that through the whole kind of process. We want to work with the administration in insuring that is going to be the case, and we are not going to get diffusion in terms of other agencies and the rest, and we are going to have responsible decisionmaking.

There may be those who differ on it. There may be those who differ in both this committee and the Congress and the Senate on it. I know there are scientists who differ.

Secretary CALIFANO. You and I do not differ on that. We are in agreement on that.

Senator KENNEDY. I think that the whole kind of process, including those kinds of protections, is very, very basic.

We will excuse you.

Secretary CALIFANO. Dr. Fredrickson is prepared to continue, Mr. Chairman.

Senator KENNEDY. Senator Chafee.

Senator CHAFEE. Dr. Fredrickson, you can go ahead.

Dr. FREDRICKSON. Senator Chafee, I have a longer statement which I would propose just to submit for the record. If I might indicate extremely briefly what that statement contains in a much shorter time——

Senator CHAFEE. You can summarize it, but there is a lot here, and I think we are interested in the gist of what you have got to say.

Dr. FREDRICKSON. I shall be very glad to do that, Senator.

I can clearly skip the portion on why we are here. I think that has been adequately covered with regard to the subject matter at hand.

In my statement I dealt particularly with the role of the Government and two organizations particularly in the development of guidelines that have now reached the stage of proposed legislation.

Those two institutions are the NIH itself and the Federal Interagency Committee which deliberated relative to implementation of those guidelines throughout all aspects of this kind of application of techniques in the country.

As you know, Senator, the history of this development begins at the Gordon Conference in 1973. It then moved on to a meeting attended by a number of international scientists at Asilomar. The two deliberations I referred to requested NIH to develop guidelines for the conduct of this research, and that those guidelines were developed by recombinant DNA molecule advisory committee and were sent to me in December 1975 for further decision.

At that time, these were reviewed locally. In February I used a committee, which is available to me, the Directors Advisory Committee at NIH, augmented with a number of members of the public who represented not only science but other disciplines, such as law, ethics, consumers, and the environment. We had a public meeting to which a number of people were specifically invited and a general public notice was given.

We had a number of statements from public witnesses. We created a hearing record and then we requested extensive comments further by mail to me.

Over a period of several months after that public hearing, we then reviewed the guidelines which had been originally submitted. We reviewed them with the committee, made certain changes, particularly with regard to implementation, and then issued those guidelines in June 1976, along with a detailed statement of the process that had been used to obtain public comment and what was done with each of those specific comments at that time.

These were published in the Federal Register, and over 40,000 copies of those guidelines have been distributed, both here and abroad, to all interested parties.

Following the issuance of the guidelines, we established an Office of Recombinant DNA Activities within the NIH to assist in the implementation of those guidelines, and then in September, after due consideration, we submitted a draft environmental impact statement on the Federal action which was the issuance of the guidelines.

We have received numerous comments on that document, and we have dealt with all of those in a redraft document which has been submitted to the Department, and it is anticipated the Department will then issue that final environmental impact statement within a reasonably short time.

So, to summarize, Senator Chafee, it was in the process of developing guidelines that we felt we had any number of ways involved the public and to a degree which is rather extra for scientific matters and to a degree which I think was fully merited by importance of the subject.

Then I will just briefly mention the activities of the Interagency Committee on Recombinant DNA Research.

This committee was established in September with the approval of then President Ford, who wrote each of the participating departments and agencies requesting that they give their full cooperation. Some 25

agencies or departments were represented, and basically these were all agencies who might be conducting or supporting such research in the Federal Government and all agencies who had any conceivable regulatory authority that might bear on those particular activities.

The committee began its sessions in November, and continued until March of this year, and its major activities after briefing itself and obtaining full information so all members might be up to seed, its major activities were to first develop from the research agencies the assurance that they could and they would all follow a single set of guidelines, NIH guidelines for federally supported research. That was achieved.

The second was to review all the existing regulatory legislation that might cover the use of these procedure.

The committee concluded and came to a full consensus that none of the existing laws that were reviewed could adequately cover this subject to its regulation, clearly not without potential of challenge to that authority in the courts.

Having concluded this, the Interagency Committee then proceeded to consider elements for possible new legislation that might be developed for extending all of the NIH guidelines to all those using these techniques throughout the United States.

In the process of that deliberation, the views of other public participants were solicited. These included agriculture and biomedical scientists, but they also included environmentalists, labor unions and industry, and these opinions and this information were then submitted to the committee so that it kept itself fully informed, not only relative to opinions outside but also made some analysis of existing bills which went in before the Congress in the March meetings of the committee.

These two pieces of legislation particularly included the S. 621 submitted by Senator Bumpers and its companion in the House submitted by Congressman Ottinger.

After detailed deliberations on March 10 and 14, the committee, on March 15, sent forward to the Secretary of HEW an interim report which discussed in detail its recommended elements for possible new legislation to cover the regulations.

Basically, Senator Chafee, what the committee is considering today, the administration bill, was constructed on those elements and on the discussion and consensus of the interagency report. Some of the aspects of that bill have been already reviewed. They are known well to you. The determination at the Department of HEW should be the locus for the regulation and use of recombinant DNA molecules, the agreement that for the present this legislation should deal only with that narrow field of use of recombinant DNA techniques, and the issues of licensing of registration and other aspects of the regulatory activity that would be required were dealt with in detail.

I should be very glad to answer any questions or enlarge upon those should it be your desire.

I think in conclusion, Senator Chafee, there is no question that the international as well as national scientific community is in agreement that until we know more about speculative potential hazard of recombinant DNA research, a common set of standards ought to be applicable everywhere insofar as it is possible to make that, not only in this country, but across the world.

The question being debated here and also in several countries within national jurisdictions is how shall we accomplish this?

First, by national law, hopefully by series of national laws that will be compatible and permit the extension of a single fabric across the use of these techniques throughout the world.

It is clear that the use of recombinant DNA techniques for research purposes now, potentially for commercial or other purposes, will continue to go on throughout all of the developed nations of the world. Virtually all of those that have any particular research capability in molecular biology now so engaged.

Many countries have already made a determination that the use of these techniques should continue. They see the benefits without weighing the speculative risks, but they all agree they must proceed with prudence and in accord with a reasonably common set of guidelines, for what happens in one part of the world clearly will be significant to the other parts of the world if any of the really significant hazards of this research, the hazards that are seen by some within it, were actually to come about.

Senator CHAFEE. Do you want to touch on the guidelines that the other countries have adopted and how they compared to those you have been operating under?

Dr. FREDRICKSON. Yes. There are three sets of guidelines that have now been issued.

The United States, in the form of the NIH guidelines, issued its guidelines at about the same time that a United Kingdom set of guidelines were issued by Britain. Britain had a parliamentary committee that considered the issue of whether this kind of research, the techniques should be used, and that Ashby committee reported to Parliaby British Medical Research Council. It developed a set of guidelines

A Williams committee was next constructed in Britain, primarily ment and said it should, it should go on with a new set of guidelines. which are relatively in accord with those in the United States in NIH guidelines.

Basically, they are "children" of Asilomar, and they are similar. The difference between United States and United Kingdom guidelines is one of exjlicitness.

Here we have an extraordinary set of explicit guidelines, deliberately developed because we have a large country and more activity than could be envisioned in Britain itself, where probably the number of laboratories using these techniques will not exceed 30 by Britain's

The NIH levels of containment, the protective devices that are the own estimate.

background of the guidelines, are in physical terms very similar to Britain. In the United States, we also had secondary stress on biological containment, the construction of defective hosts or vectors——

Senator CHAFEE. Are these on the project, on the individual or on the laboratory in our guidelines?

Dr. FREDRICKSON. Basically our guidelines are very explicit, and they deal right down to the use of these techniques in any project. They define the conditions that must obtain based on the assessment of risks, which is scaled according to the host and to the source of the foreign DNA or gene which one proposes to insert into that host.

Senator CHAFEE. Do you apply it based on the project, or based on the laboratory, the facility?

Dr. FREDRICKSON. At the present time that the guidelines are used by the NIH, by the National Science Foundation, of course the use of such techniques must be paid for. They are subsidized by the Federal Government.

Those subsidies are paid in the form of grants usually, occasionally contracts, on the basis of general plan of research that is proposed.

Here the research itself is just on the basis of its scientific potential and within the adherence to the guidelines of the proposal is also commented upon by study sections or reviewers.

An important aspect of the NIH guidelines, and one which I am sure would intend to use in the Department for regulatory purposes, is the mandatory creation of certain institutional procedures at the local institution where the laboratory is. This kind of proximal governance, I think, is absolutely essential if there is to be effective strong guidelines.

These procedures include, one, that the institution must submit a memorandum of understanding to the NIH which indicates that not only principal investigator but someone responsible in his institution agrees that this work should go on. That facilities are adequate to meet the requirements of the NIH guidelines. And that there is in place an institutional review board which reviews the capability of the institution for carrying out this research and for meeting the strict requirements of the NIH guidelines.

Senator CHAFEE. But NIH guidelines would only apply now, would they not, if you have a Federal grant involved?

Suppose somebody did not have a Federal grant?

Dr. FREDRICKSON. Well, that has long been judged to be the weakness of the whole setup, and I think that is why we are here today, Senator Chafee.

Senator CHAFEE. In other words, the reason we are here today, is because your power in NIH is only in the areas where grants are given, and absent some law, private industrial research would not be covered. Is that correct?

Dr. FREDRICKSON. That is correct.

None of our guidelines could be made applicable by something on the order of Executive order to industry or any of the other private or self-employed sector of the Government where no Federal money was involved.

Senator CHAFEE. What do they do in other countries?

There is no law in the U.K., is there?

Dr. FREDRICKSON. The United Kingdom does have a law under the British Health and Safety Authority, which is amalgamation of a number of regulatory units. It has been judged that there is authority for inspection and enforcement of standards which are set according to the U.K. guidelines. That authority then has the regulatory power and responsibility.

Other countries do not, Senator Chafee, and certainly many have not yet resolved the question of extending a common set of guidelines to all activities, both private and publicly supported in many countries.

It is a problem that they, too, are wrestling with.

Senator CHAFEE. What other countries are prominent in this research?

Dr. FREDRICKSON. Most of the countries of Europe all have some activity. I should say the area in which the largest amount of activity is going on would be Britain, France, Germany, West Germany, the Scandinavian countries, Switzerland to a lesser extent.

There is more activity in the United States than any other country.

Canada has a small amount of activity, and I neglected to complete my prior answer, and that is that the third set of guidelines are the Canadian ones which were issued only a few months ago.

Senator CHAFEE. How about the Soviets, what are they doing?

Dr. FREDRICKSON. The Soviet Union has a certain amount of activity. NIH has been in contact with them indirectly through the International Council for Scientific Unions, and directly through Professor Baya from the Soviet Union, molecular biology, who paid me a visit not long ago. He is chairman of the committee constructing the Soviet guidelines.

It was his statement to me that they would probably consist of an amalgamation of U.K. and U.S. guidelines. But research is going on in Eastern Europe—Czechoslovakia, East Germany and the Soviet Union. But probably to a somewhat lesser extent or lesser stage of development than it is in the United States.

Senator CHAFEE. You are in favor of this proposed legislation. Have you made comments on the Bumpers' legislation?

Dr. FREDRICKSON. I have not made specific comments for myself but they are contained in part in the report of the Interagency Committee. In fact, Senator Chafee, if you have no objection, I would like to offer for submission an inclusion in your record a report of the Interagency Committee and the comments of Secretary Califano upon its issuance, this document having been issued to the public on March 16.

Senator CHAFEE. Fine.

[The material referred to may be found in the files of the subcommittee.]

Senator CHAFEE. Maybe your counsel could answer this. Under the administration bill, who gets penalized if there is a criminal penalty involved, the individual, the laboratory, or the operator?

Dr. FREDRICKSON. I think I will attempt as a layman to answer your question, Senator Chafee, and the lawyers can straighten me out.

But the bill actually defines those here as persons, institutions or facilities, and undoubtedly any of those principals could be subject to either the civil or criminal penalties that are contained within the bill.

Senator CHAFEE. Does your lawyer agree?

Mr. LEVINE. Yes, whoever violates the law.

Senator CHAFEE. How do you tell?

Suppose a facility is not licensed?

Mr. LEVINE. If a facility is not licensed that is involved in DNA research, you mean?

The facility is liable, it is up to the Attorney General to institute an action.

Senator CHAFEE. Who goes to jail, the facility?

Mr. LEVINE. The operators of the facility.

Senator CHAFEE. The researcher?

Dr. FREDRICKSON. Or potentially, I should say, Senator, if that facility is an institution, by those who are responsible for the goverance

of that institution. They certainly cannot escape liability, at least automatically, as I understand the law.

Senator CHAFEE. For instance, in a university, if a university researcher exceeds the guidelines, who would be held responsible?

The president of the university, the head of the medical school, the members of the corporation?

Dr. FREDRICKSON. That, sir, has not been tested by administrative action at NIH or certainly by any action of the courts, there being no law.

I think we would have to see how that was determined in a sense by common law.

But let me answer this way.

If there were willful violation in an institution, the NIH would consider taking action against not only the investigator, but that institution if it were necessary and seemed appropriate in regard to the support of other federally supported activity.

Senator CHAFEE. I have a deep personal interest in this because I am on the Yale Corp. I want to know what our liability is.

Dr. FREDRICKSON. I think it was very clear, and as was the moral approach used in the guidelines that the investigator and his institution share this responsibility.

Senator CHAFEE. Well, we have moved into a different area from the statutes. We are now moving into the civil and criminal penalties.

Dr. FREDRICKSON. Yes, I understand.

Senator CHAFEE. I think probably that should be a little more specific and we will be talking with your counsel about this.

Doctor, thank you very much for coming. We appreciate having your views. We definitely will be talking more with you as we proceed.

Dr. FREDRICKSON. Thank you.

[The prepared statement of Dr. Fredrickson follows]:



FOR RELEASE UPON DELIVERY

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND 20014

STATEMENT BY

DONALD S. FREDRICKSON, M.D.

DIRECTOR, NATIONAL INSTITUTES OF HEALTH

ON RECOMBINANT DNA TECHNOLOGY

BEFORE THE

SUBCOMMITTEE ON HEALTH AND SCIENTIFIC RESEARCH

OF THE

SENATE COMMITTEE ON HUMAN RESOURCES

APRIL 6, 1977

I. INTRODUCTION

Good day, Mr. Chairman and other Committee members. It is a pleasure to have the opportunity to discuss with you Federal policies concerning recombinant DNA techniques. The focus of my remarks will be the activities of two organizations--the National Institutes of Health and the Federal Interagency Committee on Recombinant DNA Research.

As you know, recent scientific developments in genetics, particularly in the last four years, have culminated in the development of a powerful new tool for research--the ability to join together genetic materials in cell-free systems to form recombinant DNA molecules. I would like to emphasize the point that recombinant DNA is a tool for accomplishing the types of research that scientists have been pursuing for decades.

"DNA"--which is the shorthand way of saying "deoxryibonucleic acid"--is the material that determines hereditary characteristics of all known cells. These new techniques allow us to join together DNA segments from different sources or to rejoin the DNA from one source in a different order. This new and powerful tool of science has generated great hope and excitement and, concomitantly, many expressions of concern.

Research using recombinant DNA techniques offers great promise for better understanding and improved treatment of human diseases. Medical advances through use of this technology include the opportunity to explore complicated diseases and the functioning of cells, to better understand a variety of hereditary defects, and possibly (in the future)

to create microorganisms useful in producing medically important substances for the treatment and control of disease. Aside from potential medical benefits, a variety of other applications in science and technology are envisioned. An example is the large-scale production of enzymes for industrial use; and potential benefits in agriculture include the enhancement of nitrogen fixation in certain plants and the biological control of pests, permitting increased food production.

There may be risks in this new research area as well as anticipated benefits. A potential hazard, for example, is that the foreign DNA microorganism may alter the host in unpredictable ways. Should the altered microorganism escape from containment, it might infect human beings, animals, or plants, causing disease or modifying the environment.

Until the potential risks are better delineated and evaluated in light of developing scientific knowledge, the public should expect such research to be conducted under strict conditions ensuring safety. This was the fundamental principle that guided the National Institutes of Health and the Federal Interagency Committee in their deliberations. That is, the desire to allow this significant research to continue while protecting humans and the environment from the effects of potential hazards whose nature and the occurrence of which is as yet uncertain. I would like to review with the Committee the activities of the NIH in developing guidelines to govern this research, and then devote the rest of my testimony to the work of the Interagency Committee.

II. DEVELOPMENT OF THE NIH GUIDELINES

The first step in the development of the Guidelines was taken by the scientific community. Scientists engaged in research using recombinant DNA technology first expressed concern about the potential biohazards at the Gordon Research Conference on Nucleic Acids in July 1973. At their request, the National Academy of Sciences created a committee that called for a moratorium on certain types of experiments and for an international conference to consider this problem further. The committee also called on the NIH to establish an advisory committee to study containment procedures and draft guidelines for the conduct of this research. At the International Conference on Recombinant DNA Molecules held at Asilomar, California, in February 1975, temporary guidelines were issued, including a continued moratorium on some experiments but allowing others to proceed with appropriate biological and physical safeguards, pending issuance of NIH guidelines.

The NIH Recombinant DNA Molecule Program Advisory Committee was established in October 1974 to advise the Director of NIH. In December 1975, the Committee, after several open meetings, recommended proposed guidelines for my review and decision.

To assist me in the review of the proposed guidelines, a special meeting of the NIH Advisory Committee was convened in February 1976. Members of the Committee represented not only science but such other disciplines as law, ethics, and consumer affairs. Comments received from committee members and a number of public witnesses represented a

wide range of views. Follow-up written comments were also solicited. In April, the NIH Recombinant Advisory Committee considered these comments from the February meeting, and a number of changes to the guidelines were made. Concurrently, meetings for information exchange were held with representatives from other Federal agencies and private industry as well as with Congressional staffs. Finally, on June 23, 1976, with the approval of the Secretary of HEW and the Assistant Secretary of Health, the NIH issued guidelines to govern the research it supports involving recombinant DNA molecules. The NIH Guidelines established strict conditions for the conduct of this research, prohibiting certain types of experiments and requiring special safety conditions for other types. The provisions were designed to afford protection--with a wide margin of safety--to workers and the environment. Two weeks later, on July 7, 1976, the NIH Guidelines--together with a document indicating the basis of my decisions on principal issues--were published in the Federal Register for public comment.

Over 40,000 copies of the Guidelines have been widely distributed to foreign embassies, medical and scientific journals, NIH grantees and contractors, and major professional research societies.

III. NIH ACTIVITIES FOLLOWING RELEASE OF THE GUIDELINES

Subsequent to the release of the Guidelines, NIH initiated several actions.

A. Office of Recombinant DNA Activities

To facilitate implementation of the Guidelines, the NIH, in June 1976, established the Office of Recombinant DNA Activities: to administer and coordinate intramural and extramural activities at the NIH; to review the institutional biohazards committees; and to monitor reports and information concerning accidents, containment, and safety research innovation.

B. Published Proceedings

In August 1976, the NIH published a volume containing the transcript of the February NIH public hearing on the proposed guidelines, voluminous related correspondence, and the results of relevant meetings held prior to the release of the Guidelines in June. A second volume is planned for publication in late Spring documenting the correspondence that the NIH received on the Guidelines, the Environmental Impact Statement, and the Departmental patent policy.

C. Environmental Impact Statement

The NIH, in accordance with the National Environmental Policy Act of 1969, undertook an environmental impact assessment to review environmental effects, if any, of research that may be conducted under the Guidelines. The NIH Guidelines were released prior to the completion

of the assessment because they provide greater protection for the public and the environment than the Asilomar Guidelines or no guidelines.

A Draft Environmental Impact Statement was filed and published in the Federal Register on September 9, 1976, to afford additional public review and comment. The draft statement has been analyzed and comments received are addressed in the final Environmental Impact Statement to be published soon.

D. Department Patent Policy

In June, shortly before the release of the Guidelines, Stanford University and the University of California asked NIH to review DHEW policies relating to the patenting of inventions perfected through the use of recombinant DNA techniques and financed by NIH. Under current DHEW patent regulations, invention rights to discoveries developed under the Department's research support are normally allocated in either of two ways:

- The Department may enter into an Institutional Patent Agreement (IPA) with a university or other nonprofit institution that has adequate mechanisms for administering patents on inventions. The IPA provides the institution the first option to own all inventions made in performance of Department grants or contracts, subject to a number of conditions deemed necessary to protect the public interest.
- For those institutions that have not entered into a patent agreement with the Department, determination of ownership is deferred until an invention has been made, at which time an institution may petition the Department for ownership of the invention.

The NIH solicited opinions from a number of different groups in the scientific community and the public and private sectors concerning departmental patent policies, with respect to recombinant DNA research inventions. An analysis of the issues raised by the commentators is currently under review.

IV. THE INTERAGENCY COMMITTEE ON RECOMBINANT DNA RESEARCH

I would now like to devote the remainder of my testimony to the activities of the Interagency Committee on Recombinant DNA Research. This Committee was created, with the approval of President Ford, to address extension of the NIH Guidelines beyond the NIH, to the public and private sectors.

The specific mandate of the Interagency Committee is as follows: to review the nature and scope of all recombinant DNA research conducted in the United States, to determine the applicability of NIH standards to regulate this research nationally, to recommend mechanisms to ensure that the standards are being complied with, and to facilitate exchange of information throughout the Federal sector. The Committee is advisory to the Secretary of Health, Education, and Welfare. It includes representatives of Federal Departments and Agencies that support and conduct recombinant DNA research (or may do so in the future), and representatives of Federal Departments and Agencies that have present or potential regulatory authority in this area. At the Secretary's request, I serve as Chairman of the Committee.

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Two meetings of the Committee were held in November 1976. The first of these, on November 4, was devoted to a review of the development of the NIH Guidelines. The Committee also reviewed activities in other countries on the development of guidelines for this research. Recombinant DNA research is being conducted in a number of countries, including Canada, the United Kingdom, the Scandinavian countries, most other parts of western Europe, eastern Europe, the Soviet Union, and Japan.

In many countries, appropriate governmental or scientific bodies have reviewed the research and have agreed that it should proceed. Several of the countries have acted to establish guidelines to govern the conduct of this research, including the United Kingdom and Canada. In the United Kingdom, a parliamentary committee addressed the issue and indicated that work in this area should continue under appropriate safety conditions. Scientific advisory committees of international organizations, such as the World Health Organization, the International Council of Scientific Unions, and the European Molecular Biology Organization, have made similar recommendations.

The European Science Foundation, representing member nations from Western Europe and Scandinavia, has recommended to its members that they follow the guidelines of the United Kingdom. These guidelines are, in intent and substance, very similar to those of the National Institutes of Health. The NIH is currently working very closely with the United Kingdom and the European Science Foundation to ensure a commonality of

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standards in carrying out this research. Thus far, there has been very close cooperation and coordination among the various international and national scientific bodies, with a view to reaching a consensus on safety practices, programs, and procedures.

At the meeting of the Committee held on November 23, 1976, the Federal research agencies discussed their activities and possible roles in the implementation of the NIH Guidelines. All research agencies endorsed the Guidelines to govern recombinant DNA research. At present, the NIH, the National Science Foundation, the Veterans Administration, and the U.S. Department of Agriculture are supporting or conducting such research. The Department of Defense, National Aeronautics and Space Administration, and the Energy Research and Development Administration do not at present conduct such research, but all have endorsed the NIH Guidelines to govern future research should it be undertaken.

A. Subcommittee Review of Existing Legislation

Also at the November 23 meeting, the Federal regulatory agencies reported on their regulatory functions. Following that review, a special Subcommittee was formed to analyze the relevant statutory authorities for the possible regulation of research involving recombinant DNA technology. All regulatory agencies were represented on the Subcommittee, assisted by attorneys from their offices of general counsel.

The Subcommittee was charged to determine whether existing legislative authority would permit the regulation of all recombinant DNA research in

the United States (whether or not federally funded) and would include at least the following regulatory requirements:

(1) Review of such research by an institutional biohazards committee before it is undertaken.

(2) Compliance with physical and biological containment standards and prohibitions in the NIH Guidelines.

(3) Registration of such research with a national registry at the time this research is undertaken (subject to appropriate safeguards to protect proprietary interests).

(4) Enforcement of the above requirements through monitoring, inspection, and sanctions.

It was the conclusion of the Subcommittee that present law could permit imposition of some of the above requirements on much laboratory research involving recombinant DNA techniques, but that no single legal authority or combination of authorities currently existed that would clearly reach all research and other uses of recombinant DNA techniques and meet all stated requirements. Although there is existing authority that might be interpreted broadly to cover most of the research at issue, it was generally agreed that regulatory actions taken on the basis of any such interpretation would probably be subject to legal challenge. The Subcommittee, in reaching this conclusion, reviewed the following laws that were deemed to warrant detailed consideration:

(a) The Occupational Safety and Health Act of 1970 (Public Law 91-596)

(b) The Toxic Substances Control Act (Public Law 94-469)

(c) The Hazardous Materials Transportation Act (Public Law 93-633)

(d) Section 361 of the Public Health Service Act (42 U.S.C. 264).

In addition, several other laws were examined. The Clean Air Act, the Federal Water Pollution Control Act, the Resources Conservation and Recovery Act, and the authorities of the FDA and the Department of Agriculture.

The full Committee adopted the report of its Subcommittee and agreed that new legislation was required.

B. Interagency Committee Analysis of Elements for Legislation

In considering the elements for legislation, the Committee reviewed Federal, State, and local activities bearing on the regulation of recombinant DNA research.

Among Congressional proposal reviewed were S. 621, "The DNA Research Act of 1977," introduced by Senator Dale Bumpers, and the companion measure introduced by Representative Richard L. Ottinger in the House (H.R. 3591). The Committee also noted the resolution introduced by Representative Ottinger on January 19, 1977 (H. Res. 131), requesting DHEW to regulate recombinant DNA research under Section 361 of the PHS Act.

Among State and local activities reviewed were recommendations by the New York State Attorney General's Environmental Health Bureau for State regulation, and the Cambridge (Massachusetts) City Council's resolution on recombinant DNA research.

Several committee representatives also reported on meetings with other interested parties whose views had been solicited on legislation to regulate recombinant DNA research. Those who were contacted include agricultural scientists, biomedical scientists, environmentalists, labor unions, and private industry. At my request, the Industrial Research Institute and the Pharmaceutical Manufacturers Association are surveying their member firms to determine the scope of the research efforts in the private sector. The Pharmaceutical Manufacturers Association has endorsed the NIH Guidelines as standards for conduct of this research.

In considering elements of proposed legislation, a number of issues were raised and discussed fully by the Committee. After detailed deliberations at meetings on March 10 and 14, 1977, the Committee agreed on a set of elements for proposed legislation. The elements agreed upon and the various alternatives reviewed by the Committee were presented in an Interim Report transmitted to HEW Secretary Califano on March 15, 1977. Secretary Califano, in releasing the report on March 16, stated that "legislation in this area would represent an unusual regulation of activities affecting basic science but the potential hazards posed by recombinant DNA techniques warrant such a step at this time." He went on to say, "...I believe such a measure is necessary not just to safeguard the public but also to assure the continuation of basic research in this vital scientific area. We are not saying that research should be halted. We are urging that it should proceed under careful safeguards unless and until we have a better understanding of the

risks and benefits posed by use of recombinant DNA techniques without Government regulation."

Mr. Chairman, I would like to submit for the record the Federal Interagency Committee's "Interim Report on Suggested Elements for Legislation," along with a copy of the Secretary's press release.

With your permission, I would like to review briefly some of the major elements addressed by the Committee. The Committee determined that the Department of Health, Education, and Welfare is the appropriate locus in the Government for the regulation of the use and production of recombinant DNA molecules. In reaching this determination, the Committee took into account existing roles of certain agencies within DHEW--for example, that of the NIH in developing the Guidelines, and of the Center for Disease Control and Bureau of Biologics (FDA) in regulating infectious agents and biological products. The Committee also had before it the petition by the Environmental Defense Fund, requesting DHEW to issue regulations for recombinant DNA research.

The Committee reviewed at great length the nature and scope of regulation. Consideration was given to regulation of all laboratory research where hazardous or potentially hazardous substances were employed. There was general Committee agreement that present legislation should be restricted to recombinant DNA techniques.

However, I have established a committee at the NIH, chaired by Dr. Richard Krause, Director, NIAID, to study and recommend, if necessary, safety standards for other NIH-supported research involving actual or potential biohazards. The preliminary report is expected shortly, and I will keep the Committee informed of the progress on this NIH review.

Regulation of just the research aspects of recombinant DNA techniques presents a problem because of the difficulty in determining the border between research and pilot production. Therefore, the Committee recommends that regulation cover the production or use of recombinant DNA molecules. Such language would include research activity, and makes immaterial possible concerns whether a given activity constitutes research, pilot production, or manufacture. The Committee recommends that the Secretary, in specific instances, in consultation with appropriate regulatory agencies, be allowed to determine the nature of the activity and should defer to a regulatory body that the Secretary determines is better empowered and equipped to deal with it.

There was general agreement by the Committee that registration of projects involving the use or production of recombinant DNA molecules was necessary. The Committee also recommends that facilities be licensed and that the terms of the license include acceptance of responsibility for the particular activities and individuals at the facility. The Committee concluded that licensure of the facility and registration of projects would be more feasible and would more adequately meet the needs

for safety monitoring rather than licensure or registration of individuals engaged in research.

The Committee urges full disclosure to the appropriate regulatory body of all relevant safety and scientific information pertaining to the use or production of recombinant DNA molecules. However, the Committee recognizes the important world-wide commercial potential of recombinant DNA molecules in medicine, agriculture, and other areas of science and technology. It believes that the potential commercial uses of recombinant DNA techniques require that information of a proprietary nature and patent rights be given the appropriate protection from disclosure by the regulatory agency receiving such information as is currently provided by existing law. However, the Secretary may immediately release information if public safety requires it.

Because the potential hazards posed by the use of recombinant DNA techniques extend beyond the local to the national and international levels, the Committee recommends that a single set of national standards must govern and that, accordingly, local law should be preempted to ensure national standards and regulations. The Committee, however, took into account the activities at the State and local levels on regulation of recombinant DNA research. It was agreed that, if a State passes a law imposing requirements identical to those contained in the Federal legislation, then the Secretary may enter into an agreement with the State to utilize its resources to assist the Secretary in carrying out his duties.

Protection of workers was also considered by the Committee. Training of workers in proper laboratory techniques and long-term medical monitoring are important aspects of worker safety and were endorsed by the group.

A number of other recommendations are made, and I can discuss them further if you have questions. I would like to emphasize that the work of the Interagency Committee has been done in a most cooperative and helpful way.

DHEW will continue to cooperate and coordinate with relevant Federal Departments and Agencies in this important matter.

IV. CONCLUSION

In conclusion, this much is clear: the international and national scientific community is in substantial agreement that, until the potential hazards of recombinant DNA techniques are better understood, a common set of standards must everywhere exist for the use of those techniques. The question being debated now is how this is to be accomplished. The substance of all guidelines is sufficiently similar; how to apply them locally and nationally remains the issue.

In the United States, this question has attracted far more public attention than in other countries. Indeed, a number of local jurisdictions or States are engaged in action or debate.

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Finally, I want to note that biomedical research is entering a new era in its relationship to society. It is passing from an extended period of relative privacy and autonomy to an engagement with new ethical, legal, and social imperatives under concerned public scrutiny. NIH has responded to these concerns by requiring the formation of review boards to oversee human experimentation, animal care, and now DNA recombinant experiments. Similar bodies may soon have to oversee other hazardous laboratory work. These responsibilities are inescapable adjustments to the rising demand for public governance of science, though this need not--and, indeed, should not--go beyond what is clearly required for public safety lest we inadvertently impede successful research and hamper creativity. The progress of science will continue to depend on the initiative and insights--call it inspiration, if you like--of individual scientists.

Senator CHAFEE. The next panel is composed of Dr. Halsted R. Holman, Stanford Medical Center; Dr. Norton Zinder, Rockefeller University; Dr. Alexander Rich, Massachusetts Institute of Technology; and Dr. Harlyn Halvorson, American Society for Microbiology.

Gentlemen, we appreciate your coming. We know you will make a contribution to this testimony.

We will start with Dr. Holman. Thank you for coming all the way from Stanford.

STATEMENTS OF HALSTED R. HOLMAN, PH. D., PROFESSOR OF MEDICINE, STANFORD MEDICAL CENTER; NORTON ZINER, PH. D., PROFESSOR, ROCKEFELLER UNIVERSITY; ALEXANDER RICH, PH. D., PROFESSOR OF BIOPHYSICS, MASSACHUSETTS INSTITUTE OF TECHNOLOGY; AND HARLYN O. HALVORSON, PH. D., PRESIDENT, AMERICAN SOCIETY FOR MICROBIOLOGY, A PANEL

Dr. HOLMAN. Thank you, Senator Chafee.

I apologize in advance for handing you a copy of these comments that is marked in pencil. My time was such that I had to do the final drafting on the airplane.

Senator CHAFEE. We are glad you and the airplane both got here.

Dr. HOLMAN. So am I.

The 2 years of public debate about recombinant DNA experimentation have been illuminating and precedent setting. The public had the opportunity to learn about and, in some instances, grapple with, the enormous power of modern science and technology for social good and ill.

Some scientists and technologists, shedding protective costs and fears, have joined citizens in wrestling with the problems, and have thereby been enriched.

Political institutions have found the issues intriguing, compelling and challenging to the practice of government.

To those scientists who began the debate, and to the citizens and Government leaders who extended and deepened it, we owe a deep debt of gratitude. The debate exemplifies the best in democratic and intellectual traditions, each seeking to strengthen the other while simultaneously enlarging its own horizons.

The conclusions from the past 2 years, in my view, include the following:

(1) Estimation of both benefits and risks from recombinant research is fraught with uncertainty. The uncertainty extends to the conduct of the research itself, not just the ultimate result, because the novel organisms are created in the process of the study. This uncertainty poses issues of responsibility for consequences and consent of those who might be affected.

(2) The practical and intellectual benefits which recombinant experimentation seeks are substantial. However, recombinant technology is usually not the only means to the desired ends. In most instances, alternative means exist, though they may be more cumbersome, more tedious or less elegant. Thus the issue of choice is raised, choice among paths to the desired benefits.

(3) Agreement is widespread here and abroad that regulation of recombinant research is necessary. The issue now is the scope and character of that regulation.

(4) Whenever citizens have been given or have taken the opportunity to examine the issues posed by recombinant technology, they have displayed a remarkable capacity to learn and make sound decisions. Because the priorities chosen in recombinant research will reflect social values, and the research itself will yield social consequences, decisions concerning the research should be made by public processes. Informed citizen participation will greatly enhance those processes.

These conclusions help define the setting of this hearing. There are other relevant considerations. Public policy determination, in a situation of uncertainty about the desirability or outcome of a particular course of action, should have certain attributes. These are prudence, openness, experimentation, rigorous collection of information, and periodic reassessment.

To make these attributes specific, the experiences of responsible Government practice and of informed consent in medicine are helpful. These experiences indicate that:

(*a*) Decisionmakers must be answerable to those affected by the decision.

(*b*) Those affected must be in the position to ask pertinent questions, know to whom to direct the questions, be able to compel answers, and have the authority to choose a course of action based upon the answers.

(*c*) The goals of the action must be generally unobtainable by other less risky means.

(*d*) Benefits of the actions must outweigh risks.

(*e*) Those affected must freely assent to the action and be capable of terminating their participation.

I shall comment on the proposed legislation under three headings—containment, regulatory body, and procedures—using the aforementioned views as criteria. I believe recombinant research should continue under regulation as the only way to resolve the uncertainties and ultimately control the risks. I accept the NIH guidelines as a minimum basis for future recombinant research if they are applied to all such research whether public or private. I assume that legislation can mandate activities which will improve understanding of problems and thus improve regulation.

Physical and biological containment: Neither is flawless. The best of physical containment in the past has not fully succeeded even when the nature of the pathogen under study was known. Now the organisms emerging from recombinant experiments may be unknown.

Biological containment currently depends upon the use of a host organism, K–12 *E. coli*. While substantially enfeebled in virulence, it is nontheless a member of a family of organisms which is ubiquitous in nature and a normal inhabitant of the human intestinal tract. This creates the possibility of the direct spread of novel organisms to man.

Throughout the debate there has been little mention of epidemiology which is the discipline employed to monitor the origin, spread, fate, and consequences of the new organisms which will be created. This absence is not accidental; there are no reliable means for monitoring

recombinant organisms should they escape laboratory containment.

The legislation should therefore:

(1) Mandate intensive research to develop effective means to monitor the production and dissemination of recombinant organisms, particularly those which will be produced in large numbers.

(2) Mandate research to develop experimental host organisms different from *E. coli.* which are not common inhabitants of plants and animals, and which can be more readily tracked.

(3) Require that facilities for recombinant research, particularly those at P–3 and P–4 levels, be well isolated physically from populations particularly susceptible to infections such as hospitalized patients.

(4) Limit the number of sites in which P–3 and P–4 level experiments can be conducted until clear information is available concerning the safety of experiments. My preference would be for no more than three P–4 level facilities nationally and no more than P–3 facility per institution. It would be better if groups of universities or industries shared experimental facilities so as to combine their skills in developing epidemiological methods while reducing the chances of inadvertent infection with novel organisms.

The regulatory body: The importance of this legislative precedent in regulating an intellectual activity, and the complexities of the issues involved, require designation of a specific agency for the task. The agency should be responsible to Congress through the Secretary of HEW, and should have the power to investigate, impose sanctions, and recommend changes in both regulations and legislation. It should be composed of scientists and citizens with the latter in a majority.

It should have a limited life so that a reassessment can be made of its work and role in approximately 5 years. The agency should be charged not only with regulating recombinant research as currently envisioned, but also with establishing guidelines for genetic engineering in the future. The latter capability is certain to emerge in some form from current research.

As a model for the agency, I am attracted to the National Commission for the Protection of Human Subjects. The work of that Commission to my mind has been unique in addressing critical and contentious issues at the interface between medical science and society, and arriving at wise conclusions. I shall refer to the agency as a Commission in the remainder of this testimony.

The hallmark of the regulatory process should be openness. The work of the Commission should be public, it should disseminate information about the research and the regulatory process, and it should facilitate public participation in decisions. Other components of the legislation or the mandate of the Commission should be:

One, the Commission shall report its findings and conclusions to Congress and the public at least annually.

Two, a condition for licensing of recombinant research shall be a clear plan of the applicant agency to inform all persons involved in the proposed work of the nature of the work, the issues of potential risks, and the means of monitoring for those risks. The applicant agencies shall also be required to make available to participating workers and the surrounding communities the results of their monitoring efforts.

Three, no patents shall be granted for any form of recombinant research, or development. Patents imply secrecy and therefore run contrary to both public participation and monitoring. They also create conflicts of interest in which private gain and public good are sometimes counterposed. In this prototypic situation in which powerful techniques are being developed amidst uncertainty about social benefits and risks, openness should be a precondition for permission to proceed.

The Commission must determine liability policy where malfeasance is not involved. If recombinant research is pursued in the public interest with full adherence to public policies, individual research agency liability for harms is not necessarily a just requirement. The agencies should be required to provide all involved in the research with comprehensive health monitoring and disease management programs. Liability beyond that point however is not clearly a responsibility of the agency. This issue is a forerunner of others which will arise during exploitation of modern knowledge, and presents the Commission with an additional unusual task.

Five, Federal regulations should provide a base for local actions but not preempt them. States and localities should have the option to adopt Federal minimum standards or go beyond them. While more stringent local regulations might temporarily impede research or create hardships for some investigative agencies, this is a lesser disadvantage than inhibiting local participation and authority on matters of direct impact to the community.

Rather than discuss the bills before the subcommittee in detail, I have listed criteria whereby they or others might be evaluated. By these criteria, each bill has significant shortcomings. They all emphasize technical aspects of the regulatory process and virtually exclude issues of public participation and public review, problems created by inadequate epidemiological measures, patents, liability questions and pacing of the spread of the research to the development of knowledge about effectiveness of containment.

As a consequence, the richness of the prior debate and the implications of this regulatory precedent do not emerge. Most important, only the Metzenbaum bill establishes a Commission. By establishing a Commission with a broad mandate as previously discussed, much of the breadth and depth needed for this regulatory precedent could be captured.

On balance, I would urge adoption of the administration bill with substantial amendment along the lines that I have indicated.

The last two pages of the written testimony are answers to criticisms that have been made of some of the views expressed here. They are a bit polemical.

The first charges that these recommendations will create a cumbersome bureaucracy, restrict science, and slow the realization of benefits by emphasizing fictitious or unreasonable risks. I do not agree. The question is, whose rights and interests are involved? Viewed from the special interest of a recombinant biologist, haste may be in order. But viewed from the interests of citizens or of good government, haste may be unwise.

The goal is to achieve convergence of scientific and public interests. Time is needed for the public to understand the issues and create sound

policies. The expert who is impatient with public learning, or who seeks to circumvent the public decision process risks not only imprudent error but also intense public antagonism toward science if the expert's views ultimately prove wrong.

Society has long survived without the desired benefits of recombinant technology. A slight further delay while society catches up to science is a small price to pay for enlightenment and unity. Public comfort with this work, and support of it, is essential for the integrity both of the democratic process and of science itself.

The second criticism likens public participation in determining science policy to the anti-intellectual, destructive interventions of the Nazis, or the Soviet government through Lysenko, or Senator Joseph McCarthy into scientific and academic affairs. I believe this analogy to be quite wrong.

In each of those three instances, a powerful political and ideological force sought to usurp science for its own purposes, especially racial superiority or domination and exploitation of others. The intent of these recommendations is the opposite; they seek to involve citizens democratically in the development and direction of science and technology. Left on its own, scientific and technological knowledge could be employed for contamination of one segment of society by another.

By bringing the public into the process, the health and future of both society and science are improved precisely because a check is placed upon potential abuse of scientific knowledge, and a broad social dimension is contributed to the thinking of those who make science policy.

Thank you very much for listening.

Senator CHAFEE. Thank you, Dr. Holman.

Let me just ask one question which the panel might also comment on, and that is the assumption you start with on the first page, No. 2, that the practical and intellectual benefits which recombinant DNA experimentation seeks are substantial.

Does everybody agree with that? Is that a basic point?

Dr. ZINDER. I think there is no disagreement.

Dr. HALVORSON. Amongst us.

Senator CHAFEE. Have you heard any other disagreement on this point?

Dr. ZINDER. Yes.

Senator CHAFEE. From others?

Dr. HOLMAN. Go ahead.

Senator CHAFEE. We will find others if there are.

Dr. HOLMAN. I think that was a key point the way I wrote it "which recombinant experimentation seeks." If you say, promises, then some of us will qualify our response.

If you say, guarantees, then I don't think anyone at this table will agree. That has been the theme throughout the whole debate.

The other side of the argument is that there are alternate ways to approach realization of many of the benefits which this technology may yield us, therefore, there is the issue of choice. We are not stuck with recombinant technologies as the only way to improve agricultural yield, so that there are modulations on the point.

I do not know of anybody who says that the goals of the experimentation, the things that people think people may be able to accomplish, are going to be highly beneficial if they arrive at it.

Dr. RICH. A fundamental distinction can be made between fundamental research and amplifications. We have already seen substantial results in terms of fundamental research, new knowledge that gives us new insight from this technology.

When it comes from links, we can't be sure, we believe though, I believe, that there will be many very useful medical applications. I might name several, and I might be wrong on some.

On the other hand, I think everyone agrees that there will be substantial advances in terms of fundamental knowledge, and this is likely to be translated into practical benefits.

Senator CHAFEE. Perhaps you could comment, as each of you go forward.

I thought the make up of the Commission as proposed by Dr. Holman was interesting in that it would be composed of scientists and citizens with the citizens in the majority.

I do not know how you define a scientist. I suppose that is someone with a Ph.D., or a medical doctor. I do not know what you are thinking of when you say scientists. It is interesting that your proposal had citizens, that is, nonscientists in the majority.

Perhaps you will comment as we go along.

Perhaps Dr. Zinder could proceed now.

Thank you, Dr. Holman. That was an excellent statement.

Dr. ZINDER. Mr. Chairman, and members of the Subcommittee on Health:

I am amazed by the course of events since I testified before this committee 6 months ago. Last September I hoped that the NIH guidelines for recombinant DNA research would be given an honest chance to show their worth. I also hoped that the words "recombinant DNA" would not become a pejorative expression. But a chaotic debate has overwhelmed us. The situation has changed. Did this change occur because we have experienced a disaster? Did evidence appear for any of the horrendous scenarios that the alarmists have conjured up? No. In fact, during this past 6 month period considerable new scientific information has been discovered, and the new information confirms both the power and the likely social importance of this new technology.

I will digress a bit. Just yesterday, as I was editing my testimony, the current Journal of Science came and I offer it for the record. It contains 25 articles all on recombinant DNA. I have not yet had time to digest it, but I can tell you just from the titles that this is a treasure trove of scientific information which could have been obtained in no other manner.

Senator KENNEDY. We will have those particular articles that will be useful reviewed by the staff and we will take selected passages of the articles and make them a part of the record. We will file the magazine as a part of our files.

Dr. ZINDER. You are welcome to my copy.

I begin my testimony in this manner because I want to clarify an important scientific point. Recombinant DNA is shorthand for the techniques of splicing together DNA molecules. We are not debating these techniques. They exist, they have been applied, they are neutral, like the technique of driving a car. Instead, we are discussing two points: the kinds of molecules that may be spliced together, and the de-

liberate or accidental uses of these recombinants. We are saying that the driver should have a mechanically safe car and that the driver must understand the rules of the road.

An assessment of the various kinds of molecules that are spliced—and the possible properties of the recombinants—became the basis for the graded degrees of containment that were imposed by the NIH guidelines. Accordingly if the Congress passes legislation in this area, the law must be equally precise in its definition of "recombinant DNA". I offer the following definition for legislative purposes. "Recombinant DNA" should be defined as DNA which has been joined together, in a cell free system, such that the recombinant can be replicated in a host in which one of its components would not normally be replicated. Joining of DNA molecules from species which normally exchange DNA should be excluded from this legal definition.

Before going further, I should like to review briefly the history of this subject. In April of 1974, a group of us took an initiative that we felt would protect the public interest while also preserving the freedom of scientific inquiry. We took this initiative openly, and the subsequent debate was open. Some observers, even the most skeptical by temperament, saw what we were trying to do. For example, the Rolling Stone of June 19, 1975, said the following:

> Perhaps that was the final, foggy significance . . . a promise that the scientists who deal with the most fundamental life stuff will not sequester themselves beneath Chicago stadiums or within blockhouses in the New Mexico desert—that their work, at least as significant as the most subtle of sub-nuclear manipulations, will be done with care and public scrutiny.

Between 1974 and mid-1976, there was much discussion of the details of particular experiments and of the mechanisms and forums that might be developed to regulate this important line of work. Of course, there was always some amorphous criticism floating about. But the criticism had no focus.

Suddenly, 2 years after we began the discussion in public, a group of dissident scientists emerged. These individuals provided leadership for anyone who criticized, or misunderstood, the goals of applying the new research techniques. The scientifically trained critics made a few good debating points, most of which had already been quietly evaluated by the previous assemblies of scientists. But they also made indiscriminate attempts to frighten the public, while introducing an ad hominem tone into the argment. Why did these individuals wait so long to speak? Neither I, nor anyone else, can answer that question without exploring the psychology of these particular scientists. But we do know that frightening scenarios soon appeared in the media. None of the scare stories has any substantial basis in fact or reasoned speculation.

Recently, beginning last March, some of the critics have changed tactics, probably because it had become rather simple to refute their outrageous scenarios. The new criticism goes this way: (a) the recombinant DNA technique is not needed; (b) there are other ways to accomplish the same goals; and (c) in any event, progress in this line of research would not help those most in need because of the inequitable distribution of benefits within our society. This new line is directed, more cleverly than are the alarmist scenarios, toward the genuine concerns of sensible laymen and the public. However, it is grossly untrue

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or distorted. Until a few weeks ago, no one had seriously denied the genuine power and likely benefits of using the recombinant DNA technique. The question was whether the conjectured risks were worth the estimated benefits. Suddenly we are told that there will be no benefits at all, only risks. These gross distortions have no bearing upon the legislation in question and can safely be disregarded.

Now let me return to our main business. We are here today to attempt to convert the NIH guidelines into a wise law. If we must have legislation, I prefer a single national law rather than a crazy quilt pattern of varying laws at the State, city, and neighborhood levels. Multiple laws would only lead to confusion and, ultimately, to inadvertent violations. The national law must preempt all local laws. Otherwise, the ongoing process of basic research will be obstructed unnecessarily. Since no one can say where the next important discovery will originate, the scientific community and the general public look to the Federal Government for protection of our Nations' overall biomedical strength.

I cannot speak for all scientists on the various provisions that such a law should contain. Many investigators are angry that the situation has gone so far toward threatening perceived rights of freedom of inquiry. However, most of us could live with the basic thrust of the NIH regulations. The most important point for the active scientists in this field is that a new law be flexible: Able to take into account next year's new data, as, for example, we move to eliminate one element of risk after the other. The nature of the scientific endeavor is such that we can't predict the future and we are always moving into the unknown. The Congress has implicitly recognized this point for it has provided generous funding for basic research without specification of purpose.

I realize that one of the major arguments for comprehensive legislation is that the NIH guidelines refer only to work supported by Federal funds. Frankly, I had hoped that a special solution might have been developed so that the NIH guidelines would control Government grantees and contractors, while a specific new law would cover other segments of society. Unfortunately, this path does not seem feasible now.

In designing any new law, I favor the following general approach: The Secretary of HEW would appoint a special regulatory Commission—this is Senator Chafee's point—composed of interested citizens, competent scientists, and other professional specialists. The Commission would have the appropriate authority to monitor the pattern of research in the areas of recombinant DNA and the impacts of the guidelines affecting this research. I hope that the existing NIH Advisory Committee would be maintained to serve as the prime technical resource for the Commission to help revise and update the regulations, as it would be closest to new developments.

Advisory roles could also be assigned to such bodies as the National Academy of Sciences, to the International Council of Scientific Unions, and to other interested groups. Such a Commission would require licensing of the institutions where recombinant DNA work is being done and the registration of specific projects.

The Commission would have the power to inspect and control the work as delineated by the law. Should any of the conjectured risks

materialize, the Commission or the Secretary would have the power to enforce appropriate measures. The Commission's regulating authority could probably be delegated to the Center for Disease Control which, as I understand it, already has comparable authority over commercial testing laboratories.

There are several different bills that have been introduced, but I'll not attempt to discuss them in detail. However, I do want to remind you that we are moving into a precedent-making area—the regulation of an area of scientific endeavor—and I must plead that this be done with extreme care and without haste. The record of past attempts of authoritative bodies, either church or state, to control intellectual thought and work have led to some of the sorriest chapters in human history.

Specifically, with regard to two of the bills, the following disturbing points caught my eye: In S. 621, section 7 states that, "Persons carrying out research involving recombinant DNA shall be strictly liable, without regard to fault . . ." and so forth. Should such a provision be included, the bill could readily be reduced to one sentence that prohibits all recombinant DNA work in the United States. No scientist could obtain the necessary insurance to protect himself; the work would cease. In S. 945, section 8(b) would allow for nuisance suits. In this instance, relief could be afforded the defendant by having the plaintiff, should the suit be lost, responsible for all the costs of the litigation, including attorney's fees, plus some penalty. I cite these two examples primarily to reinforce my pleas that you move with extreme care into this new area of legislation.

At this late point in my testimony, I've a strong impulse to don my professorial robes, have a blackboard brought in and give the usual 50-minute lecture on the events of the past 3 years and my predictions of the new scientific knowledge that will be obtained using recombinant DNA. It would also certainly include this quote from an article on recombinant DNA by my colleague, Prof. Rene Dubos, eminent microbiologist and environmentalist: "I see no chance of creating, by gene recombination in the laboratory, strains of microbes more virulent than those endlessly being created by natural processes." But as this kind of a lecture is not possible, I have one last comment to make. I have been astonished, during discussions of recombinant DNA, to hear people say that there are things we are better off not knowing. As a scientists, I reject this view. As a citizen, I'd be amazed if our society would embrace such a view when our future depends on strongly acquiring such new information. Hence I remind you of the remark of Lewis Thomas, who recently wrote about recombinant DNA in the New England Journal of Medicine. Dr. Thomas said, and I paraphrase, that the greatest hubris of all is to think that you know what we ought not to know.

Thank you.

Senator KENNEDY. Very fine. We will come back to some questions, but first I will ask to have our panel complete the formal statements.

The next speaker is Dr. Alexander Rich who is a professor of biophysics at Massachusetts Institute of Technology. He has been very helpful to us on many very important matters. He has given us a great deal of his time and thought.

Dr. Alexander Rich.

Dr. RICH. I am speaking here today in two capacities. One capacity is as a member of the National Science Board and another as a faculty member at the Massachusetts Institute of Technology.

I have submitted a short statement for the Board which simply describes the activities of the Science Board. It is very similar to the statement that I delivered before the Rogers Committee.

[A statement by Dr. Alexander Rich and other material supplied follows:]

ADDITIONAL REMARKS SUBMITTED BY
DR. ALEXANDER RICH TO THE
SUBCOMMITTEE ON HEALTH AND SCIENTIFIC RESEARCH
SENATE COMMITTEE ON HUMAN RESOURCES
APRIL 6, 1977

THE NATIONAL SCIENCE FOUNDATION IS AN INDEPENDENT FEDERAL AGENCY CHARGED WITH SUPPORTING SCIENTIFIC RESEARCH AND SCIENCE EDUCATION. THE FOUNDATION, AS PART OF THIS EFFORT, SUPPORTS BIOLOGICAL RESEARCH THAT INCLUDES INVESTIGATIONS CONTRIBUTING TO FURTHERING UNDERSTANDING OF DNA AS THE PRIMARY SOURCE OF ENCODED GENETIC INFORMATION THAT DETERMINES THE PROPERTIES OF ALL LIVING ORGANISMS.

A NEW METHOD HAS BEEN DEVELOPED BY WHICH SEGMENTS OF GENETIC MATERIAL (DNA) FROM DIFFERENT SOURCES CAN BE JOINED TOGETHER OUTSIDE OF LIVING ORGANISMS. SUCH LABORATORY GENERATED MOLECULES ARE REFERRED TO AS "RECOMBINANT DNA." THROUGH THE USE OF RECOMBINANT DNA TECHNIQUES IT IS POSSIBLE IN PRINCIPLE TO TRANSFER GENETIC MATERIAL FROM ANY SOURCE TO A HOST CELL WHERE IT MAY BE ABLE TO REPLICATE AND FUNCTION. THE NSF RECOGNIZES THAT THIS METHOD POTENTIALLY OFFERS SIGNIFICANT BENEFITS IN FUNDAMENTAL AND APPLIED BIOLOGICAL RESEARCH. THE NSF ALSO RECOGNIZES THAT SUCH RESEARCH MAY INVOLVE BIO-HAZARDS. THEREFORE, IT IS ESSENTIAL THAT ALL RESEARCH INVOLVING THE USE OF RECOMBINANT DNA MOLECULES BE CONDUCTED IN A RESPONSIBLE MANNER ADHERING TO SAFETY PRECAUTIONS AND CONDITIONS DESIGNED TO PROTECT LABORATORY WORKERS, THE GENERAL PUBLIC, AND ALL OTHER LIVING ORGANISMS IN THEIR NATURAL ENVIRONMENTS.

THE FOLLOWING CHRONOLOGY HIGHLIGHTS THE NATIONAL SCIENCE BOARD ACTIVITIES IN ADDRESSING THESE ISSUES:

FOLLOWING THE REPORT OF THESE NEW TECHNIQUES, THE NATIONAL SCIENCE FOUNDATION, REPRESENTED BY DR. HERMAN W. LEWIS ON THE NIH RECOMBINANT DNA MOLECULE PROGRAM ADVISORY COMMITTEE, PARTICIPATED IN DEVELOPMENT OF NIH GUIDELINES THAT WERE PUBLISHED IN THE FEDERAL REGISTER ON JULY 7, 1976.

AT ITS MEETING ON AUGUST 20, 1976, THE NSB APPROVED A RESOLUTION ADOPTING AS POLICY OF THE NSF THE RELEVANT PORTIONS OF THE NIH GUIDELINES. A COPY OF THIS RESOLUTION IS SUBMITTED FOR THE RECORD.

IN SEPTEMBER 1976 THE SECRETARY OF HEW, WITH APPROVAL OF THE PRESIDENT, ESTABLISHED A FEDERAL INTERAGENCY COMMITTTEE ON RECOMBINANT DNA RESEARCH TO ADDRESS EXTENSION OF NIH GUIDELINES BEYOND NIH-SUPPORTED RESEARCH. NSF HAS ACTIVELY PARTICIPATED IN AND WILL CONTINUE TO SUPPORT THE EFFORTS OF THIS INTERAGENCY COMMITTEE. DR. HERMAN LEWIS AND DR. LAURENCE BERLOWITZ ARE REPRESENTING NSF IN THE DELIBERATIONS AND FORMULATION OF RECOMMENDATIONS.

THE BOARD IS PROVIDING CONTINUING OVERSIGHT IN THE AREA OF RECOMBINANT DNA RESEARCH AND ASKED DR. RICHARD C. ATKINSON, ACTING DIRECTOR OF THE FOUNDATION, TO CONVEY ITS VIEWS TO DR. DONALD S. FREDRICKSON, DIRECTOR OF THE NATIONAL INSTITUTES OF HEALTH. THEIR RECENT EXCHANGE OF LETTERS IS ATTACHED FOR THE RECORD.

THE ISSUES INVOLVED IN RECOMBINANT DNA RESEARCH ARE OF CONTINUING INTEREST TO THE NATIONAL SCIENCE BOARD. THE NSB AND THE FOUNDATION HAVE TAKEN AN ACTIVE ROLE IN REVIEWING GUIDELINES FOR SUCH RESEARCH AND NSF HAS DEVELOPED ADMINISTRATIVE PROCEDURES TO ADAPT THESE GUIDELINES FOR RESEARCH SUPPORTED BY THE FOUNDATION.

RESOLUTION
ADOPTED BY THE
NATIONAL SCIENCE BOARD
AT ITS 183RD MEETING
August 20, 1976

The Board unanimously RESOLVED that the following portions of the NIH Guidelines for Research Involving Recombinant DNA Molecules of June 1976 as published in the Federal Register on July 7, 1976, are hereby adopted as policies of the National Science Foundation to govern the activities of its staff, grantees, and principal investigators: Part II (Containment), Part III (Experimental Guidelines), Subparts A and B of Part IV (Roles and Responsibilities), and Appendix D; and REQUESTED the Director to establish appropriate administrative means, including proposal review procedures, to ensure that the award of National Science Foundation grants and activities of grantees and principal investigators are in conformance with the Guidelines for Research Involving Recombinant DNA Molecules adopted above.

NATIONAL SCIENCE FOUNDATION
OFFICE OF THE DIRECTOR
WASHINGTON, D.C. 20550

February 7, 1977

Dr. Donald S. Frederickson
Director
National Institutes of Health
Bethesda, Maryland 20014

Dear Dr. Frederickson:

As you know, the National Science Board at its August 1976 Meeting adopted as National Science Foundation (NSF) policy the relevant portions of the NIH Guidelines for Research Involving Recombinant DNA Molecules. The Board has had occasion to consider this matter further at the February meeting just concluded, and I would like to apprise you of our latest thinking on this subject.

We continue to believe that NIH should be primarily responsible for the promulgation of standards for the conduct of research utilizing recombinant DNA techniques, with the broadest possible participation of other concerned federal agencies and private groups in the process of developing these standards.

In our view, there would be great value in having a legislative affirmation of this "lead agency" role of NIH to assure that a single standard will be applicable to all investigators--regardless of source of support, or whether the sponsoring institution is academic, industrial, private or governmental. I would welcome an opportunity to explore with you at an early date the efforts we might make to pursue establishment of this legislative authority.

It is also our belief that a system for assuring accountability and conformance to accepted standards must be developed. Such a system would help to reassure the public and to avoid inadvertent exceptions to required practices, as well as to provide a means for detection and termination of deliberate non-compliance. Whatever system is developed should, in our view, be applied uniformly in every instance of use of recombinant DNA techniques. Because of the variety of circumstances

of use, establishment of a separate organizational unit concerned with compliance should be considered. Such a unit probably should be located within the agency having lead agency responsibilities in this area. It is our belief that alternative administrative arrangements might result in a discontinuity of standards and compliance assurance. I look forward to pursuing this viewpoint with you.

In any event, it is clear that our knowledge of the potential benefits and risks inherent in recombinant DNA research is severely limited by the paucity of fundamental information. Accordingly, we recognize the fluid state of the current guidelines and the continuing necessity to revise these as new knowledge accumulates. We fully share with you the intent to assure that research in this field is carried out in a responsible manner in all sectors of society. It is, of course, also important that the guidelines and system we adopt do not operate to inhibit the research thrust in this area. In my view, it is essential that NSF and NIH work cooperatively in all matters relating to use of recombinant DNA techniques.

I was delighted to learn of your reappointment. It is an eloquent testimony to the high regard in which you are held by the entire scientific community. The Board joins with me in extending to you best wishes for the future.

Sincerely yours,

Richard C. Atkinson

Richard C. Atkinson
Acting Director

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND 20014

FEB 18 1977

Dr. Richard C. Atkinson
Acting Director
National Science Foundation
Room 520
1800 G Street N.W.
Washington, D. C. 20550

Dear Dick:

I am writing in response to your letter of February 7. Thank you for informing me about recent considerations by the National Science Board concerning the NIH Guidelines for Recombinant DNA Research. As you may know, I serve as chairman of the Interagency Committee on Recombinant DNA Research that is responsible for assessing the nature and scope of this research nationally and, based on that assessment, for coming forth with recommendations on legislation and/or regulations to govern this research nationally. Your comments are most helpful and I will bring them to the attention of the Committee. Larry Berlowitz and Herman Lewis represent the Foundation on the Committee, and I would welcome any further comments on your Board's actions.

Over the past two months a special subcommittee of the Interagency Committee has been at work reviewing present statutory and regulatory authority to govern recombinant DNA research. On the basis of that review the subcommittee agreed that no present agency or Department has full authority to regulate this research. The subcommittee has been considering a bill of particulars for possible regulation or legislation and has taken into account a proposed bill by Senator Bumpers on this matter. A meeting of the Interagency Committee is scheduled for February 25 which will be devoted to a review of the subcommittee's work. In light of Senate hearings scheduled for April on this matter as well as the proposed bills in Congress, any recommendations the Committee might make on proposed regulation or legislation would be especially important prior to the forthcoming hearings. I will keep you informed on the Committee's progress.

Thanks very much for your letter.

Sincerely yours,

Donald S. Fredrickson, M.D.
Director

Dr. RICH. I would like to make a number of additional comments. I am sorry I do not have those in printed form. Many of them stem from the forum that was held at the National Academy of Sciences a few weeks ago. I had the privilege of cochairing that with David Hamburg, president of the Institute of Medicine.

We learned a great deal at that meeting. And I would like to sort of deliver to you some of the observations. This meeting represented a continued and yet another attempt to bring this issue before the public. It is an issue of great importance, one that properly should be debated.

It represents a very subtle interface between a scientific endeavor and the problems of society.

In that respect, I think the forum was quite successful. It was very well covered by the press. It generated a great deal of discussion.

A number of points remain to be emphasized. One, this is an exceedingly difficult subject for the lay subject to understand. The problem is not solely because of the technicalities of the issue, but rather because there is an apparent continuum in the discussions between at the one extreme the Andromeda strain, the fiction of the Andromeda strain, and at the other extreme, the recombinant DNA.

Unfortunately some of the discussion about new life forms gives rise in the minds of the lay public to an appreciation, an alarming appreciation of the nature of this research. We have not created new life forms with our current techniques; we can modify the DNA of an organism by one one-tenth of 1 percent up to 1 percent. It has some very interesting features. There is nothing in the research that has gone on so far that would give us justification for the kind of alarmist scenarios that have been spread about.

It is difficult then to find out where this reality-fictional interface is. One of the points made in the form, there were several speakers from the field of epidemiology, people from the Center for Disease Control, and a number of specialists. They spoke from the background of 100 years of epidemiological experience. We know a great deal about medical or microbiology. We know a great deal about how diseases spread. We know a great deal about how one should take precautions against spreading them.

They addressed themselves to the following questions:

Is it likely that one could create recombinants which were more pathogenic than existing recombinants? Their answer, similar to the one just stated by Professor DuBos, is that it is highly unlikely.

Is it likely that such organisms would spread infections, epidemics?

Again, the answer, highly unlikely. No more likely than existing pathogens with which we are familiar.

These comments should not be taken as indicating they should not have regulations in the conduct of this research. Rather it indicates something about what these experts in the subject field should represent a kind of parameter or a vantage point from which one should view these regulations.

These regulations are precautionary in nature and it is quite reasonable and sensible to take precautions but, at the same time, we should recognize that the likelihood of running into trouble is very small.

Indeed, we have had 3 years of research in this field and nothing has happened in this direction.

Another issue discussed at length was the impact of this research, of this technique and fundamental research and we have already referred to that somewhat.

One of the most exciting papers that I have read recently is a paper done by colleagues of mine both in Cambridge and at Cold Spring Harbor which describes the sequence of the gene which produces hemoglobin, the protein that carries oxygen.

What was interesting here, using recombinant DNA techniques, we could get the structure of that entire gene, not only those regions that code for the amino acids, but the regions at the beginning and end of that message that somehow regulate the production of this important protein.

The language of regulation is written there. We have it. We don't understand how it works, but it is very impressive to get that sort of information out. It clearly will give us an enormous insight into the way nature works.

For example, there is a disease, thalassemia, in which we believe construction at the beginning of the message, the regulatory part, is disordered in some way and results in a failure to produce hemoglobin. Whether indeed we will learn to treat thalassemia through this, I do not know, but it is clear that the impact in terms of fundamental knowledge is substantial.

In this form, a number of what I will call long-term ethical questions were considered. These are important subjects. They are not subjects we can dismiss politely. They refer to the question of man in relation to biological research and to society. They are difficult problems. These are questions that society must face. They require a concentrated, concerted effort involving lay people, scientists—people of all types.

Now, I think we should have in some form or another a focus place for considering these things because recombinant DNA technology is just a technology. How it is applied, really that is what society has to consider.

In terms of legislation, here I think that we are first of all using the NIH guidelines and I think they offer a protection with a substantial margin of error, so to speak, a wide margin of safety. And I think it is desirable to have uniform standards throughout the country, applicable both to industry and research institutions. The legislation, of course, is unusual because it is precautionary legislation for hypothetical theoretical risks.

The legislation, of course, is written based on our knowledge and the rate at which knowledge is changing is very rapid. We must have adaptability and flexibility and that is in the legislation. Senator Kennedy referred to this as a beginning. It is very important, indeed, that this legislation change as we learn more.

In particular, one aspect of this, as we learn more about naturally occurring recombinants, that will alter our perception of what is needed. We all know that naturally occurring recombinants occur. We have bacteria exchanging with DNA and we know this because we studied it. We do not know what happens between higher organisms and bacteria. As we clearly learn more, we clearly have to change our position. We do not want to find that the Secretary of Health, Education, and Welfare has issued regulations dealing with something that occurs naturally.

Let me focus on a few specific points relating to legislation. The legislation is, indeed, the final draft of the legislation which came out of the Interagency Committee and was recently released. The first comment deals with the definition of recombinant DNA. I am now reinforcing what Professor Zinder has said. We ought to define recombinant DNA as a DNA in which we are joining segments of DNA from different organisms that are not joined together by nature. We should not have to try to have the Secretary regulate research on processes that occur in nature.

The advantage of putting that definition in is that, of course, it will stimulate investigations as to what actually happens in nature. If we find, for example, that there is a wide interchange, a substantial interchange of DNA between man and micro-organisms, that will surely change our perception of how this legislation should be developed.

A second point concerns the discussion in section 18(4) of the bill concerning hazardous recombinant DNA. As I read the combination of part 2, italics, roman I and roman II, there is a statement there which says a hazardous recombinant DNA in which the recombinant is likely to be moved from one facility to another or to spread from such a facility.

Senator KENNEDY. That presumes a high degree of monitoring which we have not really gotten into today and I do not want to get weighted down with that. But how are we going to do that and how will we gear up to do it?

Dr. RICH. Yes, the problem is with the wording of the bill, that coupled with the fact if it is not known to be a hazard, a double negative, which says that this DNA is assumed to be hazardous DNA. That needs to be looked at because it will invite endless litigation.

Let me comment now on the question of State and local requirements. I feel strongly that local citizenry should be involved. What I would like to suggest is something concerning the manner in which they should be involved. I think it is important that an educational process, similar to the one that went on in Cambridge, Mass., be carried out. I think the citizenry can look into this area. What we have learned is if people want to spend the time studying, they can understand the issues.

I think the local involvement or State involvement should be at the level of a participating in local biohazards committee where they can play an effective role. They should also, I think, study the regulations and if, indeed, they think they would have to be changed, they should petition the Secretary for such a change. But then that change should be made, I think, nationally. If they can make a case and give reasons why our guidelines are inadequate, then I think the regulations ought to be changed nationally.

I think we would run into serious difficulties with a kind of patchwork of what can be done in Waltham cannot be done in Watertown. I think there is another danger, and here I have to take issue with my Governor Dukakis, a man who I support strongly on most issues.

The question was what would happen should a community be allowed to ban recombinant DNA research?

Now, in general, I am very much in favor of a democracy and freedom of action. In this instance, the effect of this, if there is a research university in that community, the effect of it would be to kill that

community. The research institution would cease to function. It could not recruit competent people in the field of biology. It would fold up its biology department because this area of research in the life sciences is so vigorous and so important that one could not recruit a respectable faculty.

This, I think, would have a kind of autocatalytic role and result in the destruction of that institution.

I think when you destroy this kind of community—it is very difficult to build a center of excellence in research. It can be destroyed, and once destroyed, it cannot be built up so readily. Therefore, I think we should not go into this hazardous area lightly. I think we should reflect on this. For myself, I feel that we should have national standards and we should have participation at the local level to change their standards if they can make a good argument for them, but I would not extend it to the point, as in the reading of the bill which, in fact, had "The Secretary shall change those standards."

I would at least say there "the Secretary may change those standards," because I think destroying research institution is something that is of national concern. It is something which is more than a local concern.

Senator CHAFEE. That point puts you and Dr. Zinder in opposition to Dr. Holman.

Dr. ZINDER. Strongly.

Dr. RICH. 100 percent.

Mr. HALVORSON. I join you.

Dr. RICH. Three to one.

On the question of civil versus criminal liabilities, I would say the following: I have no argument with the criminal violation, but under civil violation, I would suggest it would be more effective rather than to fine a person up to $5,000 a day, it would be more useful to offer as a penalty their loss of license to the institution.

Senator KENNEDY. I agree. I really question whether there should be any criminal aspect in this. The court is not going to put a researcher in jail in any event.

Dr. RICH. Loss of license provides for collective responsibility. It makes everyone look after everyone else. That is a more effective way to go than the other route.

Senator KENNEDY. I personally feel they simply would not put researchers in jail.

Dr. RICH. The facility should be designed as an institution. We should not have licenses to individual laboratories or subsections of an institution. The whole institution should be licensed.

Senator KENNEDY. Thank you. Your statement has been very helpful.

Dr. Halvorson.

Dr. HALVORSON. Thank you.

I am Dr. Harlyn Halvorson, president of the American Society for Microbiology and director of the Basic Medical Science Research Institute of Brandeis University, Waltham, Mass., and chairman of Brandeis University Biohazard Committee. I am speaking as the president of the American Society for Microbiology.

We have submitted two papers. The one is a position paper which the staff has, and the other is a paper given yesterday to the EPA

hearings by Dr. Ginsberg who has been chairman of the subcommittee which we do not speak about any more.

[Dr. Ginsberg's paper presented to the Environmental Protection Agency hearings on April 4, 1977, may be found in the files of the subcommittee.]

Dr. HALVORSON. The American Society for Microbiology encompasses over 25,000 scientists whose expertise ranges from molecular genetics to infectious diseases.

The American Society for Microbiology is the largest single life science society in the world. The ASM members contribute to the applied fields of infectious disease, clinical microbiology, epidemiology, industrial fermentation processes, ecological microbiology, and food technology as well as to the fundamental areas of immunology, genetics, virology, oncology, microbial physiology, environmental microbiology, mycology, and host-parasite interactions. Within our century, a third of all Nobel Prize Awards in physiology and medicine have been bestowed upon microbiologists. By virtue of its size, scope, and services, the American Society for Microbiology is a significant voice in the scientific community.

Prior to this meeting hundreds of hours have been devoted to testimony and debate presenting opinions that the NIH guidelines are appropriate and adequate, that they are too stringent, or that they are too lax. Most of those who hold the former views believe that recombinant research is critical to needed biological advances and that the benefit/risk ratio is overwhelming in favor of recombinant DNA research.

In contrast, most of those of the latter persuasion—for example, that the guidelines are not sufficiently restrictive—believe that recombinant DNA research should be forbidden, that it is too dangerous, that it is an invitation to worldwide disaster, and that suitable guidelines cannot be devised because scientists are unreliable.

Unfortunately, the debates on both sides of the aisle often have not distinguished among the many types of experiments possible, some of which are forbidden by the guidelines. And all too commonly horror scenes were predicted without scientific fact.

Parenthetically, it is of interest to point out that the major critics of this important research, and those with the most lurid predictions of forthcoming doom, are uninformed laymen and scientists who have never dealt with pathogenic organisms and appear to have little understanding of the mechanisms of spread of infectious agents. It is also noteworthy that not one expert in infectious diseases or one microbiologist accustomed to studying pathogenic organisms has spoken out against recombinant DNA research.

To obtain a professional opinion of the guidelines, Dr. Donald S. Fredrickson, Director of the National Institutes of Health, asked the American Society for Microbiology to study the guidelines and to determine whether they met essential levels of safety according to the society's professional standards.

Accordingly, I appointed a task force of microbiologists under the chairmanship of Dr. Harold Ginsberg, chairman, Department of Microbiology, College of Physicians and Surgeons, Columbia University, New York, who is present today. This task force was designed to give a broad representation, particularly in areas involving the han-

dling of pathogenic micro-organisms, epidemiology and medical microbiology, virology, industry and, in particular, members who had not served in drawing up the initial NIH guidelines, but were knowledgeable in recombinant molecule technology.

We believe that in the public interest scientific societies such as ASM have an obligation to speak out on matters where their scientific expertise is required for evaluation of the issues. The public discussions on in vitro recombinant DNA research are a case in point. This problem is fundamentally one in epidemiology: the science concerned with factors that influence the spread of disease. Yet the discussions have been characterized by uncontrolled imagination and excessive claims. The public needs to be informed if it is to have an important influence on such technical problems which involve transmission of infectious diseases, virology, epidemiology, microbial genetics, and the isolation techniques which have made possible the safe study of pathogenic micro-organisms.

As president of the only society which encompasses these disciplines, I have been deeply concerned over the inadequate technical level of this national debate.

Our committee made two unanimous recommendations, which have been accepted by the Board of Directors of the ASM:

(1) The committee recommended that the American Society of Microbiology should endorse "the NIH guidelines," and

(2) The society should assume leadership in the development of an educational program in the principles of contagion and host-parasite relationships for those engaged in recombinant DNA research but, nevertheless, untrained in these highly relevant areas of microbiology.

Much has been said about the fact that *E. coli* should not be employed as the host vector since it is a normal inhabitant of the gastrointestinal tract. But these same critics rarely make mention of the fact that the *E. coli* K–12 strain employed is a crippled organism which apparently can grow only in test tubes to which necessary ingredients are added.

In addition, Dr. Roy Curtiss is developing a host whose growth requirements are so severe that it would be unlikely to survive in the human GI tract. The cry is raised that addition of a new gene will change the *E. coli*'s character and make it a dangerous pathogen. Of course, until the experiment is made none of us can answer this seeming cry of anguish with certainty—but in my opinion, the charge is microbiological poppycock.

The notion that evolution has proceeded undisturbed by man seems without substance. Do the purveyors of this argument believe that the prevalent use of antibiotics has no effect on the evolution of micro-organisms? Do they believe that the widespread use of vaccines leaves evolution undisturbed?

Is it considered that dieting, vitamins, tobacco, alcohol, the pill, etc., are without impact on evolutionary events? Then how delicately balanced is evolution, and why would bacteria containing recombinant DNA's furnish a greater hazard to the evolutionary processes than do numerous daily events now taken for granted?

What evidence is available suggesting that the addition of extra-genetic material in a bacterium necessarily makes the organism able to cope more effectively with its environment?

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Indeed, available evidence would suggest that it may have the opposite effect and increase its division time so that it will have more difficulty in maintaining its ecological niche and surviving in the sea of organisms in man's gastrointestinal and respiratory tracts.

It seems unlikely that micro-organisms which normally inhabit the gastrointestinal tract are being continually bombarded by DNA from dying human cells. It seems likely that heterologous human DNA occasionally enters the bacterium. If such DNA can function, recombinant organisms must continually arise. If they have an evolutionary advantage they would have become evident. Although rare examples of bacteria excreting mammalian gene products, such as a hormone, have been reported, if they exist they are rare.

Our specific recommendations are:

(1) Adoption of the NIH guidelines to govern both research and use of recombinant DNA, regardless of the source of funds.

(2) Authority for licensing of facilities and determination of procedures to be regulated should be vested in the Secretary of Health, Education, and Welfare or the Director of NIH.

(3) Regulations should be flexible so that as further information becomes available the regulations can become less stringent, or if necessary, more stringent.

(4) Uniform licensing procedures should be established nationally.

(5) Licensing should be on an institutional basis. Local decision-making should be in the hands of a local biohazard committee which will have public representation, and I support Dr. Rich on this point.

(6) The Licensing Committee of the NIH should be composed only of technically trained members who represent the subdisciplines of the sciences and are familiar with accreditation procedures, and I speak to that in the document at some length.

Finally, we believe that the threat of license removal should provide the only necessary deterrent for compliance with the regulations, and this has tremendous local peer advantage which I think would, in effect, would be a stronger deterrent.

Finally, much of the controversy that has surrounded, and indeed obfuscated, the DNA recombinant field, has been generated by misconceptions and fears of the unknown. Most of the questions raised could be experimentally answered. This must be done, and groups are planning this intellectually satisfying approach. To our minds in recombinant DNA research, the major biohazard is ignorance.

Senator KENNEDY. Thank you very much. We have some strong testimony in terms of agreement by the panel members, and we have some areas, important areas where you do not agree.

I am wondering, Dr. Holman, if you could talk just a moment about the monitoring capability. What should we expect? How important is that going to be? What is the nature of the state of the art in terms of being able to monitor? What can we expect on this?

Dr. HOLMAN. I think it would be good to have the others answer.

I differ from them in another way, aside from the local control issue, and that is that they all are involved in microbiology and I am not. I am a physician. I have some understanding of it. My understanding from the standpoint of monitoring the escaped organisms, the host data will be used for the experiments from the lab—that is no real problem. Presumably you will have nothing escape from your contain-

ment facilities through air, et cetera, and you can grow these organisms and look for them in some way. It may be more tedious than with hardy organisms, but you can do it.

The issue I raised is different. Even though the breaks in containment in vitro have been very few, they have never been able to eliminate the pathogen that got out to the family. There have been no epidemics in the immediate community. Only the workers and the families are involved.

You have to find out if the organisms are present in workers or others who are in contact. This requires either growth of the organisms or identifying them by markers. It might carry an antigen. You can identify which is a specific antibody. It might produce a dye such as the organism *seraeces marsesens* and you could see the dye. There are ways of doing that.

My understanding is when we are dealing with a new organism, we do not know the genetic constitution. We do not have the circumstances of searching for the needle in the haystack for that worker or individual family member. I do not think that should prevent the worker from going on, but should face that deficiency straight up and attempt to correct it by having experimentation done within this same program to develop the methods that will permit us to monitor correctly the possible escape of organisms outside the immediate confines of the research facility.

Have I misstated the situation?

Dr. ZINDER. I would like to ask him a question because I am not quite sure what he means by escape of organisms. There are two kinds. There is first the organism which you are going to use as the host for the recombinant DNA experiment and then there is the recombinant DNA which, assuming you put in this organism. Now, what is it that you want to monitor? Certainly the other organism is going to exist all over the place. You want to monitor the specific change?

Dr. HOLMAN. The assumption on which the criticism I am making is based has to be that there will be unexpected characteristics to some of the organisms created. Now, that may not be when one does a very elegant recombinant experiment using a small segment, but when you do a shotgun experiment and introduce a large amount of DNA into a new organism, there is no way you can pick out the characteristics.

Dr. RICH. In the shotgun, you are not introducing a large amount, but many different types.

Dr. HOLMAN. You do not know them beforehand.

Dr. RICH. But it is a small amount. It is one-tenth of 1 percent, up to 1 percent, but not more.

Dr. HOLMAN. You are countering with a statistical argument. You are talking about a point of uncertainty. You do not know. No mattter how great the chance is, we do not actually know everything we put in and we certainly cannot predict all of the properties that it will impart to the organism, isn't that right?

Dr. RICH. The reason we are doing the research to——

Dr. HOLMAN. To find out.

Dr. RICH. If you have an organism and modify it slightly, you can still identify that organism. If that organism is an infectious agent,

the question is, are you going to manufacture a pathogen worse than the original host?

Let us say it was a pathogen. The discussion at the forum was rather clear. People who study pathogens for many years say the likelihood of making a better pathogen, one that is more active, is very, very small. You cannot say it is zero.

Dr. HOLMAN. I will accept that.

Dr. RICH. That is why the guidelines are there. I do not think the monitoring is a difficulty if one is looking for a pathogen. We know what tests we have to do to identify that pathogen. We know how to look and see whether a particular organism has recombinant DNA in it or not. I do not think that is a difficult problem. The problem really is one of—I do not think it differs in substance from what is standard medical microbiological practice today. We have laboratories all over in every hospital, in fact, that deal with medical microbiology. The culture organisms that cause disease. They are trained in how to handle these organisms. That is essential. The record in terms of spreading infections or making illnesses among laboratory workers is reasonably well documented and the percentage of such infections is very small—not zero, but very small.

I do not think that we have to think about work with recombinant DNA in any different way. These are still organisms. They are not new life forms. If one thinks of them as "new life forms," then you turn up your eyes and say how can we do it?

The fact is we are not creating new life forms. We are creating small variants and we contract them very effectively.

Senator KENNEDY. May I ask Dr. Holman a question? There is a sense of urgency, but not the sense of alarm that Dr. Rich and Dr. Halvorson and Dr. Zinder have posed. Are you equally——

Dr. HOLMAN. I beg your pardon? Where is the alarm?

Senator KENNEDY. Yes, are you as alarmed or less alarmed or more alarmed about the nature of this type of research program?

Dr. HOLMAN. I am not quite such that I understand that, Senator Kennedy. Am I alarmed by recombinant DNA research? No.

Senator KENNEDY. In his early statement, he said it was generally dampening down, the kinds of concerns that have been raised in terms of the risk aspects of DNA research.

Dr. Rich expressed it better obviously.

It seems that the others, at least the theme and mood of their statements would agree with that, and I am just wondering whether you would subscribe to that as well.

Dr. HOLMAN. I think we differ in the following way: I am not very alarmed about whether substantial harm will come to anybody from the pursuit of recombinant DNA experimentation under the NIH guidelines. I think they are good. I have some quarrels with aspects, but I am not alarmed. But I am not moved with that being a critical issue.

To me the critical issue involved in recombinant research is the relationship between the uses of knowledge by few experts and whatever the consequences, even if they may very unlikely be experienced by nonexperts who had nothing to do with the decisions or to use the knowledge. To me the critical issue is not whether we should do recombinant research, I would not debate the risks because they are small under prudent observation, but how it is that we in this type of

technological society are going to relate our capacities to change natural events to the opportunities for the people who will experience those changes to understand them beforehand in the sense of their being done.

I came at this very much from the standpoint of the doctor. We do have experience day after day after day of proposing changes to people. We say to them, this should be done in order to get such and such a goal. It is a bit different from the kinds of experiences my colleagues have had, and I am not saying I am any wiser for it. What I am saying is that we have had that experience. We deal with the issue of informed consent and we have come to see that it is terribly important for the patient's well-being and for the well-being of medicine in a given community, the willingness with which a community will support and allow medical practice to develop. That depends upon the confidence that the patients have in physicians and other health personnel.

We are obligated by California law now to provide any patient with whatever information that patient feels is needed so that that patient can make an informed judgment about a procedure that is proposed.

The implication of the law, and this relates to the local control issue, is that the doctors may know what is best, they may be absolutely persuaded that this is the right thing to do, but you don't do it until the patient feels he or she is comfortable to assent and that builds the bound of partnership between professionals and lay people and scientists and citizens.

What I am worried about in this particular debate is that that kind of experience and sensitivity has not developed yet in the scientific community. It is and will be.

What these guys did at a forum. Asilomar that Alex is talking about are important things. That does not change the issue, an analog of informed consent.

Senator KENNEDY. I welcome it.

Dr. HOLMAN. I apologize for the length.

Senator KENNEDY. When does that process start, the public involvement?

Dr. HOLMAN. I would ideally say it should start when you begin to develop your guidelines. It did not to the extent that I would like to have seen them, but that is all water under the bridge. The question is how to get it in now.

Dr. ZINDER. I just want to say that I do not think anyone will disagree with the idea that the public should be informed. The very fact is that over the last 2 years I have given only one lecture on the work done in my lab. I have been continuously lecturing before church groups, synagogs, and other groups on recombinant DNA. That is all I lecture about, trying to explain to them what it means. Professor Darnell at our university is doing similar kinds of things. Most of us who are involved have spent large amounts of time in the last few years lecturing to groups we never would have lectured to in the past.

Dr. RICH. Let me say that I agree wholeheartedly with that attitude and the issue really is how to bring this about, and here we have the concrete problem of legislation.

I think there should be awareness and it really goes back to education, education and awareness and public participation.

I think, though, that same ways in which this is carried out is productive and constructive and in other ways less so and that was the essence of my comment.

Dr. HALVORSON. I want to share that same point. We feel strongly about the involvement of the public in a number of places. We also feel very strongly where very technical questions have got to be resolved that the public is best served by using the expertise that is necessary to that particular question to be raised and that is why we have been concerned, for example, that the background and experience in epidemiology has not been enough in the forefront. It will continue to be when the question comes up as to regulation and supervision.

Senator KENNEDY. Those are good comments in general, but the problem is, where do you take the general kind of comments and put them into somewhat more precision?

For example, in terms of the NIH, should that only be a recommendation dealing with scientific information that is going to come over to HEW, and they will have to evaluate the protection of human subjects? Who is going to do that or do you have public participation within the NIH itself?

I get some nodding and some shaking of heads.

Dr. RICH. We already have; all of these institutions have human use committees. Any time humans are involved, this committee is concerned and, in fact, those interface with the NIH Human Use Committee. It is a requirement.

Senator KENNEDY. That would not apply to DNA research?

Dr. RICH. It applies to any research involving humans.

Senator KENNEDY. We do not know, do we? We are not there in terms of DNA research with regard to humans and we are getting into a whole new area of scientific inquiry.

You would have to ask what it does with humans, but it has not. It would now, but the impact of this in terms of humans is the thing that is on peoples' minds out there. So how do you do that? That is what we are really very much open on.

Dr. HOLMAN. My own feeling is that we need to have a specific commission to deal with this prototype situation where important basic biological research is being done that has a very high probability of having a direct implication for human use in some variant of genetic engineering.

I share your concerns. We are talking about regulating scientific activity.

Dr. ZINDER. I agree that we should have such a commission.

Senator KENNEDY. Dr. Rich?

Dr. RICH. In my comments I said I am in favor of that.

Dr. HALVORSON. We have no fault with that as long as that commission is in an agency that has the scientific expertise to monitor.

Dr. RICH. I think that the mode that you described earlier this morning, namely, one would have a regulatory agency described in this bill and sort of parallel to it, a group considering what might be called long-term implications—ethical, moral, societal, and so on. that would be more fruitful. Those are not the same organizations. They are separate functions, but one clearly relates to the other.

Senator KENNEDY. Thank you very much, very, very much. You have been very helpful.

[The position papers of Dr. Halvorson follow:]

TESTIMONY

ON FEDERAL REGULATIONS

OF RECOMBINANT DNA RESEARCH

BEFORE THE

SENATOR EDWARD M. KENNEDY COMMITTEE

HARLYN O. HALVORSON

PRESIDENT, AMERICAN SOCIETY FOR MICROBIOLOGY

DIRECTOR, ROSENSTIEL BASIC MEDICAL SCIENCES RESEARCH CENTER

PROFESSOR OF BIOLOGY

BRANDEIS UNIVERSITY

RECOMMENDATIONS

1. Adoption of the NIH guidelines to govern both research and use of recombinant DNA, regardless of the source of funds.

2. Authority for licensing of facilities and determination of procedures to be regulated should be vested in the Secretary of Health, Education and Welfare or the Director of NIH.

3. Regulations should be flexible so that as further information becomes available the regulations can become less stringent, or if necessary, more stringent.

4. Uniform licensing procedures should be established nationally.

5. Licensing should be on an institutional basis. Local decision making should be in the hands of a local biohazard committee which will have public representation.

6. The licensing committee of the National Institutes of Health should be composed only of technically trained members who represent the subdisciplines of the sciences and are familiar with accreditation procedures.

7. Threat of license removal should provide the only necessary deterrent for compliance with the regulations.

Thank you for the opportunity to appear before you today to present a summary of the views of the American Society for Microbiology regarding recombinant DNA.

My name is Harlyn Halvorson. I am a microbiologist from Brandeis University, Waltham, Massachusetts. Professionally I am Professor of Biology and Director of the Rosenstiel Basic Medical Sciences Research Center and, in addition, am Chairman of the Biohazards Committee at the University. I appear before you today as President of the American Society for Microbiology, an organization with both a great interest and expertise in this field.

To place these remarks in proper perspective, let me briefly describe the structure of ASM. The American Society for Microbiology has an active membership of over 25,000 individuals and is the largest, single, life science society in the world. The ASM members contribute to the applied fields of infectious diseases, clinical microbiology, epidemiology, industrial fermentation processes, ecological microbiology, and food technology, as well as to the fundamental areas of immunology, genetics, virology, oncology, microbial physiology, environmental microbiology, mycology and host-parasite interactions. Within our century, a third of all Nobel Prize Awards in physiology and medicine have been bestowed upon microbiologists. By virtue of its size, scope, and services, the American Society for Microbiology is a significant voice in the scientific community.

More important, we believe that in the public interest scientific societies such as ASM have an obligation to speak out on matters where their scientific expertise is required for evaluation of the issues. The public discussions on <u>in vitro</u> recombinant DNA research are a case in point. The problem is fundamentally one in epidemiology: the science concerned with factors that influence the spread of disease. Yet the discussions have been characterized by uncontrolled imagination and excessive claims. They have, unfortunately, been carried out to a large degree by individuals who are ignorant of the principles of infectious diseases. The public needs to be informed if it is to have an important influence on such technical problems which involve transmission of infectious diseases, virology, epidemiology, microbial genetics, and the isolation

techniques which have made possible the safe study of pathogenic microorganisms. As President of the only society which encompasses these disciplines, I have been deeply concerned over the inadequate technical level of this debate. Three issues are of particular importance: the chance for an epidemic, the safety of the procedures used by laboratory workers in this research, and the newness of the DNA recombinant event in nature.

Just prior to the establishment of the NIH guidelines for recombinant research in June 1976, ASM was requested by Dr. Hans Stetten of NIH to review both the adequacy of the guidelines and their recommendations for containment facilities. As President of ASM I appointed a task force of experts knowledgeable in epidemiology, virology, infectious diseases and in isolation techniques to review the NIH guidelines. This committee was under the Chairmanship of Dr. Harold Ginsberg, Chairman, Department of Microbiology, College of Physicians and Surgeons of Columbia University, New York. The committee members included Dr. Carl Johnson, Center for Disease Control, Atlanta, Georgia; Dr. Robert Huffaker, Division of Laboratory Research, New York State Health Department, Albany, New York; Dr. Jerome Birnbaum, Executive Director of Biological Sciences at Merck Sharp and Dohme, Rahway, New Jersey; Dr. Frank Young, Chairman of the Department of Medical Microbiology, University of Rochester and Microbiologist-in-Chief, Strong Memorial Hospital, Rochester, New York; Dr. Curtis Thorne, Department of Microbiology, University of Massachusetts, Amherst, Massachusetts. The committee unanimously recommended endorsement of the guidelines with minor modifications (see appendix). These recommendations were also unanimously endorsed by the Council Policy Committee of the American Society for Microbiology who expressed the conviction that these guidelines provide safe and proper procedures for the handling of recombinant DNA molecules.

We believe that the NIH guidelines provide an appropriate starting point for determining a national policy to govern both research on and the commercial use of recombinant molecules. The Department of Health, Education and Welfare is to be congratulated for the

effective and rapid manner in which it has recognized the issues and dealt with them in the current guidelines, and in the procedures adopted for applying these guidelines in NIH sponsored research. This experience is clearly an example where, when the problem has been recognized, the scientific community, through the leadership of HEW, has provided a balanced analysis and an appropriate mechanism for policing its own sponsored activities. In making this analysis a most important issue was that studies with potentially infectious microorganisms must be measured against the background of the experience in infectious diseases. Experience since 1900 has shown that potentially hazardous organisms can be handled in the laboratory under proper precautions with a very low rate of laboratory infections and virtually zero risk to the public. Experience at the Center for Disease Control, the Biological Warfare Laboratory at Fort Detrick, and numerous clinical and medical microbiological laboratories have demonstrated that infectious agents can be handled in the laboratory, extraordinarily, with very few infections and with no laboratory initiated epidemics involving more than a handful of people.

If organisms carrying certain kinds of recombinant DNA should prove to be hazardous then this hazard exists whether the work is conducted in a research laboratory or in a commercial production firm. Since it is the organism that provides the potential hazard, not the mechanism of producing it, regulations should be directed to the control of the organism. At the present time the initial research that led to the creation of an organism carrying the recombinant DNA would be covered under the guidelines but the use of the organism would not. We therefore recommend that the guidelines be extended to include all research and use of recombinant DNA regardless of the source of funding. Moreover, protection against potentially hazardous research depends ultimately upon knowledge, judgement, and training in the use of hazardous microorganisms. We would support regulation containing licensing procedures, so long as the mechanism of licensing was not so cumbersome as to impede or stifle the research itself, and provided these regulations did not encompass existing research on infectious microorganisms not associated with in vitro recombinant DNA research or on clearly harmless recombinants.

Regulations should be enforced through licensing of laboratories and, if necessary, inspection. Such regulations should be flexible to permit rapid regulation of new potentially hazardous procedures and relaxation of regulations that prove to be unnecessary. Since this is a very complex field, authority for such licensing and the determination of which procedures should be regulated should be vested in the Secretary of HEW or in the Director of the NIH. For the hazards to be seen in their proper and realistic perspective, the agency responsible for monitoring these regulations must have extensive experience with bacterial epidemics. The most appropriate federal agency is the Center for Disease Control. 4.

These same arguments make it absolutely necessary that any licensing procedures be nationally established. We, therefore, urge that the nation adopt a single system for DNA recombinant experiments. While this does not preclude local authority or responsibility in this issue it is apparent that recombinant molecules in microorganisms do not respect state or national boundaries. A uniform national procedure would preclude the expenses of establishing multiple levels of criteria, licensing, inspection and education. It would also be a step towards the goals of uniform international standards.

Local interests have an important and appropriate role to play on the institutional biohazard committee. We therefore recommend two stages of control: first, institutions should be licensed by an appropriate committee at NIH and, second, institutions should have a biohazard committee with local responsibility.

The essential question is how licensing can best be accomplished. Licensing of individual projects would be exceedingly cumbersome and unworkable because it would destroy the flexibility that is essential for creative research. We would favor licensing of an institution for work at a specified level of containment. The procedures established for radioisotope licensing by NRC provide a good model. In this, authority is given to the local biohazard committee to apply regulations at the local level. The local biohazard committee should have broad representation and be approved by NIH. Industry, non-federally-funded institutions, and federally supported institutions which may lack the necessary breadth of talent to constitute an approved biohazard committee, all present

additional problems and should be handled separately. When the location provides sufficient external expertise, external local biohazard committees should be established. In addition, federal inspection of these institutions would provide the necessary assurance of compliance.

A local biohazard committee could play a leading role in assessing individual laboratories as to the adequacy of the safety procedures, the appropriateness of levels of physical containment and biologic containment, the provision of educational materials, and review of the experimental design. In addition, quality control by local groups would be more knowledgeable of the strengths and weaknesses of any scientific colleague than governmental review agencies. This recommendation is in concert with the procedures used to protect human subjects in medical centers. All clinical studies involving human subjects must be approved by the Human Subjects Committee locally.

We have the following suggestions for procedures for local biohazard committees:

1) Eliminate non-hazardous experiments from licensure. Such an approach would permit concentration on more hazardous experiments and enhance cost effectiveness. For example, scientists have been recombining genes of E. coli in E. coli for years by established methods of genetic exchange such as transduction, transformation, and conjugation. The use of recombinant molecule technology to amplify and purify genes of an organism within the same species does not constitute an added risk. Such experiments, rated P1 EK1 in the present guidelines, are likely to be the most common ones nationally. P1 EK1 experiments could be approved by the biohazard committee but need not be certified.

2) Provide maximal local authority for decision making by biohazard committees within the boundaries of perceived risks of the experiments. This principle enables the quality control by local groups who will be more knowledgeable of the strengths and weaknesses of any scientific colleague than the governmental review agencies. In addition, this mechanism would overcome the problem of the lack of uniform educational procedures for Ph.D.'s and would address the issue of quality control. Thus the

biohazard committee could review the facilities and biological containment for P2 EK1 experiments and possibly P2 EK2 experiments and recommend approval or disapproval based on a first hand examination of the project. The decision would be registered with the appropriate governmental agency.

3) All P3 and P4 experiments regardless of EK level should first be reviewed and examined by the local biohazard committee and then their decision forwarded to the appropriate governmental agency for final action. The governmental agency would be required to respond to the question of the adequacy of the institution to perform the proposed investigations within a specified period of time.

Under the licensing procedure the public is protected by a licensing committee of the agency responsible for the regulations (NIH). Experience during the past decade has shown the ASM and other societies that certification is a complex problem. This experience, together with the technical nature of DNA recombinant research, infectious diseases and epidemiology dictate that all the members of the certifying committee be highly trained and drawn from the subdisciplines outlined earlier; in addition, the committee should include individuals who are competent in the field of certification.

Examples of certification principles could be obtained from the National Board of Medical Examiners, The American Society for Microbiology, and the American Board of Pathology. The public has an important role to play. The appropriate public representation is on the local biohazard committee. We are concerned that federal legislation not impose unreasonable restraints on open inquiry and research. For example, we are deeply concerned over some of the sections of the recently introduced bill by Senator Bumpers,"DNA Research Act of 1977, S621". Section 7 of this bill dealing with civil liberties provides for liability without regard to fault for injury to persons or property covered by such research.

The only precedent is that of malpractice suits in the medical profession. Malpractice is based on the violation of practices currently accepted in the medical community. Research is innovative, frequently involving new areas of investigation. It is therefore difficult to compare the standard practice of medicine with innovative aspects of research. One could define liability in terms of use of the appropriate physical and biological levels of containment. This would ensure that the investigators are using the appropriate experimental procedures but does not embody the concept of experimental error. To err is human and liability for error is difficult to establish. At present we have not had similar liabilities attached to other major segments of our economy such as auto mechanics, lawyers and morticians. I would find it difficult to envision liability applied to basic research. In addition, the insurance that is available to physicians would probably not be applicable to basic scientists. If there were great liability there would be extreme constraints on universities and individuals. An inability to protect from liability by an insurance mechanism could preclude undertaking this important research. Thus the effect of section 7 of the Bill S621, or related legislation, would be to discourage or forbid recombinant research in many of the academic institutions in this country.

If such restrictions had been applied in the last 70 years we would have effectively blocked the success we have had in dealing with infectious disease organisms. We are vigorously opposed to the insidious control of scientific inquiry by these punitive means. Threat of removal of license from an institution or organization has proven a successful deterrent with radioisotopes and should be sufficient with DNA recombinant molecules.

Finally, although the widely perceived risks are undoubtedly much greater than the true ones (no one has yet been shown to have lost a day of work because of infection with a product of DNA recombinant research), caution should be exercised until more is known about what hazards, if any, exist in working with bacteria containing recombinant DNA.

The public furor over DNA research has now progressed to the point where some federal regulation is inevitable. However, as further information becomes available the regulations will have to be modified. It is essential that any proposed legislation be equally flexible. The interim report of the Federal Interagency Committee on Recombinant Research: Suggested Elements for Legislation of March 15, 1977, provides many useful guidelines for proposed legislation. One can be too careful: advances in science are necessary for the health and well-being of our country, and such advances should not be discouraged by unreasonable restrictions.

One of our main concerns is the apparent intemperate rush to establish legislation in this field. We must not compromise these advances in medical science. Also we must not establish a dangerous precedent by moving so rapidly that legislation is drafted without the best possible information, provided through consultation with the appropriate qualified scientific and medical experts.

December 1, 1976

Dr. DeWitt Stetten, Jr.
Department of Health, Education and Welfare
Public Health Service
National Institutes of Health
Bethesda, Maryland 20014

Dear Dr. Stetten,

As you recall, during your visit to the American Society for Microbiology at its annual meeting last May in Atlantic City, you requested our Society to support the biohazard guidelines on DNA recombinant research published by the National Institutes of Health. In accordance with that request, on behalf of the Council Policy Committee of the American Society for Microbiology, I appointed a review committee under the chairmanship of Dr. Harold S. Ginsberg, Chairman of the Department of Microbiology, College of Physicians and Surgeons of Columbia University, to head this review of the NIH Guidelines. The committee members include Dr. Carl Johnson, Center for Disease Control, Atlanta, Georgia; Dr. Robert Huffaker, Division of Laboratory Research, New York State Health Department, Albany, New York; Dr. Jerome Birnbaum, Executive Director of Biological Sciences at Merck Sharp and Dohme, Rahway, New Jersey; Dr. Frank Young, Chairman of the Department of Medical Microbiology, University of Rochester, Rochester, New York; Dr. Curtis Thorne, Department of Micribiology, University of Massachusetts, Amherst, Massachusetts. The above Committee was designed to give a broad representation, particularly in areas involving the handling of pathogenic microorganism, epideniology and medical microbiology, virology, industry and, in particular, members who had not served in drawing up the initial NIH Guidelines.

The Committee has unanimously recommended the endorsement of the Guidelines with the following changes or reservations listed below. These recommendations have been unanimously approved by the Council Policy Committee of the American Society for Microbiology at its November meeting in Annapolis.

We understand that the NIH Guidelines are under continuous review and upgrading. We suggest that the following changes or reservations in the Guidelines be considered by your Committee in their continuing deliberations:

1. Standard practicen, page 5, Paragraph 2 should have the following concluding statement: "Persons with immunological deficiencies or other compromised host defense machanisms, whether genetic or iatrogenic, should not participate in research with recombinant DNA molecules. Moreover, because there is an increased possibility of colonization and hence, transfer of plasmid vectors in persons whose normal bowel flora has been disturbed (e.g., by antibiotic therapy), such persons must not work with DNA recombinants formed with any E. coli K-12 host vector system during the therapy

period and for seven days thereafter. Persons who have achlorhydria or who have had surgical removal of part of the stomach or bowel should avoid such work with E. coli K-12, as should those who require large doses of antacids." (These latter statements relating to colonization were transferred from page 22, Paragraph 2, because it was considered desirable to be stated early in the guidelines.)

2. Containment: a) P_2 facilities should contain a biological safety cabinet.

b) In P3 facilities in general exhaust air should be filtered or incinerated and discharged outdoors. Recirculation of the exhausted air should not be permitted.

c) The current requirements of a P4 facility are satisfactory according to the equipment presently available, but the Guidelines should permid development of P4 facilities with other characteristics which will still furnish the high level of containment required. New types of high level physical containment consisting of new easily manipulatable and comfortable suits are being developed under the aegis of the Center for Disease Control.

3. Experimental guidelines: a) (Page 19, Paragraph 1, last sentence should be altered as follows:

We do not propose that experiments with Class 3-5 agents be immediately permitted. However, specific experiments in these catagories may be of sufficient direct societal benefit that their development may proceed if special biological containment precautions and equipment are used and provided that these experiments are expressly approved by the Recombinant DNA Molecule Program Advisory Committee and the Director of the NIH.

b) Footnote 4, page 54, line 9: "where applicable" should be deleted. Both techniques (i.e., hybridization and restriction endonuclease fragmentation analysis) should be required to demonstrate the particular molecular fragment used.

On behalf of the ASM we appreciate the opportunity of contributing to the continuing discussions of the National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules. In an area of research which concerns both the technology and training in the handling of potentially dangerous infectious agents, the American Society for Microbiology with its long history of interests and concerns offers its continuing support and availability in an area of public interest to all of us.

Sincerely yours,

Harlyn O. Halvorson

Harlyn O. Halvorson, President
American Society for Microbiology

HOH:jmp

cc: R. Acker, J. Joseph, D. Cox, F. Rasmussen, H. Ginsberg

RECEIVED NOV 15 1976

College of Physicians & Surgeons of Columbia University | *New York, N.Y. 10032*

DEPARTMENT OF MICROBIOLOGY 630 West 168th Street

November 11, 1976

Dr. Harlyn O. Halvorson
President
American Society for Microbiology
Brandeis University
Waltham, Massachusetts 02154

Dear Harlyn:

Enclosed please find the report of the Ad hoc Committee you appointed to review the NIH guidelines on recombinant DNA molecules. This report received the unanimous endorsement of the committee members. I do not believe that there is any need for me to attend the meeting of the Council Policy Committee when this report is presented. It is relatively straightforward and I believe easily understood. If you have any questions, please call me.

With warm personal regards, I remain

Yours very truly,

Harold S. Ginsberg, M.D.
Chairman

HSG/j

Enc.

cc: Dr. R.F. Acker
Dr. J.M. Joseph

Report ASM ad hoc committee on NIH guidelines
for recombinant DNA

Date of meeting: September 28, 1976

Committee members present: Drs. J. Birnbaum, R. Huffaker, C. Johnson, C. Thorne, F.E. Young, and H.S. Ginsberg (Chairman)

The committee's deliberations consisted of a detailed consideration of each section of the "National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules" (June, 1976). Based on President Halvorson's charge to the committee, discussion was not entertained concerning the moral issues that this area of research have raised. The committee unanimously recommends that the American Society of Microbiology should endorse the "Guidelines" with the following changes or reservations:

1. Standard practices, page 5, Paragraph 2 should have the following concluding statement: "Persons with immunological deficiences or other compromised host defense mechanisms, whether genetic or iatrogenic, should not participate in research with recombinant DNA molecules. Moreover, because there is an increased possibility of colonization and, hence, transfer of plasmid vectors in persons whose normal bowel flora has been disturbed (e.g., by antibiotic therapy), such persons must not work with DNA recombinants formed with any E. coli K-12 host vector system during the therapy period and for seven days thereafter. Persons

who have achlorhydria or who have had surgical removal of part of the stomach or bowel should avoid such work with E. coli K-12, as should those who require large doses of antacids." (These latter statements relating to colonization were transferred from page 22, P 2, because it was considered desirable to be stated early in the guidelines.)

2. Containment: a) P_2 facilities should contain a biological safety cabinet.

b) In P3 facilities the general exhaust air should be filtered or incinerated and discharged outdoors. Recirculation of the exhausted air should not be permitted.

c) The current requirements of a P4 facility are satisfactory according to the equipment presently available, but the Guidelines should permit development of P4 facilities with other characteristics which will still furnish the high level of containment required. New types of high level physical containment consisting of new easily manipulatable and comfortable suits are being developed under the aegis of the Center for Disease Control.

3. Experimental guidelines: a) (Page 19, P 1, last sentence should be altered as follows:

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may proceed if special biological containment precautions and equipment are used and provided that these experiments are expressly approved by the Recombinant DNA Molecule Program Advisory Committee and the Director of the NIH.

b) Footnote 4, page 54, line 9: "where applicable" should be deleted. Both techniques (i.e., hybridization and restriction endonuclease fragmentation analysis) should be required to demonstrate the particular molecular fragment used.

The committee makes two additional recommendations:

1. The ASM should assume leadership in the development of an educational program in the principles of contagion and host-parasite relationships for those engaged in recombinant DNA research but untrained in these highly relevant areas of microbiology.

2. The ASM should seek council from individuals qualified in evaluation of the adequacy and feasibility of the containment of plant systems. The committee considered itself without sufficient expertise in this field.

Respectfully submitted,

J. Birnbaum
R.H. Huffaker
C. Johnson
C.B. Thorne
F.E. Young
H.S. Ginsberg (Chairman)

TESTIMONY ON FEDERAL REGULATION OF RECOMBINANT DNA RESEARCH

UNITED STATES SENATE SUBCOMMITTEE ON HEALTH

April 6, 1977

Halsted R. Holman, M.D.
Professor of Medicine
Stanford Medical School

The two years of public debate about recombinant DNA experimentation have been illuminating and precedent setting. The public has had the opportunity to learn about and, in some instances grapple with, the enormous power of modern science and technology for social good and ill. Some scientists and technologists, shedding protective coats and fears, have joined citizens in wrestling with the problems, and have thereby been enriched. Political institutions have found the issues intriguing, compelling and challenging to the practice of government. To those scientists who began the debate, and to the citizens and government leaders who extended and deepened it, we owe a deep debt of gratitude. The debate exemplifies the best in democratic and intellectual traditions, each seeking to strengthen the other while simultaneously enlarging its own horizons.

The conclusions from the past two years, in my view, include the following: 1. Estimation of both benefits and risks from recombinant research is fraught with uncertainty. The uncertainty extends to the conduct of the research itself, not just the ultimate result, because the novel organisms are created in the process of the study. This uncertainty poses profound issues of responsibility for consequences and consent of those who might be affected. 2. The practical and intellectual benefits which recombinant experimentation seeks are

substantial. However recombinant technology is usually not the only means to these desired ends. In most instances alternative means exist, though they may be more cumbersome, more tedious or less elegant. Thus the issue of choice is raised, choice among paths to the desired benefits. 3. Agreement is widespread here and abroad that regulation of recombinant research is necessary. The issue now is the scope and character of that regulation. 4. Whenever citizens have been given or have taken the opportunity to examine the issues posed by recombinant technology, they have displayed a remarkable capacity to learn and make sound decisions. Because the priorities chosen in recombinant research will reflect social values, and the research itself will yield social consequences, decisions concerning the research should be made by public processes. Informed citizen participation will greatly enhance those processes.

These conclusions help define the setting of this hearing. There are other relevant considerations. Public policy determination, in a situation of uncertainty about the desirability or outcome of a particular course of action, should have certain attributes. These are prudence, openness, experimentation, rigorous collection of information and periodic reassessment. To make these attributes specific, the experiences of responsible government practice and of informed consent in medicine are helpful. These experiences indicate that: A. Decision makers must be answerable to those affected by the decision. B. Those affected must be in the position to ask pertinent questions, know to whom to direct the questions, be able to compel answers, and have the authority to choose a course of action based

upon the answers. C. The goals of the action must be generally unobtainable by other less risky means. D. Benefits of the actions must outweigh risks. E. Those affected must freely assent to the action and be capable of terminating their participation.

I shall comment on the proposed legislation under three headings - containment, regulatory body and procedures - using the aforementioned views as criteria. I believe recombinant research should continue under regulation as the only way to resolve the uncertainties and ultimately control the risks. I accept the N.I.H. guidelines as a minimum basis for future recombinant research if they are applied to all such research whether public or private. I assume that legislation can mandate activities which will improve understanding of problems and thus improve regulation.

PHYSICAL AND BIOLOGICAL CONTAINMENT

Neither is flawless. The best of physical containment in the past has not fully succeeded even when the nature of the pathogen under study was known. Now the organisms emerging from recombinant experiments may be unknown.

Biological containment currently depends upon the use of a host organism, K12 E. Coli. While substantially enfeebled in virulence, it is nonetheless a member of a family of organisms which is ubiquitous in nature and a normal inhabitant of the human intestinal tract. This creates the possibility of the direct spread of novel organisms to man.

Throughout the debate there has been little mention of epidemiology

which is the discipline employed to monitor the origin, spread, fate and consequences of the new organisms which will be created. This absence is not accidental; there are no reliable means for monitoring recombinant organisms should they escape laboratory containment.

The legislation should therefore:

1. Mandate intensive research to develop effective means to monitor the production and dissemination of recombinant organisms, particularly those which will be produced in large numbers.
2. Mandate research to develop experimental host organisms different from E. coli which are not common inhabitants of plants and animals, and which can be more readily tracked.
3. Require that facilities for recombinant research, particularly those at P3 and P4 levels, be well isolated physically from populations particularly susceptible to infections such as hospitalized patients.
4. Limit the number of sites in which P3 and P4 level experiments can be conducted until clear information is available concerning the safety of experiments. My preference would be for no more than three P4 level facilities nationally and no more than one P3 facility per institution. It would be better if groups of universities or industries shared experimental facilities so as to combine their skills in developing epidemiological methods while reducing the

chances of inadvertent infection with novel organisms.

THE REGULATORY BODY

The importance of this legislative precedent in regulating an intellectual activity, and the complexities of the issues involved, require designation of a specific agency for the task. The agency should be responsible to Congress through the Secretary of H.E.W., and should have the power to investigate, impose sanctions, and recommend changes in both regulations and legislation. It should be composed of scientists and citizens with the latter in a majority. It should have a limited life so that a reassessment can be made of its work and role in approximately five years. The agency should be charged not only with regulating recombinant research as currently envisioned, but also with establishing guidelines for genetic engineering in humans in the future. The latter ~~is a~~ capability is certain to emerge in some form from current research.

As a model for the agency, I am attracted to the National Commission for the Protection of Human Subjects. The work of that Commission to my mind has been unique in addressing critical and contentious issues at the interface between medical science and society, and arriving at wise conclusions. I shall refer to the agency as a Commission in the remainder of this testimony.

PROCEDURES

The hallmark of the regulatory process should be openness. The work of the Commission should be public, it should disseminate information about the research and the regulatory process, and it should facilitate public participation in decisions. Other components of the legislation or the mandate of the Commission should be:

1. The Commission shall report its findings and conclusions to Congress and the public at least annually.
2. A condition for licensing of recombinant research shall be a clear plan of the applicant agency to inform all persons involved in the proposed work of the nature of the work, the issues of potential risks, and the means of monitoring for those risks. The applicant agencies shall also be required to make available to participating workers and the surrounding communities the results of their monitoring efforts.
3. No patents shall be granted for any form of recombinant research or development. Patents imply secrecy and therefore run contrary to both public participation and monitoring. They also create conflicts of interest in which private gain and public good are sometimes counterposed. In this prototypic situation in which powerful technologies are ~~microbiology is~~ being developed amidst uncertainty about social benefits and risks, openness should be a precondition for permission to proceed. where malfeasance is not involved.
4. The Commission must determine liability policy. If recombinant research is pursued in the public interest with full adherence to public policies, individual research agency liability for harms is not necessarily a just requirement. The agencies should be required to provide all involved in the research

with comprehensive health monitoring and disease management programs. Liability beyond that point however is not clearly a responsibility of the agency. This issue is a forerunner of others which will arise during exploitation of modern knowledge, and presents the Commission with an additional unusual task.

5. Federal regulations should provide a base for local actions but not prompt them. States and localities should have the option to adopt federal minimum standards or go beyond them. ~~should their collective wisdom dictate.~~ While more stringent local regulations might temporarily impede research or create hardships for some investigative agencies, this is a lesser disadvantage than inhibiting local participation and authority on matters ~~which might affect them directly~~ of direct impact to the community.

Rather than discuss the bills before the Subcommittee in detail, I have listed criteria whereby they or others might be evaluated. By these criteria, each bill has significant shortcomings. They all emphasize technical aspects of the regulatory process and virtually exclude issues of public participation and public review, problems created by inadequate epidemiological measures, patents, liability questions and pacing of the spread of the research to the development of knowledge about effectiveness of containment. As a consequence, the richness of the prior debate and the implications of this regulatory precedent do not emerge. Most important, only the Motzenbaum bill establishes a Commission. By establishing a Commission with a broad mandate as previously discussed, much of the breadth and depth needed for this regulatory precedent could be captured. On balance, I

would urge adoption of the administration bill with substantial amendme

In conclusion, I should like to respond to two criticisms which have been made of the views expressed herein. The first charges that these recommendations will create a cumbersome bureaucracy, restrict science, and slow the realization of benefits by emphasizing fictitious or unreasonable risks. I do not agree. The question is, whose rights and interests are involved? Viewed from the ~~parochial~~ special interest of a recombinant biologist, haste may be in order. But viewed from the interests of citizens or of good government, haste may be ~~an enemy~~ unwise. The goal is to achieve convergence of scientific and public interests. Time is needed for the public to understand the issues and create sound policies. The expert who is impatient with public learning, or who seeks to circumvent the public decision process ~~because he or she already knows what's right,~~ risks not only imprudent error but also intense public antagonism toward science if the expert's views ultimately prove wrong. Society has survived ~~long~~ long without the desired benefits of recombinant technology, ~~and~~ a slig further delay for Evil, Internecine and virility. while society catches up to science is a small price to ~~has other paths to the same end at its command~~. Public comfort with this work, and support of it, is essential for the integrity both of the democratic process and of science itself.

The second criticism likens public participation in determining science policy to the anti-intellectual, destructive interventions of the Nazis, or the Soviet government through Lysenko, or Senator Joseph McCarthy into scientific and academic affairs. I believe this analogy ~~concept~~ to be quite wrong. In each of those three instances, a powerful political and ideological force sought to usurp science

for its own purposes, especially racial superiority or domination and exploitation of others. The ~~third~~ initial of these recommendations is ~~exactly~~ the opposite; they seek to involve citizens democratically in the development and direction of science and technology. Left ~~to~~ on its own, ~~devices, export~~ could scientific and technological knowledge ~~can~~ be employed for domination of one segment of society ~~to achieve a superiority of power and a dominating capacity over society not unlike those of the three examples listed~~ by another. By bringing the public into the process, the health and future of both society and science are improved precisely because a check is placed upon potential abuse of scientific ~~power~~ knowledge, and a broad social dimension is contributed to the thinking of those who make science policy.

Thank you for permitting the expression of these views.

Senator KENNEDY. For our next panel of witnesses we have Mr. Daniel Hayes, former mayor, city of Cambridge, chairperson, Cambridge Experimentation Review Board, and David Clem, member of the Cambridge City Council.

Both gentlemen are intimately involved in the controversy in Cambridge.

There is convincing evidence that time and energy given to complex issues may affect people's lives who deal with it substantively and in a fruitful manner.

We are delighted to have you here, Mr. Hayes, and we will start with you.

STATEMENT OF DANIEL HAYES, FORMER MAYOR, CITY OF CAMBRIDGE, MASS., AND CHAIRPERSON, CAMBRIDGE EXPERIMENTATION REVIEW BOARD, AND DAVID CLEM, MEMBER, CAMBRIDGE CITY COUNCIL

Mr. HAYES. Mr. Chairman and Senator, last week we presented a statement and sent it to your office. I am going to read that statement. There are some additional comments I would like to make at the end.

Mr. Chairman and Senator, when our board was formed, one of its duties was a review and assessment of the adequacy of the NIH guidelines. A challenge to one's eyesight was well as Webster's dictionary. Both of which failed me on many occasions.

In our study we found areas of the guidelines that could be improved. Harvard University and MIT both adopted policies that were in excess of the NIH requirements.

A major concern was the fact that the guidelines could only be enforced on NIH-supported research. It is that concern that brings us to Washington and the effort of Congress to bring all recombinant DNA research under Federal regulations.

Our board has reviewed five pending bills as well as the interagency report. Rather than comment on the merits of these proposals, we felt that it would be more appropriate to submit our own recommendations for consideration.

Our recommendation is that all biological research and production involving recombinant DNA technology should come under the control of two commissions:

(I) A Commission to regulate all biological research and production using DNA technology. Membership: The membership should be broad based with a minimum of 12, no more than 4 to be actively engaged in biological research. The public and the media as well as the medical and legal professions shall be represented.

The Commission should be funded and empowered to (1) establish regulations.

It is assumed that the current NIH guidelines would be used as a basis for the regulations. Changes would have to be made to cover private industry as well as items covered in this regulation.

(b) Health monitoring: Guidelines shall be established for monitoring the health of all participants in recombinant DNA research on a continuing basis.

(c) Registry: There shall be established and maintained a registry of all workers participating in this research for the purpose of long-term epidemiological studies.

(2) *Licenses.*—Facilities: A license or certificate would be issued to an institution, firm, or other agency, if the Commission is satisfied that all requirements set forth in the regulations can be met.

(5) Researchers: A license, certificate, or registration shall be issued to ensure that all participants in the research are qualified through education, training, and experience to perform this research.

(3) *Permits.*—No recombinant DNA research or production shall be undertaken without a permit from the Commission. A risk assessment must be undertaken prior to the granting of a permit.

The Commission shall be able to make additional requirements over and above the general regulations for any individual project.

The Commission shall further have the power to waive the licensing and permit requirements if, in their judgment, ample evidence is available to show that a level of or type of experiment is sufficiently safe.

(4) *Inspection.*—A staff shall be established to make inspections of facilities to insure compliance with regulations, permits, licenses, and health monitoring programs.

They shall have power to stop a project and close a facility. The Commission shall have the power to revoke licenses and permits as well as initiate court action for violations. Protection shall be given to any individual cooperating with the Commission in assuring that the requirements are carried out.

(5) *Public Informatiton.*—The Commission shall hold public hearings prior to making any regulatory or policy changes. An annual report shall be made of all activities of the Commission as well as pertinent information relative to the progress of the research. A list shall be published in the Federal Register and a national publication—such as the magazine Science—of all licenses and permits granted or revoked.

(6) *Safety Office.*—There shall be established and maintained a safety office that would collect, assess, and immediately distribute all information pertinent to safety in recombinant DNA research.

(7) *Applications for Patents.* Shall be reviewed by the Commission for compliance with all requirements.

(8) *Research.* The Commission shall sponsor and fund research in the areas of biological safety and health monitoring.

State and local options. No NIH classified P–4 level of research shall be allowed without approval of the local governing body. State and local authorities may enact additional requirements subject to the approval of the Commission.

(II) A Commission to study the broad social issues raised by genetic research and to make recommendations relative to the use and application of the results of all DNA or genetic research.

Membership: The Commission shall be broad based with a wide range of public and professional representation.

Public Forums: The Commission shall conduct public forums and solicit opinions from all segments of our society. They shall periodically issue reports for public comment.

Report: The Commission shall issue a report containing but not limited to recommendations and guidelines for the future use and application of all genetic research. In issuing this report, it shall take into consideration such matters as limits, safeguards, social effects, industrial and clinic applications, and ethical issues.

These recommendations are made in their briefest form. Time does not permit a full discussion on each element. We recognize that additional elements would have to be added to make these Commissions consistent with other regulatory and study commissions as well as relationships with established government agencies.

I might add that you have asked two questions this morning in your statement. The answer to one is obvious. We do believe in the regulations that would be nationwide.

Second, you asked a question about flexibility. We agree that there should be flexibility because new knowledge will be forthcoming and the regulations should be able to adapt immediately to getting the knowledge.

I would like to add two questions. Is it practical and reasonable? The first is, is it practical in that it is legislation that can be enforced, and reasonable that it would be enforced?

One of the things we found in the testimony, we found that this type of research is different from others in that it is fairly easy to do. It can be done off campus and the more restrictive you become with regulations, the more likely you will have it done off campus, off the grounds and thereby create a more integrated hazard.

Senator KENNEDY. Those are very thoughful comments.

Mr. HAYES. By the way, I did submit a comparison of our recommendations with the administration's recommendations.

Senator KENNEDY. Well, it is very, very helpful, very comprehensive.

I do not find any difficulty with anything that you proposed. It seems to me it makes a lot of sense.

Your comparison will be made a part of the record at this point.

[The following was received for the record:]

CERB	ADMINISTRATION
Commission	Secretary -- HEW
Regulations Can Change NIH Public Hearings Health Monitoring Health Registry	Standards Can Change NIH
Licenses Facility Participants	License Facility
Permits Meets all regulations as well as any additional requirements	Registration Secretary sets requirements
Inspection Power to close	Inspection May seize material
State & Local May add subject to approval No P 4 without local approval	State & Local May add subject to approval
Public Information Hearings Annual Report Publication - Licenses & Permits	
Safety Office	
Patents Applications reviewed	
Research Safety & Health Monitoring	Training Safety
	Expires in five years

Senator KENNEDY. David Clem?

Mr. CLEM. Thank you, Mr. Chairman. I apologize for not having my comments typed in advance.

I think it is important for us to reflect on why this Senate subcommittee is considering legislation to regulate the conduct and development of recombinant DNA research. I suspect that the events in Cambridge are largely responsible. Therefore, I would like to outline what actually happened in Cambridge so that you might better understand the utility of the Cambridge experience.

It is my contention that an important lesson to be derived from the Cambridge experience has been largely ignored or perhaps overlooked in the draft legislation before Congress.

I will confine my remarks to this omission. What is the lesson to be learned from the Cambridge experience?

I believe it is simple, straightforward and sound. The lay public has a right to be involved in the decisions affecting their general health and safety and given adequate information and meaningful power, the lay public can address complex issues and resolve them equitably. The proof is in the pudding.

In June of 1976, the Cambridge City Council became aware of an intense and sometimes acrimonious debate within the Harvard scientific community. The issue was the proposed construction of a P–3 laboratory at Harvard to facilitate recombinant DNA research. I think it is fair to say in spite of the wide coverage of the NIH process to articulate research guidelines within the scientific journals, the overwhelming majority of the Cambridge City Council had never heard of recombinant DNA research. None of us understood the profound implications of conducting such research.

We asked to be informed, not a radical demand, and the Mayor convened two public hearings on the issue. What the council learned was genuinely disturbing.

First, we discovered that several prominent scientists were opposed to allowing recombinant DNA research to continue. The original call for limits to inquiry came not from the Cambridge City Council, but from the academicians themselves.

Second, we learned that the issue was not new. For over 2 years, the scientific community debated safety aspects of continuing recombinant DNA research and had even imposed a voluntary moratorium for certain types of experiments. Therefore, the concept of a moratorium came not from the Cambridge City Council, but from the scientists themselves.

Third, the city council learned that the NIH had promulgated guidelines for conducting recombinant DNA research. However, we soon discovered that these guidelines only applied to NIH-funded projects and were not applicable to industrial research, private, nonprofit research, or research funded by other Federal agencies.

Assuring guidelines were warranted, the NIH guidelines, released the day of our first public hearing, were not comprehensive. Indeed, the first call for broader acceptance of the guidelines came not from the city council, but from the Director of the National Institutes of Health.

Fourth, the city council was informed that recombinant DNA experiments at the P–1 and P–2 levels were already under way at

Harvard and at MIT and, in spite of the requirements of the Environmental Protection Act of 1970, the Director of the National Institutes of Health had released the guidelines prior to the completion of our environmental impact statement.

The city council did not charge the National Institutes of Health with violation of public policy if not law; the Director of the National Institutes admitted violations because of compelling health issues and the need for uniform safety standards.

Five, the Cambridge City Council gradually understood that the National Institutes of Health guidelines were basically developed by scientists performing recombinant DNA research and directly interested in recombinant DNA research.

We discovered that monitoring of compliance to the guidelines was left to the institutions performing the work and that the only penalty for noncompliance was removal of funding executed through the "courts" of the National Instituites of Health. The researchers were their own judge and jury. The city council did not conjure up a violation of the principle of self-interest; it was self-evident and self-imposed.

So what did the Cambridge City Council do? Did we ban all recombinant DNA research? No.

Did we stop any scientists from continuing research already under way? No.

Did we prohibit Harvard from building their laboratory? No.

Did we force MIT to withdraw its petition to the National Institutes of Health for laboratory certifications? No.

Did we ask for a good-faith moratorium on P–3 and P–4 research—research contemplated but not underway? Yes.

Did we allow Harvard to begin construction of their laboratory subject to regular building and health inspections to insure normal compliance to State building laws and the NIH guidelines? Yes.

Did we create a Cambridge Experimentation Review Board consisting of lay citizens completely removed from recombinant DNA research to review the NIH guidelines and report back to the council on recommendations for action? Yes.

Did we divorce this study group from opportunistic political interference and give them complete latitude in tackling this difficult and confusing and complex issue? Yes.

That's what the city council did—a housewife, a student, a security guard, a pharmacist, a college administrator, a welfare mother, a rubbish disposal contractor, a court clerk, and a tax collector—nine city councilors in Cambridge, Mass. That's what we did because Congress left us no choice. A failure to act forced us to act.

And what were the results of our action?

The Cambridge Experimentation Review Board produced a coherent, readable report recommending the continuation of recombinant DNA research——

Senator KENNEDY. Do you think we should ask if they could do it in all local communities?

Does it make sense to put out Federal law?

Mr. CLEM. Yes.

We were worried about the comprehensive nature of the guidelines which we did not have the authority to impose. The Cambridge Ex-

perimentation Review Board produced a coherent, readable report recommending the continuation of recombinant DNA research, subject to five additional safety requirements.

The report recommended a municipal ordinance to impose these guidelines on all recombinant DNA research in Cambridge, thereby closing the most obvious loophole in the weakness of the NIH guidelines.

The Cambridge Experimentation Review Board recommended continuing citizen review and the creation of a Cambridge Biohazard Committee to oversee the work of the institutional biohazards committee and to impose stringent health monitoring systems, not only for the public, but for the principal investigator, the lab technician, and the janitor. Not an unreasonable report not a rhetorical diatribe against the "experts" from "the people."

For almost 6 months, eight Cambridge citizens volunteered two nights a week to learn, to question, to debate, and to decide ultimately unanimously. The report has been acclaimed by proponents and opponents alike. The lay public proved they were up to the test.

The Cambridge City Council, a political body elected by the citizens of Cambridge, let the lay public decide. In spite of strong personal disagreements with the conclusions of the Cambridge Experimentation Review Board Report on both sides of the issue, the Cambridge City Council unanimously adopted a local ordinance implementing the Cambridge Experimentation Review Board Report.

I urge the Senate not to exclude any vehicle for lay review of scientific decisionmaking that may impact the public health. The Cambridge model is ample proof that the public is prepared to accept risks as long as they are included in the decisionmaking process.

There should be minimal national standards, but there should also be local review.

Issues of scientific expediency and convenience should not outweigh informed consent at the local level.

In conclusion, I would like to offer a personal position, reached after many hours of thought and introspection.

The real issue before us is not recombinant DNA research. The basic issue is the right of the public to know and the right of the public to decide.

Our institutional models for decisionmaking falter when complex issues such as recombinant DNA arrive. Public health versus private rights of inquiry represent a very delicate balance. I submit that the Federal dictate is necessary but is insufficient.

We must experiment with ways to achieve a comprehensive consensus on the profound issues of our time.

The Cambridge model is one example. It is probably not perfect or even adequate, but it is a beginning; and I urge the Senate not to preempt local attempts to inform citizens and build consensus at the local level.

The Cambridge experience has shown local consensus is needed and it can be achieved.

Thank you very much.

Senator KENNEDY. Well, I want to say that that is a very interesting commentary, the commentary of Mr. Hayes and your commentary about the evolution of this process and what the issues were that were

before you and how you approached them and came up with the conclusions.

I think it is of great use to our committee and it will be useful to hear about these in some detail.

I suppose the real question that remains is this:

When you are going to permit local groups to effectively enjoin the research in various areas of the country, you are going to give them that kind of power and authority; and you have to weigh that in your decision.

It is a question we asked the earlier panel and you have related in your statements and comments your views; but maybe you should just serve that up one more time.

I am playing the devil's advocate here.

Say something comes out of some laboratory, and given the attitude, the fear that people have about these kinds of issues—the legionnaire's disease is the obvious case for it just struck an enormously responsive chord in people's minds—there is a great deal of fear about these issues.

Say that this incident happened out in Dubuque, Iowa, and then next week Hoffman-LaRoche comes in and says we want to do some recombinant DNA research? And the committee gets up in arms.

Could not this veto bring to a standstill the research in an area which with all its imperfections, offers, as everyone states, potential for enormous good and some risk; and how should we approach it?

I think you do not want my own personal view, but that is something that will be asked and debated on in this committee and on the floor of the Senate, in people's minds, and at the universities.

How would you answer that?

Mr. CLEM. The example you mentioned, the legionnaire's disease, took place during relatively the same period as our local representation review board was weighing the issue of a P–3 laboratory at Cambridge.

I think that probably did have some impact in terms of how they approached the problem with large amounts of skepticism, but I don't think we can argue with the bottomline conclusion; and I do not think that will be banned in every city and down throughout the country. I think there are certain areas such as Cambridge which are prepared to accept the risk of certain types of research.

I would think in keeping with the recommendations that Mr. Hayes presented to you today that a minimal standard might be to allow P–1, P–2, and P–3 research which most people recognize as offering relatively small risks as a reasonable, minimal standard throughout the country, but the more dangerous research relating to P–4 be confined in the short run to a few facilities that are highly monitored, highly controlled to contribute to our understanding of recombinant DNA research in general.

Some of the legislation proposed offers a specific limit to the number of facilities. I am a Ph. D. student and I am sensitive to the issues of academic freedom, but research does not have to be site specific. People have the opportunity to travel to facilities to do research. We are not stifling inquiry. We are simply trying to control it and the only justification for controlling it is public health.

I do not think that the whims of any political referendum should control that type of research; but I think that the communities are prepared not to take the issue lightly but to work very hard to try to educate themselves and make that decision. The Cambridge experience demonstrates that an equitable and fair and responsible document and conclusion can be reached.

The interesting thing to me is that the opponents of recombinant DNA research in many ways spun their own web when they came to us and said, let the people decide; don't let those experts decide; and the fact is that the people did decide and suggested that the research continue.

Once we have that consensus, then we have swept away the foundation of any criticism about allowing research that may affect the public health.

Senator KENNEDY. Mr. Hayes, is there anything you would like to add to that?

Mr. HAYES. Gentlemen, Senator, one of the problems as I read those bills in the Senate about this local option is the fear I have that the issue will come before a local governing body, whether it is a town meeting or city council, and that is an issue that should be in a public forum, whether it was on the basis of a health commission or the health department, where people do have an opportunity to study and make an evaluation.

The important statement in there is that that risk assessment must be made and that is an assessment made before anything is even started.

Hopefully, that assessment is such that research would continue because it is considered. That which is considered risky would be placed in the higher levels, such as P–5.

The local option should be there. They should decide. It wouldn't be any hindrance to overall DNA research.

Senator KENNEDY. I want to thank you very much.

Your statements have been very helpful, very, very persuasive, and I thank you again.

The subcommittee stands in recess until 2 o'clock this afternoon.

[Whereupon, at 1:20 p.m., the subcommittee recessed, to reconvene at 2 p.m. this same day.]

AFTERNOON SESSION

The Subcommittee on Health and Scientific Research of the Senate Committee on Human Resources was reconvened at 2:46 p.m., Senator Edward M. Kennedy presiding.

Senator KENNEDY. We will come to order.

Our next witness is an old friend of this committee and members of the committee, Joe Stetler, who is president of the Pharmaceutical Manufacturers Association. And he indicated in the last hearing before this committee on the DNA issue his support of PMA for the guidelines, which was, I think, a very responsible attitude for the industry. And I think it was very instructive.

We are glad to have you. We apologize for the delay.

STATEMENT OF C. JOSEPH STETLER, PRESIDENT, PHARMACEUTICAL MANUFACTURERS ASSOCIATION, ACCOMPANIED BY DR. JOHN G. ADAMS, PMA VICE PRESIDENT, SCIENTIFIC AND PROFESSIONAL RELATIONS, AND BRUCE J. BRENNAN, PMA VICE PRESIDENT AND GENERAL COUNSEL

Mr. STETLER. Thank you, Mr. Chairman.

I am accompanied today by John Adams, who is vice president of PMA Scientific and Professional Relations, and Bruce Brennan, who is general counsel of our association.

We are pleased to have the opportunity to come before the committee on the three bills that are pending before you, S. 621, S. 945, and S. 1217.

We have indicated in our prepared statement some comments on all three bills. I am going to make just a couple of general comments, talk on S. 1217 briefly and not go into the others in any detail.

And then I would like to comment on the questions that you had in the Congressional Record when you introduced S. 1217.

Recombinant DNA research, which is the subject of all three of these bills, in our opinion, offers great promise in many fields, including the treatment and prevention of various diseases. For this reason we feel that any legislation adopted should encourage as well as regulate such research.

As you indicated, last September we testified before the subcommittee on the involvement of PMA member firms in recombinant DNA research. At that time we commented on the NIH guidelines that were issued in July of 1976, pointing out that with some minor modifications the industry would voluntarily comply with them.

Since that time we have continued to work with State and Federal legislative and regulatory bodies as they have weighed the need for public involvement in this frontier field of science. We officially commented to the Department of HEW in November on the NIH guidelines and reiterated the intention of PMA member firms to voluntarily comply. We have also sought from NIH a clarification of the confidentiality of information aspects of the guidelines.

Early in March of this year, we again surveyed PMA member firms to determine the current involvement of their companies in this type of research. Contrary to some allegations that the drug industry is heavily engaged in such research, our survey found that only three PMA member firms are now so engaged in their own facilities in the United States. Three other firms are sponsoring such research through grants or contracts. Additional details of the survey have been provided to NIH, and we would be pleased to make it available to the subcommittee too, if you wish.

Although we are concerned about certain provisions of the pending proposals, we agree that legislation should be enacted in order to provide adequate safeguards, enforcement mechanisms, and research encouragement. In order to satisfy these interests, uniform national standards should be established for all recombinant DNA research, whether conducted under public or private auspices.

Individual facilities at which such research is carried on should be regulated by the Department of HEW. The Secretary of that agency

should also be given authority to inspect those facilities for compliance with safety and other requirements. We also feel that Federal legislation should require the establishment of institutional review committees.

With respect, specifically, to S. 1217, called the Recombinant DNA Regulation Act, we feel that the basic regulatory framework set forth in that bill is sound and would provide effective controls while not interfering excessively with the research process. We agree that the Secretary of HEW should promulgate standards concerning the production and possession of recombinant DNA.

Despite this general support, we are troubled by certain of the specific provisions of the bill. The interagency report, which the bill purports to implement, would apply the concept of licensing by the Federal Government to facilities which produce or possess recombinant DNA and subject the actual research projects only to a registration or notification requirement. Yet the actual provisions of S. 1217 would appear to create a different situation.

We would suggest that the language be clarified to make sure that the licensing provisions of the bill be limited to the licensing of facilities. It is appropriate for the Secretary of HEW to be assured that a facility used for recombinant DNA research is in compliance with Federal standards and is meeting necessary safety requirements.

To go beyond that point and require preapproval by the Secretary of research projects would create substantial impediments to the conduct of the research. In effect, it would place the Secretary in the position of directing all such research conducted within the United States.

As we understand the recommendations of the Federal Interagency Committee on Recombinant DNA Research and the testimony of Dr. Fredrickson before the House—particularly on March 31—the administration would also support this approach.

We do not find any specific provisions in S. 1217 which would protect the confidentiality or trade secret status of proprietary information submitted to the Secretary as part of the reporting process or as part of an application for a license. The pharmaceutical industry supports the submission of sufficient information to the Secretary to describe research projects which are being undertaken in this critical area.

At the same time, if that research is to be given eventual commercial application and if private resources are to finance this research, there must be some protection given to trade secret data. We would like to offer an amendment to the bill which we believe would provide that sort of protection and not impede the regulatory process or interfere with the Secretary's understanding of the scope of the research being conducted. Our amendment is modeled after similar provisions in the Federal Nonnuclear Energy Research and Development Act.

Section 11 of the bill proposes a system under which an employee, who was discharged or feels discriminated against because of testimony or other actions taken by him in a proceeding against his employer, could petition for relief to the Secretary of Labor. After an investigation, the Secretary of Labor could issue an order to reinstate the employee, pay compensation, including back pay, and assess compensatory and even exemplary damages against the employer.

This proposed system for handling employee/employer discrimination complaints seems to be unnecessary. Certainly there is a related but less punitive—currently, there is a related but less punitive procedure available under section 11(c) of the Occupational Safety and Health Act of 1970.

We recommend that any provision in recombinant DNA legislation, intended to safeguard against reprisal by employers, be consistent with those provisions.

The provisions of the bill with respect to enforcement would authorize the Secretary of HEW to directly assess civil penalties for violations of the act other than those relating to employee protection. We do not oppose a system of civil penalties being associated with violations of the bill. However, we recommend that an action for civil penalties be initiated by the Government in a Federal court proceeding rather than in an administrative proceeding before the Secretary.

We note that there is a provision for judicial review regarding the promulgation of standards under the act. However, there is no provision for judicial review of actions of the Secretary concerning the issuance or revocation of a license. We would hope that this is merely an oversight by the drafters of the proposal. Judicial review of licensing requirements is a matter of due process which is protected by the Constitution and should be included in this bill.

Finally, on the bill, we would suggest that the preemption section be amended to provide that the Secretary "may" rather than "shall" exempt a State or subdivision from the provisions of section 10(a).

As I indicated, the balance of our statement deals with the other two bills, which I will skip and just ask to be included in the record.

Mr. Chairman, I would like to comment on the 11 questions that you had, very briefly. The 11 questions that were in your statement of April 1.

You suggest a redefinition of DNA research. We have discussed that proposed new definition with various people in the industry, and have no problem with it. A suggestion was made that possibly it could be stated somewhat more clearly, and I did hear this morning one of the scientific witnesses suggest a statement, which I think was intended to do just that.

But we have no problem with enlarging the definition, as it is now in the bill.

The business of a commission to participate in DNA research divisions: We would advocate the creation of an Advisory Council, composed of experts not only in the field of recombinant DNA research and bioethics, law, and other nonscientific disciplines. We think the DNA expertise should cover biomedical, agricultural, and other scientific disciplines.

You commented on the scope and the nature of statutory standards. It was our belief that the use of the term "production or possession" was intended to regulate all activities involving recombinant DNA, including research and commercial development, and of course, therefore, would have no objection to including the term "research," which would then cover research, production, and possession.

With respect to standards dealing with the qualifications of the individuals conducting the research, we have reservations. It could result in government control of scientific qualifications and the estab-

lishment of a Federal roster of persons authorized to engage in such research. If that was the result, we think it would be a bad one.

Breach of Standards as grounds for revocation of license. We agree with that point. Our reading of section 5 of the bill to us seems to provide that authority at this present time.

A licensure contingent on adequate safety standards. We agree.

Continuing research or continuing study of ethical, societal, and legal implication of DNA research. We agree.

Public right to know who, where, and under what conditions the research is being conducted. We agree.

You asked a question about funding and staffing. I think it is impossible to estimate that without some additional details.

Senator KENNEDY. Before we leave the question of licensing of various facilities, I think what is important is that we pinpoint responsibility. And I do not know how we can pinpoint responsibility just by withdrawing a license from a facility. Who are they going to hold responsible? I mean, do they just hold the facility responsible? Do you hold a president of a drug company? Do we bring in Bob Clark down here?

Mr. STETLER. No.

Senator KENNEDY. Who do you hold responsible? Is it the people that are working in that facility who have responsibility for the facility, and who were making judgments in terms of that facility? I think this is the question. I mean, we are trying to get some responsibility—there is wiretap legislation, you know, where they just say that it is the inherent power of the White House. Somebody authorized; we never knew who authorized the 26 wiretaps on newsmen. Somebody down there; it may have been the President, or it may have been Kissinger. No one knows, even to this day, who did it.

We want to get somebody who assigns that factor to be accountable and to be responsible. And we are going to know who that is. By getting that, you are going to make sure that that person, that individual, is going to exercise responsible judgment in making those decisions.

Mr. STETLER. No problem with that, sir.

Senator KENNEDY. In protecting the public interest.

Mr. STETLER. Yes.

Senator KENNEDY. And so I am just wondering how we wrestle with this issue, besides just the facility. The facility, after all, is not doing the research, it is the people in it.

Mr. STETLER. I think when we say "facility" we would contemplate the identification of a responsible party in charge. But we do not want the licensure feature to get to is the business of each project being subject to preclearance or licensure. We do not think that all the individual people that are going to be involved in these various phases of research should be subject to governmental clearance. But when we say "facility" we would contemplate the identification of a responsible party.

I think that is what you are after.

Senator KENNEDY. Well, yes. But I mean maybe it is just a question of words, about how many or who these people are going to be in order to get accountability. You know, as someone who has taken the position of being opposed to criminal penalties, I do, however, sup-

port civil penalties. So we have got to know who is accountable. Under your proposal, we are just talking about the laboratory or the facility. Who is going to be subject to the civil penalties?

Mr. STETLER. Well, I do not mean to say that we are talking only facility. As I just said, when we talk facility we are talking about the responsible party in charge. Which we have to do on many things in which we relate to government. There are responsible people who are identificable in our operations. And certainly in terms of this type of very specialized research, that person with the company is identificable.

Mr. ADAMS. Senator, we are already doing that in a related area, biological manufacturing, in order to obtain a license application from the Bureau of Biologics, a responsible party must be named. So that kind of an appartus is already in place.

Senator KENNEDY. He is the guy that goes to the can.

[Laughter.]

Senator KENNEDY. Not really, I mean, it pays to find the man.

Mr. STETLER. Yes.

Senator KENNEDY. Old Jim Jones, he has gone away again. OK. Well, I do not mean to be facetious about it. But you know what the problem is. We are going to have to deal with it.

Mr. STETLER. I do.

Senator KENNEDY. Okay.

Mr. STETLER. Just three more questions you had. One, should there be a limit on the number of facilities? We think the answer to that is no.

Should there be Environmental Impact Statement? Yes, we think there should.

Senator KENNEDY. You think there should?

Mr. STETLER. What is that?

Senator KENNEDY. You think there should be?

Mr. STETLER. Yes, sir.

Senator KENNEDY. Which would you rather have, environmental impact or local community veto?

Mr. STETLER. I wish I were not limited to just that choice. I think maybe——

[Laughter.]

Mr. STETLER. They are separable.

Senator KENNEDY. Separability?

Mr. STETLER. Sounds like beating the wife.

Senator KENNEDY. I know. Well, you have indicated support for the environmental impact.

Mr. STETLER. That is right.

Senator KENNEDY. Which I think is——

Mr. STETLER. We would support that. I realize that although anybody that is involved in this would like to escape from two layers of controls.

Senator KENNEDY. Yes.

Mr. STETLER. At the present time there is so much interest in it, and the problem is so severe that what we might like to see in the way of exemption provision in the bill, that is probably not likely, so I do not know that we are going to be able to escape from local involvement, and it is there already.

I think you know that the few companies that we have that are involved in it are in cities where they are now relating—despite what I heard somebody say this morning—those companies are relating to local committees. And I do not think that is going to change.

Senator KENNEDY. No, that really was not—well, I mean—but how do you stand on the question of the local veto?

Mr. STETLER. If I had my preference, I would like to see a strong Federal provision in the statute that would govern this type of research and not leave it to 100 or several hundred jurisdictions.

Senotor KENNEDY. All right. Now we have got the strong provision, and we permitted the States to do add-ons. If, according to the administration's legislation, they have to interpret that as to be an add-on, the proposal must be a strengthening provision; but the Administration does permit it. Say that they interpret the provision to be that those citizens in a local community, like Cambridge, Mass., would be able to in their own good judgment to put a moratorium on it. Would you still support the legislation?

Mr. STETLER. Our companies are prepared to live with that.

Senator KENNEDY. You could live with that?

Mr. STETLER. Our companies are now, and I presume they expect they will.

Senator KENNEDY. Well, that is a very impressive commentary and statement. I mean, we heard some of the researchers, three out of the four researchers this morning were unwilling to live with it. To have the industry themselves prepared to live with it, I think is fine.

Mr. STETLER. I think we are a little more used to that sort of control and supervision than individual researchers are. It may not come as hard for us as it does for them.

[Laughter.]

Senator KENNEDY. OK.

Mr. STETLER. Lastly, information to be submitted to the Government and disclosed to the public. We believe the name and address of the facility, the names of the research personnel involved, and an outline of the research projects should certainly be submitted to the Government and, if deemed desirable, be made available to the public.

We also believe that facilities should be subject to Government inspection and reporting requirements. In any case, however, confidential proprietary information submitted should be given protection under the specific provisions of the implementing legislation, along the lines of an amendment which I indicated we would like to be able to submit.

Mr. Chairman, I think that is sufficient.

Senator KENNEDY. Yes; that is fine. Just on the area of the trade secrets, I mean obviously it is a question of balance between public information and protecting, you know, the particular innovativeness of a company's research on this.

OK, how do you assess the need for public disclosures versus need for trade secret protection?

Mr. STETLER. I think we are prepared to recognize that, given this particularly unique area of research and the problems that may exist, there has to be a good deal of leveling and disclosure with the public, so they can be part of the decision, and know what is going on.

Nevertheless, even if you believe that, as I do, and you comply with that, there are still some information that is going to develop as a

result of some very extensive and expensive research, which we think should not be divulged—not so much as a public problem as a problem as far as competitors are concerned.

Now, as you know, there are statutes that deal with trade secret information. Some of the recent decisions in that area are pretty broad, and I think the statute does provide that one type of information that is going to be protected is any type that is recognized in an implementing statute. That, of course, is why we would like to see the committee look at that and see if there could be carved out an agreeable appropriate area for proprietary research which could be protected in this statute, rather than left to the general law with respect to trade secrets and confidential data.

Senator KENNEDY. How has industry been able to work out its problems with the current NIH guidelines?

Mr. STETLER. Well, there have been changes recently; for many, many years there really was no problem. We had some question with the way in which the law was implemented by Food and Drug. As a matter of fact, we instituted a lawsuit to try and get an interpretation that we felt was reasonable under the act, and we lost that.

So right now we are subject to the interpretation or the reading of the law as the Commissioner of Food and Drug or HEW sees it.

I would say, to this juncture I do not think we have been hurt seriously by that. But the protection is not really there, it is a matter of implementation and decision on an individual basis.

Senator KENNEDY. But they have been able to move ahead, haven't they, that is, the industry?

Mr. STETLER. Oh, yes.

Senator KENNEDY. Have you remarked on how many pharmaceutical companies are now engaged in the research?

Mr. STETLER. According to our survey, in the United States, three in their own facilities and three are doing it through contract work, outside of their facility. Now, some are very interested, as I think they indicated to you, but those are the only ones that are really involved, according to the reports they gave us.

Senator KENNEDY. Do you have any problem about having public members on the review boards and also trying to attempt to protect trade secrets?

Mr. STETLER. No, sir.

Senator KENNEDY. What about the patent boards? Has the industry got some position on the patent boards, on what changes ought to be made?

Mr. STETLER. Yes; we think the patent law, as it applies to other results of our research, should apply to this. We have not asked for any special patent privileges, nor do we think there should be less.

I think, and I know part of this legislation, one of these other bills, would limit or restrict the ability to patent results of this type of research. We think that falls more in terms of a punitive provision, and certainly anyone that would encourage research in this area, I would say we would foresee the same application of the patent laws to this research as to our other research.

Senator KENNEDY. Yes; would you not prefer to have it under one governmental regulatory agency rather than scattered around?

Mr. STETLER. Yes; we would. HEW would be our preference.

Senator KENNEDY. OK. I think that is important testimony on it. I think that makes the most sense. Otherwise we are going to have, I think, a proliferation of different rules, regulations, all of which are going to make it more complex.

Mr. STETLER. And probably multiple life insured procedures, which are going to be delayed.

Senator KENNEDY. OK.

Thank you very much.

Mr. STETLER. Thank you.

[The prepared statement of Mr. Stetler and accompanying material follows:]

C. Joseph Stetler, President
Pharmaceutical Manufacturers Association

Before the

Subcommittee on Health and Scientific Research
Senate Committee on Human Resources

on

S. 621, S. 945 and S. 1217, 95th Congress

April 6, 1977

Mr. Chairman and Members of the Committee:

I am C. Joseph Stetler, President of the Pharmaceutical Manufacturers Association, an organization composed of 129 member firms that discover, develop, manufacture and market most of the prescription drugs and a large percentage of the medical devices and diagnostic products available in the United States. Accompanying me are Dr. John G. Adams, PMA Vice President, Scientific and Professional Relations and Bruce J. Brennan, PMA Vice President and General Counsel. We are pleased to accept the Subcommittee's invitation to present our views on S. 621, S. 945 and S. 1217, 95th Congress.

Recombinant DNA Research, the subject of all three bills, offers great promise in many fields, including the treatment and prevention of various diseases. For this reason we feel that any legislation adopted should encourage as well as regulate such research.

Last September, we testified before your Subcommittee on the involvement of PMA member firms in Recombinant DNA research. At that time, we commented on the NIH guidelines of July, 1976, pointing out that with some minor modifications, the pharmaceutical industry would voluntarily comply with them.

SINCE THAT TIME, WE HAVE CONTINUED TO WORK WITH STATE AND FEDERAL LEGISLATIVE AND REGULATORY BODIES AS THEY HAVE WEIGHED THE NEED FOR PUBLIC INVOLVEMENT IN THIS FRONTIER FIELD OF SCIENCE. WE OFFICIALLY COMMENTED TO THE DEPARTMENT OF HEW IN NOVEMBER ON THE NIH GUIDELINES AND REITERATED THE INTENTION OF PMA MEMBER FIRMS TO VOLUNTARILY COMPLY WITH THEM. WE HAVE ALSO SOUGHT FROM NIH A CLARIFICATION OF THE CONFIDENTIALITY OF INFORMATION ASPECTS OF THE GUIDELINES.

EARLY IN MARCH OF THIS YEAR, WE AGAIN SURVEYED PMA MEMBER FIRMS TO DETERMINE THEIR CURRENT INVOLVEMENT IN RECOMBINANT DNA RESEARCH. CONTRARY TO SOME ALLEGATIONS THAT THE DRUG INDUSTRY IS HEAVILY ENGAGED IN SUCH RESEARCH, OUR SURVEY FOUND THAT ONLY THREE PMA FIRMS ARE NOW SO ENGAGED IN THEIR OWN FACILITIES. THREE OTHER FIRMS ARE SPONSORING SUCH RESEARCH THROUGH GRANTS OR CONTRACTS. ADDITIONAL DETAILS OF OUR SURVEY HAVE BEEN PROVIDED TO NIH, AND WE SHALL BE PLEASED TO MAKE THEM AVAILABLE TO THE SUBCOMMITTEE IF YOU WISH.

ALTHOUGH WE ARE CONCERNED ABOUT CERTAIN PROVISIONS OF THE PENDING PROPOSALS, WE AGREE THAT LEGISLATION SHOULD BE ENACTED IN ORDER TO PROVIDE ADEQUATE SAFEGUARDS, ENFORCEMENT MECHANISMS, AND RESEARCH ENCOURAGEMENT. IN ORDER TO SATISFY THESE INTERESTS, UNIFORM NATIONAL STANDARDS SHOULD BE ESTABLISHED FOR ALL RECOMBINANT DNA RESEARCH, WHETHER CONDUCTED UNDER PUBLIC OR PRIVATE AUSPICES. INDIVIDUAL FACILITIES AT WHICH SUCH RESEARCH IS CARRIED ON SHOULD BE REGULATED BY THE DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE. THE SECRETARY OF HEW SHOULD ALSO BE GIVEN AUTHORITY TO INSPECT THOSE FACILITIES FOR COMPLIANCE WITH SAFETY AND OTHER REQUIREMENTS. WE ALSO FEEL THAT FEDERAL LEGISLATION SHOULD REQUIRE THE ESTABLISHMENT OF INSTITUTIONAL REVIEW COMMITTEES.

S. 1217, 95TH CONGRESS

THE LEGISLATIVE PROPOSAL TO WHICH WE WOULD LIKE TO DIRECT OUR PRINCIPAL ATTENTION IS S. 1217, THE "RECOMBINANT DNA REGULATION ACT". WE FEEL THAT THE BASIC REGULATORY FRAMEWORK SET FORTH IN THAT BILL IS SOUND AND WOULD PROVIDE EFFECTIVE CONTROLS WHILE NOT INTERFERING EXCESSIVELY WITH THE RESEARCH PROCESS. WE AGREE THAT THE SECRETARY OF HEW SHOULD PROMULGATE STANDARDS CONCERNING THE PRODUCTION AND POSSESSION OF RECOMBINANT DNA.

DESPITE THIS GENERAL SUPPORT, WE ARE TROUBLED BY CERTAIN OF THE SPECIFIC PROVISIONS OF THE BILL. THE INTERAGENCY REPORT, WHICH THE BILL PURPORTS TO IMPLEMENT WOULD APPLY THE CONCEPT OF LICENSING BY THE FEDERAL GOVERNMENT TO FACILITIES WHICH PRODUCE OR POSSESS RECOMBINANT DNA AND SUBJECT THE ACTUAL RESEARCH PROJECTS ONLY TO A REGISTRATION OR NOTIFICATION REQUIREMENT. YET, THE ACTUAL PROVISIONS OF S. 1217 WOULD APPEAR TO CREATE A DIFFERENT SITUATION.

ONE OF THE NECESSARY PREREQUISITES FOR OBTAINING A LICENSE UNDER THE BILL IS AN AGREEMENT AND A DETERMINATION BY THE SECRETARY THAT PRODUCTION OR POSSESSION OF RECOMBINANT DNA WILL ONLY OCCUR AS A PART OF A REGISTERED PROJECT. UNDER THE TERMS OF SECTION 6 OF THE BILL, THE SECRETARY WOULD REGISTER THE PROJECT ONLY IF THE REGISTRATION REQUEST IS ACCOMPANIED BY INFORMATION ADEQUATELY DESCRIBING THE PROJECT. WHEN THESE TWO SECTIONS ARE READ TOGETHER, THEY SEEM TO REQUIRE NOT ONLY PRECLEARANCE, THAT IS LICENSURE OF THE FACILITY BUT ALSO OF THE RESEARCH PROJECT.

WE SUGGEST THAT THE LICENSING PROVISIONS OF THE BILL BE LIMITED TO THE LICENSING OF FACILITIES. IT IS APPROPRIATE FOR THE SECRETARY OF HEW TO BE ASSURED THAT A FACILITY USED FOR RECOMBINANT DNA RESEARCH

IS IN COMPLIANCE WITH FEDERAL STANDARDS AND IS MEETING NECESSARY SAFETY REQUIREMENTS. TO GO BEYOND THAT POINT AND REQUIRE PREAPPROVAL BY THE SECRETARY OF RESEARCH PROJECTS WOULD CREATE SUBSTANTIAL IMPEDIMENTS TO THE CONDUCT OF THE RECOMBINANT DNA RESEARCH. IN EFFECT, IT WOULD PLACE THE SECRETARY IN THE POSITION OF DIRECTING ALL SUCH RESEARCH CONDUCTED WITHIN THE UNITED STATES. AS WE UNDERSTAND THE RECOMMENDATIONS OF THE FEDERAL INTERAGENCY COMMITTEE ON RECOMBINANT DNA RESEARCH AND THE TESTIMONY OF DR. FREDERICKSON BEFORE THE HOUSE SUBCOMMITTEE ON SCIENCE, RESEARCH AND TECHNOLOGY ON MARCH 31, THE ADMINISTRATION WOULD ALSO SUPPORT THIS APPROACH.

WE DO NOT FIND ANY SPECIFIC PROVISIONS IN S. 1217 WHICH WOULD PROTECT THE CONFIDENTIALITY OR TRADE SECRET STATUS OF PROPRIETARY INFORMATION SUBMITTED TO THE SECRETARY AS PART OF THE REPORTING PROCESS OR AS PART OF AN APPLICATION FOR LICENSE. THE PHARMACEUTICAL INDUSTRY SUPPORTS THE SUBMISSION OF SUFFICIENT INFORMATION TO THE SECRETARY TO DESCRIBE RESEARCH PROGRAMS WHICH ARE BEING UNDERTAKEN IN THIS CRITICAL AREA. AT THE SAME TIME, IF THAT RESEARCH IS TO BE GIVEN EVENTUAL COMMERCIAL APPLICATION AND IF PRIVATE RESOURCES ARE TO FINANCE THIS RESEARCH, THERE MUST BE SOME PROTECTION GIVEN TO TRADE SECRET DATA. WE WOULD LIKE TO OFFER AN AMENDMENT TO THE BILL WHICH WE BELIEVE WOULD PROVIDE THAT SORT OF PROTECTION AND NOT IMPEDE THE REGULATORY PROCESS OR INTERFERE WITH THE SECRETARY'S UNDERSTANDING OF THE SCOPE OF THE RESEARCH BEING CONDUCTED. OUR AMENDMENT IS MODELED AFTER SIMILAR PROVISIONS IN THE FEDERAL NON-NUCLEAR ENERGY RESEARCH AND DEVELOPMENT ACT.

SECTION 11 OF THE BILL PROPOSES A SYSTEM UNDER WHICH AN EMPLOYEE, WHO WAS DISCHARGED OR FEELS DISCRIMINATED AGAINST BECAUSE OF TESTIMONY OR OTHER ACTIONS TAKEN BY HIM IN A PROCEEDING AGAINST HIS EMPLOYER

COULD PETITION FOR RELIEF TO THE SECRETARY OF LABOR. AFTER AN INVESTIGATION, THE SECRETARY OF LABOR COULD ISSUE AN ORDER TO REINSTATE THE EMPLOYEE, PAY COMPENSATION, INCLUDING BACK PAY, AND ASSESS COMPENSATORY AND EVEN EXEMPLARY DAMAGES AGAINST THE EMPLOYER. THIS PROPOSED SYSTEM FOR HANDLING EMPLOYEE/EMPLOYER DISCRIMINATION COMPLAINTS SEEMS TO BE UNNECESSARY. CURRENTLY, THERE IS A RELATED BUT LESS PUNITIVE PROCEDURE AVAILABLE UNDER SECTION 11(c) OF THE OCCUPATIONAL SAFETY AND HEALTH ACT OF 1970 (29 USC, SEC. 660(c)). WE RECOMMEND THAT ANY PROVISION IN RECOMBINANT DNA LEGISLATION INTENDED TO SAFEGUARD AGAINST REPRISAL BY EMPLOYERS BE CONSISTENT WITH THOSE PROVISIONS.

THE PROVISIONS OF THE BILL WITH RESPECT TO ENFORCEMENT WOULD AUTHORIZE THE SECRETARY OF HEW TO DIRECTLY ASSESS CIVIL PENALTIES FOR VIOLATIONS OF THE ACT OTHER THAN THOSE RELATING TO EMPLOYEE PROTECTION. WE DO NOT OPPOSE A SYSTEM OF CIVIL PENALTIES BEING ASSOCIATED WITH VIOLATIONS OF THE BILL. HOWEVER, WE RECOMMEND THAT AN ACTION FOR CIVIL PENALTIES BE INITIATED BY THE GOVERNMENT IN A FEDERAL COURT PROCEEDING RATHER THAN IN AN ADMINISTRATIVE PROCEEDING BEFORE THE SECRETARY.

WE NOTE THAT THERE IS A PROVISION FOR JUDICIAL REVIEW REGARDING THE PROMULGATION OF STANDARDS UNDER THE ACT. HOWEVER, THERE IS NO PROVISION FOR JUDICIAL REVIEW OF ACTIONS OF THE SECRETARY CONCERNING THE ISSUANCE OR REVOCATION OF A LICENSE. WE WOULD HOPE THAT THIS IS MERELY AN OVERSIGHT BY THE DRAFTERS OF THE PROPOSAL. JUDICIAL REVIEW OF LICENSING REQUIREMENTS IS A MATTER OF DUE PROCESS WHICH IS PROTECTED BY THE CONSTITUTION AND SHOULD BE INCLUDED IN THE BILL.

FINALLY, WE WOULD SUGGEST THAT THE PRE-EMPTION SECTION BE AMENDED TO PROVIDE THAT THE SECRETARY "MAY" RATHER THAN "SHALL" EXEMPT A STATE OR SUBDIVISION FROM THE PROVISION OF SECTION 10(A).

S. 621, 95TH CONGRESS

S. 621, THE "DNA RESEARCH ACT OF 1977", WOULD AUTHORIZE THE SECRETARY OF HEW TO PROMULGATE GUIDELINES APPLICABLE TO EITHER PUBLIC OR PRIVATE ORGANIZATIONS. THE BILL WOULD ALSO IMPOSE CERTAIN RESTRICTIONS ON PATENTS BY LINKING THE GRANTING OF PATENTS TO ADHERENCE TO THE GUIDELINES, AS WELL AS REQUIRING FULL DISCLOSURE OF INFORMATION WITH RESPECT TO PROCESSES AND ORGANISMS. THE HEW SECRETARY WOULD BE AUTHORIZED TO ISSUE LICENSES FOR RESEARCH AND TO SEEK AN INJUNCTION AGAINST A RESEARCH FACILITY BELIEVED BY THE SECRETARY TO BE A SIGNIFICANT HAZARD TO THE PUBLIC HEALTH. FEDERAL AGENTS COULD CONDUCT INSPECTIONS IN ORDER TO DETERMINE WHETHER RESEARCH FACILITIES WERE BEING OPERATED IN COMPLIANCE WITH THE ACT, THE GUIDELINES, AND THE TERMS OF ANY LICENSE. PERSONS DOING RESEARCH WITH RECOMBINANT DNA WOULD BE HELD STRICTLY LIABLE WITHOUT REGARD TO FAULT FOR ANY INJURY CAUSED BY SUCH RESEARCH.

THE PRINCIPAL REGULATORY TOOL IN THIS BILL IS THE LICENSING SYSTEM. WE BELIEVE ITS TERMS ARE TOO BROAD AND NEED TO BE REDEFINED. FURTHER, WE NOTE THAT THE FEES TO BE CHARGED FOR SUCH LICENSES COULD BE UTILIZED TO ENFORCE THE SECRETARY'S GUIDELINES. IF THAT WERE CONSTRUED TO MEAN PAYING THE SALARIES AND EXPENSES OF INSPECTORS AND THE CENTRAL OFFICE FORCE WHICH WOULD REVIEW ALL OF THE INSPECTION REPORTS, THE RESULTANT HIGH LICENSING FEES COULD, IN EFFECT, BAN USEFUL RESEARCH BY SMALL LABORATORIES.

TWO OTHER PROVISIONS OF THIS BILL WARRANT COMMENT. THE RESTRICTIONS ON PATENTS WHICH THE BILL WOULD ESTABLISH ARE UNNECESSARY AND COULD WORK AS A DISSERVICE TO THE PUBLIC BY DISCOURAGING RESEARCH. WE ARE GENERALLY OPPOSED TO SPECIFIC LIMITATIONS ON THE GENERAL PATENT LAWS, SUCH AS THE ONE IN SECTION 6, WHICH WOULD PRECLUDE THE GRANTING OF A PATENT UNLESS ALL GUIDELINES HAVE BEEN STRICTLY FOLLOWED. WE BELIEVE

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THERE IS ALREADY SUFFICIENT AUTHORITY IN THE LICENSING AND OTHER PROVISIONS TO PRECLUDE MISUSE BY PRIVATE PARTIES.

THE OTHER PATENT RESTRICTION RELATES TO COMPLETE DISCLOSURE OF PROCESSES AND ORGANISMS IN THE PATENT APPLICATION. WE ASSUME THAT THIS PROVISION SUGGESTS SUBSTANTIALLY GREATER DISCLOSURE OF INFORMATION THAN WOULD ALREADY BE REQUIRED IN THE NORMAL PATENT APPLICATION. WE FEEL THAT THE DEMAND FOR SUCH DISCLOSURES WOULD ACTUALLY FORCE PERSONS HOLDING INVENTIONS TO GUARD THE RESULTS OF THEIR RESEARCH BY AVOIDING THE PATENT SYSTEM AND RELYING ON THEIR COMMON LAW TRADE SECRET PROTECTIONS. INSTEAD OF MAKING THEIR INVENTION KNOWN TO THE EXTENT THAT THE PATENT LAWS REQUIRE, AND, THEREBY AIDING OTHER INVESTIGATORS OR INVENTORS IN THE PROCESS, RESEARCHERS OR INDUSTRIAL FIRMS HAVING SUCH KNOWLEDGE WOULD CIRCUMVENT THE PATENT SYSTEM.

AS TO DISCLOSURE OF INFORMATION DEVELOPED IN THE RESEARCH PROCESS, WE OUTLINED OUR RESERVATIONS WITH THE DEPARTMENT OF HEW WITH RESPECT TO THE GUIDELINES ON RECOMBINANT DNA RESEARCH WHICH WERE PUBLISHED LAST SUMMER. WE ACCEPT AND SUPPORT THE CONCEPT OF INFORMING THE SECRETARY OF HEW THAT A FIRM OR INDIVIDUAL IS ENGAGING IN RECOMBINANT DNA RESEARCH AND OF PROVIDING INFORMATION AS TO THE TYPE OF PROJECT THAT IS BEING UNDERTAKEN AS WELL AS ITS GENERAL NATURE. IT IS IMPORTANT, AT THE SAME TIME, TO MAINTAIN THE CONFIDENTIAL AND TRADE SECRET STATUS OF PROJECTS OR DATA WHICH DESERVE THAT STATUS. WITHOUT THAT FORM OF PROTECTION, IT WOULD BE DIFFICULT FOR PRIVATE FIRMS TO JUSTIFY THE KINDS OF INVESTMENTS NECESSARY TO FINANCE THIS KIND OF RESEARCH.

THE NO-FAULT, STRICT LIABILITY PROVISIONS COULD VERY WELL CAUSE THE INSURANCE INDUSTRY TO REASSESS ITS ENTIRE COVERAGE FOR PHARMACEUTICAL COMPANIES OR OTHER FIRMS. IN LIGHT OF THE EXPERIENCE

WHICH THE INDUSTRY ALREADY HAS IN DEALING WITH THESE AND OTHER SUBSTANCES IN RESEARCH ENDEAVORS, WE FEEL THERE IS NO EVIDENCE TO SUPPORT THIS TYPE OF REQUIREMENT. PERSONS INJURED AS A RESULT OF RECOMBINANT DNA RESEARCH WOULD CLEARLY BE COVERED UNDER EXISTING INSURANCE PROGRAMS. THIS WOULD SEEM TO BE TRUE ALSO IN ACADEMIC INSTITUTIONS. FINALLY, WE QUESTION WHETHER A PROVISION OF THIS SORT SHOULD BE ENACTED IN VIEW OF THE VARIETY OF STATE STATUTES AND DECISIONS WHICH WOULD PROVIDE RELIEF UNDER LIABILITY CIRCUMSTANCES.

S. 945, 95TH CONGRESS

THE RECOMBINANT DNA STANDARDS ACT OF 1977, S. 945, TAKES A SOMEWHAT DIFFERENT APPROACH TOWARD REGULATION. THE BILL WOULD AUTHORIZE THE SECRETARY OF HEW TO PROMULGATE STANDARDS RELATING TO SUCH RESEARCH. IT WOULD ALSO AUTHORIZE HIM TO CONDUCT INSPECTIONS IN ORDER TO DETERMINE WHETHER A RESEARCH FACILITY WAS BEING OPERATED IN COMPLIANCE WITH THE VARIOUS PROVISIONS OF THE ACT. AGAIN, THE ATTORNEY GENERAL, ON A DETERMINATION BY THE SECRETARY THAT A FACILITY'S RECOMBINANT DNA RESEARCH WAS ENDANGERING THE PUBLIC HEALTH, COULD BRING SUIT TO ENJOIN THAT ACTIVITY. THE BILL WOULD ALSO AUTHORIZE INDIVIDUALS TO INITIATE A LAWSUIT FOR AN INJUNCTION ON GROUNDS THAT THE CONTINUATION OF RESEARCH AT A PARTICULAR FACILITY WOULD BE A SIGNIFICANT HAZARD TO THE PUBLIC HEALTH. IF THE CITIZEN SUCCEEDED IN OBTAINING AN INJUNCTION, THE OFFENDING FACILITY WOULD BE ASSESSED ATTORNEY'S FEES, WITNESS FEES, AND OTHER REASONABLE COSTS OF LITIGATION.

WITH RESPECT TO PENALTIES, THE BILL PROVIDES THAT A VIOLATION OF ANY REGULATION PROMULGATED UNDER THE ACT WOULD BE A MISDEMEANOR AND WILLFUL VIOLATIONS WOULD BE CLASSIFIED AS FELONIES.

BOTH S. 621 AND S. 945 TREAT VIOLATIONS OF THE ACT AS CRIMINAL OFFENSES. IN ADDITION, BOTH BILLS WOULD IMPOSE CRIMINAL PENALTIES AGAINST EMPLOYERS WHO DISCHARGE OR DISCRIMINATE AGAINST EMPLOYEES BECAUSE OF THE EMPLOYEE'S INVOLVEMENT IN PROCEEDINGS AGAINST THE EMPLOYER. WE FEEL THESE PENALTY PROVISIONS ARE TOO SEVERE FOR THE MANY TYPES OF ACTIVITIES WHICH THEY WOULD EMBRACE. WE SUGGEST A MORE DEFINITIVE SET OF PENALTIES THAT WOULD PERMIT INJUNCTION OR PERHAPS CIVIL FINES FOR MINOR OFFENSES AND RESERVE CRIMINAL PENALTIES FOR MORE SERIOUS OFFENSES OR IN THE CASE OF WILLFUL VIOLATIONS OF LICENSING OR STANDARDS PROVISIONS. CRIMINAL PENALTIES SHOULD NOT BE IMPOSED UPON EMPLOYERS SIMPLY BECAUSE OF EMPLOYMENT DECISIONS, EVEN WHERE SUCH DECISIONS ARE DETERMINED TO BE DISCRIMINATORY. THERE ARE ALREADY SUFFICIENT ENFORCEMENT PROCEDURES UNDER THE OCCUPATIONAL SAFETY AND HEALTH ACT OF 1970.

TITLE II OF S. 945, WOULD ESTABLISH A NATIONAL COMMISSION FOR THE STUDY OF RECOMBINANT DNA RESEARCH AND TECHNOLOGY, WITH MEMBERS TO BE APPOINTED BY THE SECRETARY OF HEW. THE COMMISSION WOULD STUDY THE APPROPRIATENESS OF CONTINUING RECOMBINANT DNA RESEARCH; DEVELOP GUIDELINES FOR ITS CONDUCT AND ADVISE THE SECRETARY CONCERNING ADMINISTRATIVE ACTION TO PUT SUCH GUIDELINES INTO EFFECT. IN VIEW OF THE WORK ALREADY COMPLETED BY THE NATIONAL INSTITUTES OF HEALTH AND THE INTERAGENCY COMMITTEE ON RECOMBINANT DNA RESEARCH, WE QUESTION THE PRACTICALITY OF ANOTHER STUDY COMMISSION. FROM OUR READING OF THE MARCH 15 INTERIM REPORT OF THE INTERAGENCY COMMITTEE, IT WOULD SEEM THAT THE SECRETARY ALREADY HAS A WELL QUALIFIED ADVISORY BODY. THIS GROUP HAS SOUGHT THE COUNSEL OF EXPERIENCED EXPERTS FROM ACADEMIA AND FROM INDUSTRIAL CONCERNS. WE WOULD HOPE THAT IT WOULD CONTINUE TO DO SO AND WOULD THEREBY SATISFY THE OBJECTIVDS OF TITLE II OF S. 945.

MR. CHAIRMAN, THAT CONCLUDES OUR TESTIMONY. WE WILL BE PLEASED TO SUPPLY THE COMMITTEE WITH SPECIFIC AMENDATORY LANGUAGE COVERING THE SUGGESTIONS WE HAVE MADE. WE WILL ALSO BE PLEASED TO RESPOND TO ANY QUESTIONS WHICH YOU MIGHT HAVE.

Responses to Propositions and Questions In Preamble to S. 1217

1. Definition of Recombinant DNA Research

We have discussed the proposed new definition of recombinant DNA research with industry experts and have been informed that it is acceptable. The new definition differs technically in the introduction of the term "molecule" in addition to the term "segments of molecules". The new definition also includes research as distinguished from the more limited definition of the recombinant DNA molecule. It appears to refer to that research which involves the synthetic or unnatural recombination of DNA segments or DNA molecules. We have no objection to this redefinition but suggest that it could be stated somewhat differently for purposes of clarity.

2. Commission to Participate in DNA Research Decisions

We would advocate the creation of an "Advisory Council" composed of experts in the field of recombinant DNA research and bioethics, law and other nonscientific disciplines. DNA expertise should cover biomedical, agricultural and other scientific disciplines.

3. Scope and Nature of Statutory Standards

It was our belief that the use of the term "production or possession" was intended to regulate all activities involving recombinant DNA, including research and commercial development. We would, therefore, have no objection to including the term "research". Accordingly, the scope should include research, production and possession.

With respect to standards dealing with the qualification of the individuals conducting the research, we have reservations. It could result in governmental control of scientific qualifications and the

ESTABLISHMENT OF A FEDERAL ROSTER OF PERSONS AUTHORIZED TO ENGAGE IN SUCH RESEARCH.

4. Breach of Standards - Grounds for Revocation of License

We agree with this point. Our reading of Section 5 of the bill provides necessary authority.

5. Licensure Contingent on Adequate Safety Standards

We agree.

6. Continuing Study of Ethical, Societal and Legal Implication of DNA Research

We agree.

7. Public Right to Know Who, Where and Under What Conditions

We agree.

8. Funding and Staffing

Impossible to estimate without additional details.

9. Limit on Number of Facilities

No.

10. Environmental Impact Statement

Should be required.

11. Information to be Submitted to Government and Disclosed to Public

We believe the name and address of the facility, the names of the research personnel involved, and an outline of the research project should be submitted to the government and, if deemed desirable, be made available to the public. We also believe that facilities should be subject to government inspection and reporting requirements. In any case, confidential proprietary information submitted should be given protection under specific provisions of the implementing legislation.

Senator KENNEDY. We appreciate it. We appreciate your patience and your testimony.

All right. Jacqueline Warren of the Environmental Defense Fund.

STATEMENT OF MS. JACQUELINE M. WARREN, ENVIRONMENTAL DEFENSE FUND, ACCOMPANIED BY LESLIE DACH, STAFF SCIENCE ASSOCIATE

Ms. WARREN. Mr. Chairman, I am Jacqueline Warren of the Environmental Defense Fund, and with me this afternoon is Leslie Dach, a science associate on the staff.

The Environmental Defense Fund has been actively involved in the recombinant DNA issue for some period of time.

In a hearing that you held at the end of last year, Dr. Burke Zimmerman, formerly of our staff and presently with Congressman Rogers' Health Subcommittee staff, testified on the concern that our organization feels, that thorough education and training must be required for all personnel who are presently, or in the future will be, working with recombinant DNA techniques, and that rigorous monitoring and enforcement of safety procedures must be required for all research in this area.

We have also petitioned the Department of HEW, and it currently has before it a petition to immediately apply the NIH guidelines to all recombinant DNA research around the country.

I would like to address myself today to the specific issues that Congress needs to consider if effective legislation is to be passed.

Our concern is that sufficient incentives have to be built into any regulatory system through outside supervision and also self-policing, to make sure that the regulations will be complied with. And also that the public will have adequate access to information concerning research on recombinant DNA.

When I received a copy of the administration bill, I did not realize that it was the administration bill, and have referred to it as a subcommittee draft; but I have—although we generally support the regulatory scheme that is outlined there, we have several serious reservations.

We would agree that the Secretary of HEW or whoever will be given the authority to control this program should promulgate the NIH guidelines right away. And it is our understanding that the final environmental impact statement on those guidelines is due out at any time. If that environmental impact statement is adequate, I would agree that there does not need to be a second environmental impact statement when the Secretary promulgates those regulations.

And the same is true for the reference to the APA. But I would certainly think, once those guidelines are out and once final guidelines go into the work, there ought to be public hearings on those guidelines under the APA, and the public should have an opportunity to comment on all the issues that are involved in that, some that the NIH did not really consider, such as whether recombinant DNA research should be undertaken at all at this time, and whether E. coli bacteria should be used at all.

We also would want an impact statement on the final regulation, and would want the regular application of the APA.

We also believe that—I would disagree with the PMA on this point—that both institutions that are authorized to conduct recombinant DNA research and particular projects should be licensed separately through the principal investigator on each project. Simply licensing the facility and then registering the projects to be conducted is not sufficient to insure that proper safety precautions will be maintained.

And I notice in the administration bill that any application for registration is required to be registered, it says that the Secretary shall register. I do not think that this is going to provide any adequate assurance of safety.

We also would disagree with the statement in the administration bill that licensing requirements be transferred—the authority for that be transferred to the States or localities. I think that would give rise to a tremendous variation in standards all around the country, and I also think local boards and State boards may be very much subject to pressure than a single Federal licensing authority.

So we would support strict licensing requirements by the Federal Government, with substantial civil penalties to be maintained in order to assure proper protection of the public health.

And in our statement we have suggested language which would require licensing of both the authorized facility and the specific projects.

With respect to the——

Senator KENNEDY. What about the researchers?

Ms. WARREN. I think that if, when you license a specific project—we have proposed in our testimony that the principal investigator specify right in the application for a particular project the identity, qualifications, and training of all personnel who would be engaged in the project, and the facilities and materials to be used in such project. And also an assessment of the potential risks of the project.

In our suggested proposal for licensing the facility, we suggested that it be required that a Biohazards Committee be established right at that institution to supervise compliance with the regulations, that training programs be established, and that every single person who is going to be in any way involved in that have to go through that training program. And that when a particular project is applied for, that there has to be certification from the Biohazards Committee at the institution, specifying that the facilities have been—have met all Federal requirements and that they are approved by the Biohazards Committee, and that everybody involved in the project is trained.

I do not see how you are going to be able to maintain the kind of control that this sort of research would require. I mean, this Federal licensing of radioactive materials today, and from everything I have ever heard about that, it is a complete farce. You simply write in for a license and you get it. And there is no monitoring of the disposal of the materials, there is no careful regulation; and this is in a university setting. These regulations would be applying outside of universities, but throughout the whole private sector; but because of the possible harm that could result from this kind of research as such, I think that it requires a unique set of very stringent regulations.

And, as I said before, enough incentives built into the system, both through Federal regulations and also self-policing, so that the regulations would be complied with.

I think there should be sanctions. A project could lose its license for violations. The whole facility could lose their authorization to have that kind of research conducted, if enough violations took place. Perhaps even Federal funding for research at the facility. Enough so that everybody involved knows that if they do not comply, the whole project may have to be stopped, or the whole facility may not be allowed to continue with that.

For this reason we would also want the Biohazards Committee to be the entity to which violations are reported, and that violations of the regulation should have to further be reported to the Secretary of HEW.

It sounds like somewhat rigorous policing, but I just do not see how you are ever going to be able to enforce the kind of strict regulations that need to be established for this sort of research and have any guarantee whatever that you are going to be able to assure safe research going on.

We would also want, when a facility applies for the approval to conduct this research, and also when a particular project applies for a license, that that application be published in the Federal Register, with a description of what is being asked for, so that the public has some opportunity to comment, first of all, of whether they want that facility in their locality and whether they want the particular project. Not that their views would be dispositive, but some of the bills that I have seen provide for publication after the fact, it presents the public with a fait accompli. I do not know particularly what redress they would have at that point. At least if they know when the application is received that an opportunity will be given to them to present their views in opposition to it, and then, of course, if the final decision is something they do not like, they could have other remedies at law.

But just to simply say the license has been issued really just tells them, without giving them any opportunity to comment.

We support the inclusion of provisions regarding inspections and enforcement by the Federal Government.

And, as I said before, we would like to see a bill that has revocation of licenses applicable not just to the facility but also to the particular project.

We would also like to authorize the particular entity at HEW that is going to be in charge of this to be able to immediately get an injunction to restrain ongoing research, if the Secretary has reason to believe that this poses a serious or substantial hazard to public health.

The administration bill states that a district court may restrain recombinant DNA—I forgot the language exactly—recombinant DNA research or activities. But I think that to stress the point that care has to be taken, there should be a special authorization for immediate injunctions against particularly hazardous work.

And we also believe that individuals should be entitled to go to court and ask for injunctions of the same sort, if they have reason to believe that particular activities are posing a serious hazard to public health.

Of course, it would have to be on the basis of a proper showing.

As a public interest organization engaged in litigation, EDF also urges that language be included in the bill to provide for attorney's fees, covering the fees and costs of experts and litigation expenses in actions which are brought pursuant to this act. I mean, provisions such as this are being routinely included in many acts these days, and

I just wanted to mention that, if there is a role for the public under this act, that attorney's fees should be considered and included.

We also suggested that a recombinant DNA Advisory Committee be created in HEW to make recommendations to the Secretary with respect to the final regulation——

Senator KENNEDY. You may be interested to know that the President has announced, about an hour ago, support for S. 270.

Ms. WARREN. Oh, really?

Senator KENNEDY. Which reaches these attorneys' fees question, so that will be helpful in terms of the governmental agencies.

Ms. WARREN. Yes; that is the one that goes across all the agencies?

Senator KENNEDY. Yes.

Ms. WARREN. Oh, that is good to know.

Well, as I was saying, we are recommending that an advisory committee be created at HEW to make recommendations to the Secretary with respect to the final recombinant DNA guidelines and regulations, which will be promulgated. And also to make recommendation with respect to the—to all other matters that are related to the effective administration of this act.

And that the legislation should also specify what the makeup of the local biohazards committee should be, saying what kind of expertise should be represented on those committees.

One point I want to mention again, about the advisory committees, that they should have a majority of persons who are not engaged in or do not have a financial interest in recombinant DNA projects, in order to bring an outside lay input into the question of how the act would be administered.

I have also an interest in the question of trade secrecy, which has come up under other statutes, most recently the Toxic Substances Act; but I would agree with the PMA that there has got to be a lot more disclosure to the public in the area of recombinant DNA research than has traditionally been afforded. The statutory, the general statutory constraint on release of trade secret information, which is 18 U.S.C. 1905, and is generally referred to in many other statutes where there is a reference to prohibiting disclosure of confidential information—1905 is very broad. It deals with information that concerns or relates to trade secrets, processes, operations, style of work, apparatus, identity, confidential statistical data. I mean, it will exclude absolutely everything. If you simply say that the confidential data will be withheld in accordance with 18 U.S.C. 1905, you are going to be, by statute, excluding all information.

I think that there ought to be a specific exemption from this statute in any bill that this committee comes out with, and that they should make an effort to define what a trade secret is going to be considered in this area. Even though I know it is a difficult thing to do, but this ought to be a line drawn between the public interest and the narrow private interest in protecting competitive disadvantaged in matters which could have such a gross effect on the public health if something were to go wrong.

And the only way that you can get public input is to advise the public of who is doing what, and for what purpose.

I think that specific trade secret processes could still be protected in this way, and still allow the public the disclosure.

One proposal might be to provide for patenting of the process, so that the information is public, but the person who developed it has the exclusive right to it.

I have been told that this is possible under American patent law, but not under European. But I am not a patent lawyer, and I do not really know.

All I do know is that when you give a general protection for trade secrets, you deprive the public of much more information than legitimately they should be deprived of. And particularly when you are talking about matters that affect the public health.

That concludes my statement.

One of the things that you mentioned in terms of your statement is about the advisory committee for the Department of Health. The matter that concerns me is how, you institutionalize on one hand the public's participation in terms of establishing the ethical-moral questions, which I think the Protection of Human Subjects Panel probably can do, and on the other, the need in terms of evaluating the various scientific developments and their applicability; and, I wonder whether that is going to be left strictly in terms of the research departments of the community, or whether it includes the public.

I am just not quite sure if we leave it just in terms of research community and then put another advisory group whether we are going to have it so diffused and so fractionated that—we are not really going to serve much of purpose.

My inclination is that, the public ought to be involved closely with the scientific and research group, in terms of making the recommendations and in terms of the scientific decisions, let alone the ethical and moral questions.

I am just interested in what your own reaction is on this question.

Ms. WARREN. I think that the——

Senator KENNEDY. You see the point I am driving at?

Ms. WARREN. Yes; I do.

I think that having the President's commission deal—do a study or have a separate Commission do a study on the ethical and moral considerations——

Senator KENNEDY. Well, it is a continuing kind of oversight, a continuing process.

Ms. WARREN. Yes.

Senator KENNEDY. I think they will have to do it, would you not?

Ms. WARREN. Yes. I do. But in terms of strengthening, broadening or otherwise modifying the NIH guidelines, or coming out with final regulations that are going to govern this research, I think that I would—I am afraid that if it is just the Secretary of HEW, that it is going to be just NIH again, and that the guidelines are going to be the same. And that was why we recommended an Advisory Committee that would be made up of——

Senator KENNEDY. Well, why not sort of an advisory group—why not, whatever that panel is that is going to make those recommendations, why not have them include public people?

Mr. DACH. I think that was our suggestion, that that advisory committee would include a majority of nonscientists.

Senator KENNEDY. Well, yes, but——

Ms. WARREN. It would not have to be an advisory committee. It could be an entity inside HEW—is that what you are suggesting?

Senator KENNEDY. Yes.

I do not—maybe it is just a question of the organization. I can see a scientific group down here and then an advisory group for the scientific group, you know. And if it is given the weight that most people would give to the advisory group, it would not be a hell of a lot. And I am just wondering if that is a sufficient kind of protection, or whether, in actuality the scientific group that is going to make the recommendations, in terms of science information and technology, whether you would not lay people on that. That is basically my suggestion.

Ms. WARREN. I think you should. I think that many advisory committees presently advising on technical matters, for example, under the Safe Drinking Water Act there is, by statute, a Safe Drinking Water Advisory Council, which includes a certain number of technical people, but specifies in the statute that there has to be representation of consumer, environmental, and other outside groups. So that they do, even the outside people have input into the technical decisions.

Senator KENNEDY. OK.

Thank you very much. Very, very helpful.

Ms. WARREN. Thank you.

[The prepared statement of Ms. Warren follows:]



Environmental
Defense
Fund

1525 18th Street, NW, Washington, D.C. 20036 • 202/833-1484

STATEMENT BY THE ENVIRONMENTAL DEFENSE FUND ON

THE NEED FOR REGULATION OF
RECOMBINANT DNA RESEARCH

BEFORE THE SENATE HEALTH SUBCOMMITTEE
OF THE
COMMITTEE ON HUMAN RESOURCES

APRIL 6, 1977

PREPARED BY: JACQUELINE M. WARREN, LESLIE DACH,
AND
JOSEPH H. HIGHLAND, PH.D.

Mr. Chairman, members of the committee, my name is Jacqueline Warren. I am a staff attorney with the Environmental Defense Fund (EDF), a non-profit membership organization dedicated to the preservation and improvement of both the human and natural environment. Throughout the past 10 years, EDF has represented its members' views on a variety of socially important issues including: the regulation of potentially toxic and harmful materials, the establishment of environmentally sound policies with respect both to the development and use of our energy resources and the creation of responsible programs to preserve and protect our natural environment.

For more than a year now, EDF has been actively engaged in efforts to ensure that research and product development involving the use of recombinant DNA techniques is properly and carefully regulated. Both Dr. Burke Zimmerman, formerly a staff scientist with EDF and currently on the staff of the Subcommittee on Health and the Environment of the Committee on Interstate and Foreign Commerce, and Dr. Joseph Highland, Chairman of EDF's Toxic Chemicals Program, worked closely with Dr. Donald Fredrickson, Director of the National Institutes of Health, and his staff, during the development of the NIH guidelines for recombinant DNA research. On September 22, 1976, Dr. Zimmerman presented testimony to this Subcommittee in which he stressed our concerns that thorough education and training must be required for <u>all</u> personnel involved in work employing recombinant DNA techniques and that rigorous monitoring and enforcement of safety procedures must be required for <u>all</u> work in this area. Currently, the Department of Health,

Education, and Welfare (HEW) is considering a petition filed jointly by EDF and the Natural Resources Defense Council requesting that the Secretary of HEW, by the authority vested in him under the Public Health Service Act, immediately promulgate the NIH guidelines as regulations to govern all recombinant DNA research. The petition also includes a request for public hearings on the substance of the NIH guidelines including the broader issues of whether or not recombinant DNA research should be undertaken at all at this time, and if so, whether or not any strain of the colon bacillus E. coli can be considered a safe subject for these experiments.

Today I would like to address myself to the issues that must be considered if the Congress of the United States is to pass legislation designed to regulate recombinant DNA research effectively. Since November 11, 1976, when our petition to HEW was filed, several bills have been introduced into the House and Senate, and it is my understanding that others are still to be introduced. Most of these bills propose a regulatory mechanism for dealing with both research and product development involving recombinant DNA techniques. Clearly, any legislation enacted must deal both with the potential problems associated with academic research and those relating to industrial research, development, and production.

After a thorough review of the legislation proposed so far and careful consideration of the issue, we believe that any legislation developed by this Subcommittee should contain provisions such as the following:

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1. Standards:

a) §4(a)(1) should provide as follows:

Within 90 days of enactment, the Secretary of HEW shall promulgate as interim standards, applying to the production or possession of recombinant DNA, the Recombinant DNA Research Guidelines [41 Fed. Reg. 27901 (1976)], with any strengthening modifications he deems necessary.

b) §4(a)(2) of the draft subcommittee bill should also provide for public hearings. Issues to be considered should include but not be limited to: the appropriateness of conducting work at this time involving recombinant DNA techniques and the sufficiency of the interim standards to insure adequate safety not only for those individuals involved directly in recombinant DNA research but the general public as well. Specifically the question of whether or not the colon bacillus E. coli should be used for any experiments should be discussed.

c) §4(a)(4) should read as follows:

Periodically, but no longer than at intervals of one year, the Secretary of HEW after consultation with the Recombinant DNA Advisory Committee described below, should reaffirm the adequacy of the existing standards or amend the standards in a manner which strengthens the protection of public health.

2. Licensing and Registration

It is our belief that both the institution at which recombinant DNA research is conducted, be it an academic facility or an industrial one, and the principal investigator on each project, must be properly licensed by the Secretary of HEW. Simply licensing the

facility and registering the projects to be conducted, is not sufficient to insure that proper safety precautions will be maintained. Neither is it acceptable for the Secretary of HEW to transfer his authority for licensing to any state or local agency. Strict licensing requirements with substantial civil penalities must be maintained in order to assure proper protection of public health and well being.

We therefore suggest that §§5(a), (c), and (d) should be deleted and replaced by language such as the following:

a) Effective one hundred and eighty days after enactment, no person may produce or possess recombinant DNA unless such person holds a license authorizing them to engage in such research project or is under the direct supervision of a person holding such a license and unless the proposed project is to be carried out in a facility approved by the Secretary of HEW.

b) A facility may be granted approval as an institution for recombinant DNA work if the appropriate officials directing such an institution supply the Secretary of HEW with an application in such form and manner as may be prescribed by the Secretary, which indicates that:

1) The official(s) submitting the application are aware of their personal liability and that of their institution in cases where the provisions of this approval or those described in the licenses provided to individual researchers in their institution are violated;

2) That within 90 days of approval being granted, a local biohazard committee will be established, whose functions

and composition will be in accord with provisions described below, and

3) That within 90 days of approval being granted, a training program will be established that will be required of all employees who will in any way engage in activities connected with the production or possession of recombinant DNA. Such a program will be given free of cost and entail no loss of salary to employees who it is deemed must take this course. The curriculum of this course will be in accord with provisions proposed by the Secretary of HEW.

Any approval (or license) granted to an institution shall be subject to periodic review and approval must be reauthorized no later than 3 years after the initial approval has been granted. Reauthorization by the Secretary of HEW will be deemed appropriate providing that the Recombinant DNA Advisory Committee, described below, has had a reasonable opportunity to provide the Secretary with its recommendations on the issue.

d) Upon the issuance or renewal of any authorization for a facility, the Secretary of HEW shall publish in the Federal Register a notification of his/her action which will include but not be limited to, a description of the conditions and limitations pertinent to such authorization.

3. Section 6 of the Subcommittee draft should be deleted and the following language substituted:

a) A license authorizing a particular recombinant DNA project shall be granted to the principal investigator only upon an application made by such person in such form and manner as may be

prescribed by the Secretary of HEW. An application for such a license shall include:

1) A detailed description of the research project to be authorized by the license including: the identity, qualifications, and training of all personnel to be engaged in such project, and the facilities and materials to be used in such project;

2) Assurances satisfactory to the Secretary that the research project will conform to all requirements prescribed under the standards to be established for such work; and

3) An affirmation by the individual seeking the license that he/she is aware of the personal liability and that of their institution, in cases where the provisions of the license to be granted are violated.

b. Any license granted an individual shall be subject to periodic review and revision and shall be granted solely for the project defined. Any modification of a project that entails a higher level of risk as defined by the guidelines both in terms of biological or physical requirements will require a new license. A license shall be granted for the time necessary to complete the proposed project but in no case longer than two years. The Secretary of HEW may renew an expired license provided that the Recombinant DNA Advisory Committee, described below, has had a reasonable opportunity to provide the Secretary with its recommendations on the issue.

c. Upon the issuance or renewal of any license, the Secretary of HEW shall publish in the Federal Register a detailed description

of the sources of the biological materials to be used, the physical and biological containment requirements which are applicable and the objectives of such work.

4. Inspections: EDF supports the subcommittee's proposals for inspection of recombinant DNA research facilities. We would suggest, however, inclusion of the following language in §7:

No inspector shall be required to obtain a search warrant from any judicial officer prior to entering a facility in order to conduct an appropriate inspection.

5. EDF supports §§8, 9, 10, 11, and 12 of the subcommittee draft bill dealing with records and samples, reports, effect on state and local requirements, employee protection, and consultation with other agencies.

6. Enforcement: EDF supports §13(a) through (d) but believes that the same language should be added to provide for revocation of an institution's authorization to conduct recombinant DNA research, and, where appropriate, loss of federal funds for research, in cases where repeated project license violations have occurred.

7. Emergency Procedures for Hazardous Recombinant DNA and Administrative Restraint or Seizure: EDF strongly supports §§14 and 15 of the subcommittee draft bill as essential for safeguarding the public health. We recommend, however, that the following language be added to §14:

At anytime that the Secretary of HEW has reason to believe that a continuation of any activity at a facility conducting work involving recombinant DNA would constitute a significant hazard to

the public health, the Attorney General shall, at the Secretary's request, bring suit in the name of the United States in the district court for the district in which the facility is situated or any district in which such firm, institution or individual is doing business, to enjoin the continuation of such activity and upon a proper showing, a temporary injunction or restraining order against continuation of such activity pending a final order may be granted without bond.

At anytime any person has reason to believe that a continuation of any activity at a facility conducting work involving recombinant DNA constitutes a significant hazard to the public health, that person may bring suit in the United States District Court in which such a facility is located to enjoin continuation of such activity and upon proper showing, a temporary injunction or restraining order against continuation of such activity pending issuance of a final order may be granted without bond.

8. Attorney's Fees; Fees and Costs of Experts; Litigation Expenses

As a public interest organization engaged in litigation, EDF urges that language such as the following, consistent with provisions in many other recent statutes, be included: Notwithstanding any other provision of law, any party or party intervenor in a civil action shall be entitled to recover reasonable attorney's fees, fees and costs of experts, and other reasonable costs of litigation, including taxable costs, from the defendant if:

1) The court affords such a party the relief sought in a substantial measure; or

2) The court determines that such action served an important public purpose.

Reasonable attorney's fees, fees and costs of experts, and other costs of litigation awarded should be based on prevailing market rates for the kind and quality of services furnished.

9. License Revocation: EDF supports the language of §5(e) as it applies to the revocation of an institutional authorization but believes it should also be applicable to the licensing of a research project.

10. Recombinant DNA Advisory Committee: EDF urges the inclusion of a provision establishing an advisory committee and recommends adoption of the following language:

There is established the Recombinant DNA Research Advisory Committee to make recommendations to the Secretary with respect to the approval of applications for the issuance or renewal of licenses and authorizations under this act, promulgation of standards, and other matters related to the effective administration of this act. The advisory committee shall have a majority of members who are not engaged in or have a financial interest in recombinant DNA research projects.

11. Biohazards Committees: EDF recommends that institutional biohazards committees be established to ensure compliance with the requirements of this Act and the specific conditions of the institutional authorization and the project license(s). The activities of the committees shall include but not be limited to inspection of containment facilities, certification of employee training programs, and other activities deemed appropriate by the Secretary. The membership of the committees should include representation of all personnel involved in the research and in

maintenance of containment facilities.

We appreciate this opportunity to present our views to the Subcommittee and hope the foregoing suggestions will assist in the development of legislation to regulate recombinant DNA research.

Senator KENNEDY. We have two more witnesses. I might ask if they would be good enough to come up together.

Pamela Lippe of Friends of the Earth, and Jeremy Rifkin of People's Business Commission.

If they would be kind enough to come together.

STATEMENT OF MS. PAMELA LIPPE, FRIENDS OF THE EARTH, AND JEREMY RIFKIN, PEOPLE'S BUSINESS COMMISSION

Ms. LIPPE. Mr. Chairman, thank you for the opportunity to testify here today.

My name is Pamela Lippe, and I represent Friends of the Earth, a national environmental group with 25,000 members and sister organizations in 13 foreign nations.

The recombinant DNA technology is acknowledged by many as the most powerful technique ever available to science. Its ease of deployment, increased precision and speed will insure overnight proliferation in an unfettered and supportive environment. The irreversibility of the consequences, the acknowledged state of ignorance, and Murphy's law caution against this.

Friends of the Earth calls for an immediate moratorium on all research that would produce novel genetic combinations. This will allow for careful and deliberate development of our recombinant DNA policy, based on risk assessment studies and with full public participation and input.

We now have the technological capability to change the very essence of the natural—from microorganism to man. This is long before we are able to adequately assess the long-term consequences of this unprecedented power, long before we can formulate goals for its use, and without serious consideration of the moral and ethical problems that will eventually demand resolution. Public input into this discussion and participation in the decisionmaking must be given the highest priority if we wish to avoid rapid polarization and an emotional debate which will serve no one's purposes adequately.

Salvador Luria, Nobel laureate in physiology and medicine, has said:

> The progress of science is so rapid that it creates an imbalance between the power it places in the hands of man and the social conditions in which this power is exerted. Then neither warnings of scientists, nor breadth of public information, nor wisdom of citizens may compensate for inadequacies of the institutional framework to cope with new situations.

We have no satisfactory mechanisms for negotiating the conflicts of interest between the scientific community and the society at large. In fact, this may be the most important task before us. Recombinant DNA will certainly not be the last issues of this magnitude that our institutions will have to deal with.

Why must we rush ahead? There are no immediate benefits to be lost. Deferring most of these experiments for a few more years will allow for deliberate and long-range planning, based on risk assessment studies rather than best guesses. This seems like a much more sensible approach than policy-by-patchwork, but it requires time and resources, and a firm decision now before the economic and intellectual investment becomes greater and closes out a variety of options.

Almost everyone agrees that the public should be involved, but no one knows exactly how this could be accomplished. There will be no easy solution; we need to be bold, original, creative, and constructive. Excuses such as increased cost or bureaucracy should not be allowed to stand in the way of developing these sorely needed mechanisms. Friends of the Earth's general recommendations in this regard are:

We support, in principle, the creation of a commission to study the recombinant DNA issue in full. Its mandate must be broad and should include a consideration of the social, political, and ethical issues. It should be free to determine whether the recombinant DNA technique should be used at all, and, if it is to go on, under what public direction. All available options should be considered, including ban, full federalization, or, at minimum, determining priorities of research to be carried out first—development of alternative host organisms to *E. coli*, or risk assessment studies, for example. Friends of the Earth feels that a moratorium is necessary to insure that the best course of action is not prematurely foreclosed.

The makeup of this commission or any advisory committee must be carefully constituted to fairly represent the public and the various scientific concerns potentially affected by this new technology—for example, ecologists, immunologists, virologists, public health professionals, epidemiologists, and pediatricians. To avoid conflicts of interest, practitioners of this technique are best limited to supplying information rather than setting the standards which govern their own research. This is one of the major problems with the existing NIH recombinant DNA Advisory Committee, and has tended to make the public ill at ease with the adequacy of the guidelines.

The NIH DNA Molecule Program Advisory Committee must be reconstituted if it is to be involved in continued policy formation and standard setting on this issue.

Any promulgation of new guidelines or revision of existing guidelines must be open to wide public input. This could be accomplished by holding hearings not only in Washington, but regionally, preferably in areas such as Cambridge or San Diego. This will allow for new faces with potentially new information based on experience to have input into the process. The present attempt by the existing recombinant DNA Advisory Committee to revise the guidelines without any provision for public input until after the fact is an affront to the concept of public participation. This is a natural and logical place for the process to be opened up.

State and local governments should be encouraged to hold their own hearings and be allowed to promulgate regulations stricter but not more lax than Federal law.

Creative and unprecedented use of the mass media should be encouraged. It is necessary for the public to be fully informed before they can decide.

The following comments will be addressed to the specific bills so far introduced.

S. 945. My remarks will be concerned here only with the commission part of this bill, since it is, in principle, the only part we could support. In general, the bill is well drawn; its mandate is broad. We would make the following suggestions:

Section 202(1)(A). The committee should study the appropriateness and the ethical and scientific principles underlying both research and production using recombinant DNA technology. The industrial exploitation of this new technique poses special, perhaps more serious, problems which have yet to be adequately addressed.

Section 202(1)(B). The committee should also study mechanisms for public education and participation in the decisionmaking process. This seems the logical body to be charged with this responsibility.

The Commission could be empowered to educate the American public to the recombinant DNA issue through organizing and funding of regional and national forums, or by arranging prime-time in-depth media coverage. Creative media strategies should be developed similar perhaps to the Advocate series jointly produced several years ago by WGBH in Boston and KCET in Los Angeles.

I would like to introduce for the record a description of the Advocate series.

S. 621

While we wish to commend Senator Bumpers' foresight and quick action on this important issue, we must reiterate that any regulatory bill is clearly premature, and based on insufficient information. This bill sets up a licensing procedure, but is extremely vague. There are no criteria, no guidelines, no provisions for public input, and no detailed enunciated goals of Federal regulation. We could not support any guidelines promulgated without full public participation. Ninety days is simply not long enough. Nevertheless, we strongly support the concept of full liability without regard to fault, and complete public disclosure. For further comments I will include my testimony before the House Health Subcommittee in which I critiqued more fully the House counterpart, H.R. 3592.

S. 1217

Section 2. Findings. They appear to be more optimistic than the facts would suggest. Recombinent DNA may offer many potential benefits. This is very speculative now.

Subsection 4. Regulation has not always been a very effective way to minimize risk, particularly in numerous, decentralized, difficult to monitor sites, for example, clean air and water provisions.

Section 4. Standards. Who will in actuality be setting these standards, with what mechanisms for public input?

Subsection 1. There is no sound reason for Congress to exempt the NIH guidelines from the National Environmental Policy Act. These regulations will be in effect for at least a year and a half, and exemptions such as this set a dangerous precedent. Furthermore, recombinant DNA is precisely the type of potential danger that NEPA was designed to cover. In fact, this issue, perhaps more than any other, requires extensive thought regarding environmental impact. What kind of testing will be required to insure that these organisms will not disrupt the delicate ecological balance?

We need a NEPA review now. The NIH draft EIS is totally inadequate. If we do not know enough to assess the environmental impact of this technology, then perhaps we should not be moving ahead so quickly.

I would like to introduce into the record two sets of comments on the NIH draft environmental impact statement, one by Marcis Cleveland of the Natural Resources Defense Council, and one by Burke Zimmerman, formerly with the Environmental Defense Fund.

Furthermore, there are no provisions for public participation in the standard setting. This is a logical point to open the process up, and there would be time to implement these procedures.

Section 10(b). It should be made clear in this section that it is the Secretary's duty to permit stricter standards to be in force if the State sets them. His role should be nondiscretionary. He must let stricter regulations stand, and not set up any procedures to discourage the States from exercising this prerogative. Furthermore, is this needed at all, or does it just set up another administrative hurdle for the States?

Section 12. This is a logical point to mandate an all-citizens' advisory committee to consult with the Secretary. The public perspective is otherwise not adequately represented.

Section 16. Appropriate training in the safe handling of recombinant DNA must be mandated for all, from project director to bottle washer.

This law effectively limits both the public and the Congress from having any input into the regulation of recombinant DNA research. It suggests that the Congress leave all discretion in standard-setting in the hands of the Secretary. There are no guidelines regarding what the Secretary should do under sections 4, 5, and 6; that is, no goals he must meet, no minimum standards he must adhere to. Nothing in this act constrains him from promulgating guidelines weaker than what exists now. We feel that the lack of standards in this bill and all proposed so far support our position for immediate moratorium to avoid rushing into premature and inadequate regulation.

Furthermore, to whom will the Secretary delegate his authority? While the NIH and its Recombinant DNA Advisory Committee may seem the logical place, we must not forget our recent experience with the Atomic Energy Commission. Congress must avoid repeating this serious error. One agency cannot promote and adequately regulate.

The bill's apparent attitude toward the public increases our concern over its lack of standards. It has no provision for permitting public participation. Its judicial review is limited to section 4, and then only for 60 days to persons "adversely affected" without defining those words. There are no requirements for rulemaking, for notice-comment procedures, or for hearings on the record. All that is left to the discretion of the Secretary, and again we do not know to whom the actual authority will fall. There is no requirement to follow the Administrative Procedures Act, no guarantee for public participation, no citizen suit provisions, no sunshine provisions, no intervenor funding.

In conclusion, we feel that the vagueness of these bills, and their lack of public participation mechanisms, support our call for an immediate moratorium.

And finally, we have heard quite a bit about the misconceptions of the public today, I would like to have submitted for the record several more pages regarding what we perceive to be some of the serious misconceptions of the scientific community. I will submit them with my written testimony.

Thank you.

Senator KENNEDY. OK. We will be glad to receive those additional inclusions.

Mr. Rifkin.

Mr. RIFKIN. Senator Bumper, in his written statement this morning, said that there were two major issues under consideration. The first is the issue of public safety in recombinant DNA research. The second is the issue of the implications that the recombinant DNA research has for genetic engineering and manipulation of human life.

These hearings today, from what I can gather, and I have been here since around 11 this morning, have spent over 90 percent of the time on the first issue, safety.

I would like to address my remarks to the second issue, because I think it is the predominant issue that should be under consideration for legislation. That is the effect of this research on genetic engineering and manipulation of human life.

With the discovery of DNA and the process of recombination, man has unlocked the mysteries of life itself. It is now only a matter of a handful of years before scientists will be able to irreversibly change the evolutionary wisdom of billions of years with the creation of new plant strains, new animals and new forms of human life.

I believe the precedents are being set right now which could lead all of us inextricably into the kind of "brave new world" that Huxley fantasized some 40 years ago.

Although the biologists prefer not to talk about it, they know what their research can and will inevitably lead to, just as the physicists knew but refused to acknowledge what their research in splitting the atom could and would inevitably lead to. Like the atomic physicists, the biologists are now attempting to hide from the ultimate consequences of their individual experimentations in this field. By reducing their work to specialization on one or another particular genetic process, they pretend not to see the contribution of each and every discovery to the overall process of redesigning the evolutionary order and eventually man as well.

Now I would like to address myself to a specific issue that has come up over and over again.

Many of the biologists that are working in this field maintain that recombinant DNA experimentation——

Senator KENNEDY. Would I assume from what you have said that you would say that the development of nuclear energy has been more of a negative factor, than it has been a plus in terms of our society?

Mr. RIFKIN. I believe if we took a balance sheet from 1942 to 1977 and asked ourselves if we had more benefits than we have had harm as a result of nuclear technology, I would say that any rational person would have to say that it has been more detrimental to the human community on this planet, than it has been beneficial.

And I would like to make one more parallel between the nuclear question and the genetic question of DNA. Many of the scientists are saying that recombinant DNA and genetic engineering, for example, are two different and unrelated issues. This is pure nonsense. They are as related as the splitting of the atom is to the development of a nuclear bomb. In fact, even the House Subcommittee on Science, Research, and Technology has acknowledged that it is impossible to

divorce recombinant DNA experimentation from genetic engineering. They are one and the same.

And for that reason, the House committee report in 1976 is entitled "Genetic Engineering, Human Genetics, and DNA Recombinant Research."

I would like, Senator Kennedy and the staff here, to get a feel for what these scientists are talking about, some of the critics who have not been here to speak, so I would like to give some comments from some of the scientists in the community who are critical to this research.

John Beckwith and some of his colleagues at the Harvard Medical School have decided not to use this technology at all under any circumstances. Their reasons go far beyond the current controversy over whether or not laboratory procedures can be made safe enough to prevent deadly viruses from escaping into the environment, causing illness and death.

Beckwith, who has been doing research in the bacterial genetics for 12 years, argues that recombinant DNA research is leading directly to genetic engineering and the modification of the human species.

In a world riddled with strife, inequities and programed institutional violence, Beckwith asks this question: Can any reasonable person really believe that human genetic engineering will not be used to domesticate entire populations, classes and races, once it is available?

Ethan Signer, a biologist at MIT has echoed Beckwith's concern at the recent National Academy of Science forum.

This research, he said, is going to bring us—this is a direct quote—is going to bring us one more step closer to genetic engineering of people. That is where they figure out how to have us produce children with ideal characteristics.

Last time around, says Signer, the ideal children had blue eyes, blond hair, and Aryan genes.

Signer's concern that recombinant research and genetic engineering could lead to a mass eugenics movement here in this country is based on the writing of many leading proponents of this research. The fact is that top geneticists, working in the area of recombinant DNA, have warned that the human gene pool is deteriorating at such a rapid rate that an aggressive positive eugenic program must now be initiated if we are to upgrade the human race.

I suggest that members of this Senate subcommittee examine the hundreds and hundreds of position papers, symposium transcripts and essays in which leading geneticists and molecular biologists have officially gone on record as favoring the kind of genetic alteration of human life that Hitler envisioned and that Huxley warned of.

These documents can be found here in Washington, D.C., at the Kennedy Institute of Bio-Ethics at Georgetown University.

Senator KENNEDY. I am familiar with them.

Mr. RIFKIN. I have personally looked through the reams of documents in this library. I hope the Senator's staff will as well. I can assure members of this subcommittee that the American public has every reason to be alarmed about the implications of what some of America's leading geneticists are advocating; and that this Kennedy Center of Bio-Ethics can provide some very good documentation.

Senator KENNEDY. It is very interesting that you bring up the Kennedy Center, because one of the reasons for the Kennedy Center,

which was supported by the Foundation, has been to deal with mental retardation, and there are a number of kinds of issues that are related to the retarded. One of them, of course, is the whole question of consent, informed consent. And we have seen that in terms of the sterilization of the Relph[?] girls, the use of D propobara[?] in terms of the mentally retarded down in Tennessee, the whole kind of question about who is going to provide informed consent.

And then there are a lot of ethical kinds of questions that are involved.

But also in terms of mental retardation there is an aspect which relates to the genes.

Mr. RIFKIN. Right.

Senator KENNEDY. The alteration and the change of genes may be one of the causes of mental retardation. So the question is the following: You talk about the modification and you raise quite rightfully, I think, the concern the public would have in terms of behavior and the modification of genes, and the abuses of what that kind of device could take.

There is also the potential, real potential, for some very important kinds of progress in this area, and I think it is entirely appropriate to raise the dangers, the risks, as well as the benefits.

If you remember, the first question I asked Secretary Califano was to give us the evaluation of the risk and benefits, and the first question I asked the head of the NIH was to give us that as well. I think we are being naive not to try and evaluate what the enormity of the risk is as well as understanding the benefits.

And we welcome, obviously, your comments and views, that you make on it.

Then we try in terms of legislation to see, in areas of potential risk and danger, what kinds of insulations and protections can be established. I imagine you would say, well, no matter what you do institutionally, the potential dangers are so great that the power which is going to be behind them to release them are going to be so significant, that it is not going to make much of a difference in terms of institutional kinds of protections.

But, you know, as you mentioned, the Kennedy Center there, which is basically a combination of Yeshiva Medical School and Harvard Divinity School, is concerned with the ethics of these various factors. I mean there are important ethical and moral questions in terms of many of the issues that you relate to here, and certainly in trying to bring at least some commonality of viewpoint on it.

But, as anyone who would be listening through the testimony or do the reading, which obviously you have, it is a troublesome area. But I mean it is also a hopeful area. And these are the challenges which are presented to us.

Mr. RIFKIN. In reference to what you were saying about the benefits versus their problems, there is a thin line of transition between what scientists call negative eugenics and what they call positive eugenics. And negative eugenics is what you referred to, Senator, in terms of defects, diseases that are hereditary, that can be cured, mental retardation, et cetera.

What I am suggesting in my research at the Kennedy Bio-Ethics Center and in talking with some of the people in the field, that there

now is a move from negative eugenics to positive eugenics philosophically within the molecular biology community. And I think this is where we have to seriously consider what is going to happen with this research in the long run.

I would like to take a few minutes to quote some of the leading geneticians in the field, who are doing the breakthrough research. I think it would be helpful to get a little of their philosophy, because a few times this forum has talked about their philosophy.

Senator KENNEDY. You have got about 10 or 12 minutes, and then I must leave. So whichever way you would like to handle it, fine.

Mr. RIFKIN. OK. Fine.

Geneticist James Danielli, director of the center of theoretical biology at the State University of New York at Buffalo, argues that "from the point of view of genetics, man is a barbarian, clad in the trappings of a civilization in which he is ill at ease and barely able to contend."

Danielli said that "most men contribute little to civilization other than maintenance services, if that, and are consumers rather than originators of civilization."

According to Danielli, with human genetics as it is, the possibilities of improving man's condition by improving human institutions and environments, "is a very dubious proposition." Danielli believes that it is now time to "consider other possibilities if civilization is to persist," and he suggests that those other possibilities lie in genetic engineering.

Nobel Prize-winning biologist, Joshua Lederberg, who is one of the undisputed leaders in this field, has gone a step further. In reference to cloning, which we have heard something about, the artificial creation of genetically identical individuals with carefully chosen physical and mental traits, Lederberg says:

> If a superior individual is identified in society, why not copy it directly, rather than suffer all the risks of recombinational disruption, including those of sex? Leave sexual reproduction for experimental purposes.

"When a suitable type, meaning person, is ascertained," Lederberg believes we should "take care to maintain it by clonal propagation."

Even as many of the leading proponents in this field deny any intent of fundamentally altering human life through genetic engineering, it is clear by their unpublicized remarks——

Senator KENNEDY. Now, I am not sure that Lederberg—I know Lederberg very well. I am not sure that he would take—I do not know how those particular phrases are used on it, but I would be interested if he was opposed either to this legislation or opposed to the research on that.

Mr. RIFKIN. Senator, I would be very pleased to provide this committee with many statements by Joshua Lederberg in this regard.

Senator KENNEDY. He is on our panel to review the whole saccharin study. He is a very distinguished person. He is also on our advisory committee, the science advisory committee on the Foundation that I mentioned earlier. So I am very familiar with him.

But I would be surprised—and I will make sure I find out. He is a very, very distinguished researcher and scientist. I know him very well, and I would just be surprised that the utilization of these statements, or the context they should be used in.

But if you have more of Lederberg, I would be interested.

Mr. RIFKIN. I would be glad to provide the committee with hundreds of pages of transcripts of what Joshua Lederberg has said about positive eugenics and moving into that area of cloning.

Senator KENNEDY. Fine.

Mr. RIFKIN. By the way, I think that the fact that he is part of these committees even gives me more question to be disturbed about the nature of his research and what is happening in this area.

Senator KENNEDY. This is just on the saccharin study.

Mr. RIFKIN. Well, let me continue there. Dr. James Bonner of Cal Tech lays out another possible scenario. He says, and this is written in the Ames Aerospace Research Center publication. He says,

> One suggestion has been to remove genetic material from each individual after birth and then promptly sterilize that individual. During the individual's lifetime, records would be kept of accomplishments and characteristics. After the individual's death, a committee decides if the accomplishments are worthy of procreation into other individuals. If so, genetic materials would be removed from the depository and stimulated to clone a new individual. If the committee decides that the genetic material is unworthy of procreation, it is destroyed.

Bonner concludes his remarks with this quotation: "The question indeed is not a moral one, but a temporal one—when do we start?"

The question here is how close are we to the kind of reality that Bonner and Lederberg and Danielli and other prominent scientists are talking about. For those on this subcommittee who hope that such developments, like cloning are far enough off in the future to delay any immediate consideration by this committee, they should heed the prediction of the experts in the field.

Dr. Watson, who is a Nobel Prize winner in his field, says we are within 20 years of cloning the first human on this planet.

Joshua Lederberg, who we mentioned earlier, a distinguished scientist, says that we are within 0 to 15 years of cloning the first individual.

The specter of mass genetic engineering is about to leap out from the pages of science fiction and become a competing reality in this world. Nevertheless, proponents and critics continue to limit this recombinant DNA issue to questions of appropriate safety guidelines over the laboratories and laboratory experimentations. Not one member of the Senate has even mentioned the social, moral and theological implications of genetic engineering in the long term on human life, over the next 25 years. I have not heard it in any of the committee hearings thus far.

If Congress refuses——

Senator KENNEDY. Well, of course—were you here for my opening statement this morning?

Mr. RIFKIN. I came in at 11, and I heard the questions.

Senator KENNEDY. Well, we started at 9:30 on that, and that obviously is the view of others of my colleagues in terms of the protection of human subjects panel, the purpose of which is to include the ethicists, physicians, scientists, researchers, public participation to guide us in terms of a variety of different kinds of health issues that involve those implications, and we are strongly concerned about it, I emphasized and stressed it.

You know—most of your thrust is just some questions about being opposed to genetic engineering, and you say that this recombinant

technology is where it is going to bring us. And then you spell out what your reasons are against that particular kind of question.

But that really is a complex issue—and you just go right into that, you draw those lines very, very clearly, which other researchers, trained researchers, and others have been much more cautious in terms of drawing lines, even those that have reservations about this basic kind of approach. You do not appear to have these similar kinds of reservations.

But I think, quite frankly, both the researchers that were here this morning and others, I know, have been extremely sensitive to the ethical and moral kinds of questions that have been raised. We certainly are. I think the members of this committee are. The administration, I know, has been. Mr. Califano commented on that.

So I would not treat that lightly. I know you place a higher emphasis, a higher priority—a high priority on it, but I do not know whether it is any higher than some of those that support this legislation.

Mr. RIFKIN. Senator, I think what I am trying to get at, and perhaps not as articulately as I should, is that in hindsight, let us go back, for example, to 1942, at the University of Chicago, and let us say we had some Senators and Congress people sitting up here and we had some scientists sitting down here. And we are talking about the whole question of the future of atomic energy.

The scientists were talking on and on about splitting the atom and the technical aspects of that. I would wonder would the Senator who was questioning the scientists, would you in hindsight have asked them about what the implications of splitting the atom would be to the potential of nuclear holocaust, which was only 3 years after they were able to do that, that first act.

What I am saying is that there is a natural progression here, from the beginning of recombinant DNA research, which has been a tremendous breakthrough and has allowed scientists to advance much faster in the area of genetic engineering. There is a natural progression from that to what the New York Times editorial a few weeks ago called the ultimate conclusions of this research.

For that reason, I think that it makes little sense to go ahead now with regulations over this research, but allow it to continue before there has been any public discussion of what the implications of the research are. Because once the research begins, it is going to be difficult, if not impossible, to halt its progression. We saw that in the question of nuclear power.

Senator KENNEDY. Well, of course, the fact of the matter is, in 1942 we were in the middle of a war, and it is an entirely different kind of an issue than we are at a time of peace. And also you have got the track record of the scientific community themselves in terms of the Asilomar Conference. The first time in any kind of research in an important area of endeavor that the scientists themselves were prepared to say stop, look and listen. I mean, you can argue about sort of the makeup of the Asilomar Conference, whether they had enough people, the consumer interest input and all the other kinds of input.

But you cannot get away from, I think, the very kind of responsible effort that was made by the scientific community in this area.

And as one who is attempting to try and insulate the decisionmaking process with, you know, the public and consumer interest in this area, I do think that we do a disservice to the scientific community not to recognize that they are living on the same planet with us, and they have got many of the same concerns that I am sure that you have.

You know, I just am not prepared to quite overgeneralize as far as you might in terms of this particular kind of issue.

Mr. RIFKIN. Let me just conclude my remarks and skip over a lot of this material here.

I would just like to go on record, and I know it will not mean much in terms of the hearing, in going on at this rate.

But if I could go on record, because I think in a few years from now the Congress people here and the Senators in this body are going to look back at their decisions to continue with this research before the American public know about its long-rang implications, they are going to look back on that, I think, with the same sense of concern as they did when they passed the Gulf of Tonkin resolution 12 years ago, which led to the escalation of the war.

Because I believe when the American public begins to know more about the long-range implications of this research on human life itself, that we are going to see a maelstrom of protests in this country in which every American is going to ask of their elected representatives: What was your stand on this issue, and why was it allowed to go ahead before there was any public dialog as to what are the implications?

And I think the pro and con, I believe that all the benefits should be put out so that people can assess both sides and make a critical judgment before we continue. I think, had we done that in the nuclear area, we might be much better off now, had we tried to look at this as a long-range technology with long-range consequences.

So, in conclusion, the Peoples Business Commission is calling for a 4-year moratorium on this research, and during that time we hope that the Congress will set up a commission of lay people, and I know that you, Senator Kennedy, are very interested in getting public involvement in this, a commission made up of lay people, a cross section of the American public be empaneled to commission studies, subpena witnesses, hold hearings, and present all the options of this research, pro and con, to the American public over the next 4-year period, with even the option of having prime-time television, small segments of it to be used to explain this.

Because I think this is the most important issue ever to face the human family. It is a turning point in history.

At the end of that 4-year period, I would hope that this public commission would draw up all of those possible options for a national referendum or at least have them as part of the Presidential election of 1980, so that the American public can put this issue within the context of the over-all political process in which we are functioning.

Now, I know that some Senators and Congress people, and the press, will scoff at this as unrealistic and naive and generalistic and sensational. On the other hand, my own concern as a citizen of this country is that we are on the verge of a mass positive eugenic movement in this society, that is my opinion after looking at this; and second, that the long-range implications of genetic engineering can have ir-

reversible consequences for human life as we know it, which leaves me, third, to believe that 3 or 4 years in the long course of history is not too long a time to take a breath, to hold the research, and to discuss all of the implications involved in it.

I believe that we owe that to ourselves and the rest of life on this planet.

Senator KENNEDY. Well, I want to thank you for your statement. I do not minimize the political dynamite in the issue. I mean, I do not question that. As one who has seen the reluctance to add fluoride in the water in communities in my own State, which I like to believe is an enlightened State, where you get half the kids in Charlestown, Mass., having 13 cavities or more, and you can go out in areas of Brookline or Newton, which have fluoride in their water, and you rarely find a cavity. Let alone the cost and human distress and the lack of treatment for any of those kids, one sees the reluctance of Congress to even come to grips with an optional plan. So I do not question that there may be a red-hot political kind of problem in this issue, I do not question that.

The question is whether what we are trying to do is right; is it the correct kind of question? And I do not underestimate the ability of people to demagog this issue. I certainly want to make it clear that I think your presentation has been extremely responsible, both you and Ms. Lippe, and particularly in the urging of public involvement and discussion. I mean, I cannot myself accept the moratorium, but I think you are stressing the emphasis of it.

I, of course, have been encouraged by the fact that in a pretty typical community, Cambridge, Mass., a really rather typical group, the city council made a judgment to move ahead on allowing the research.

Now, if you know Cambridge—do you know Cambridge?

There are probably not many communities that have a more difficult town-gown relationship and in so many instances there are those forces within the community that are prepared to go after the university. I have seen that many times.

And yet, you know, you have a preliminary almost sort of an advisory opinion from a group of thoughtful citizens that got into this in a very comprehensive way. Not that that is going to or should end or resolve this issue of recombinant DNA; of course it should not.

Maybe if they had had the presentation on the Gulf of Tonkin, they would not have made the decision that we made in the Senate on it.

But, nonetheless, these are tough and difficult questions, and I think you have raised some important factors that we ought to consider.

I want to thank both you and Ms. Lippe for your presentation here.

[The prepared statements of Ms. Lippe and Mr. Rifkin along with other information referred to, and additional material supplied for the record, follows:]

TESTIMONY OF FRIENDS OF THE EARTH
SUBCOMMITTEE ON HEALTH AND SCIENTIFIC RESEARCH
SENATE COMMITTEE ON HUMAN RESOURCES

April 6, 1977

Mr. Chairman, thankyou for the opportunity to testify here today. My name is Pamela Lippe, and I represent Friends of the Earth, a national environmental group with 25,000 members and sister organizations in thirteen foreign nations.

The recombinant DNA technology is acknowledged by many as the most powerful technique ever available to science. Its ease of deployment, increased precision and speed will ensure overnight proliferation in an unfettered and supportive environment. The irreversibility of the consequences, the acknowledged state of ignorance, and Murphy's law caution against this.

Friends of the Earth calls for an immediate moratorium on all research that would produce novel genetic combinations. This will allow for careful and deliberate development of our recombinant DNA policy, based on risk assessment studies and with full public participation and input.

We now have the technological capability to change the very essence of the natural - from microorganism to man. This is long before we are able to adequately assess the long-term consequences of this unprecedented power, long before we can formulate goals for its use, and without serious consideration of the moral and ethical problems that will eventually demand resolution. Public input into this discussion and participation in the decision-making must be given the highest priority if we wish to avoid rapid polarization and an emotional debate which will serve no ones' purposes adequately.

Salvador Luria has said:

> "the progress of science is so rapid that it creates an imbalance between the power it places in the hands of man and the social conditions in which this power is exerted. Then neither warnings of scientists, nor breadth of public information, nor wisdom of citizens may compensate for inadequacies of the institutional framework to cope with new situations."

We have no satisfactory mechanisms for negotiating the conflicts of interest

between the scientific community and the society-at-large. In fact, this may be the most important task before us. Recombinant DNA will certainly not be the last issue of this magnitude that our institutions will have to deal with.

Why must we rush ahead? There are no immediate benefits to be lost. Deferring most of these experiments for a few more years will allow for deliberate and long-range planning, based on risk assessment studies rather than best guesses. This seems like a much more sensible approach than policy-by-patchwork, but it requires time and resources, and a firm decision now before the economic and intellectual investment becomes greater and closes out a variety of options.

We are making premature decisions based on insufficient information without the much needed participation of all responsible and concerned parties. Almost everyone agrees that the public should be involved, but no one knows exactly how this could be accomplished. There will be no easy solution; we need to be bold, original, creative, and constructive. Excuses such as increased cost or bureaucracy, should not be allowed to stand in the way of developing these sorely-needed mechanisms. Friends of the Earth's general recommendations in this regard are:

1. We support, in principle, the creation of a commission to study the recombinant DNA issue in full. Its mandate must be broad and should include a consideration of the social, political, and ethical issues. It should be free to determine whether the recombinant DNA technique should be used at all, and if it is to go on, under what public direction. All available options should be considered including ban, full federalization, or at minimum determining priorities of research to be carried out first - development of alternative host organisms to *E. coli*, or risk assessment studies, for example. Friends of the Earth feels that a moratorium is necessary to ensure that the best course of action is not prematurely foreclosed.

2. The make up of this commission or any advisory committee must be carefully constituted to fairly represent the public and the various scientific concerns potentially affected by this new technology (for example, ecologists, immunologists, virologists, public health professionals, epidemiologists, and pediatricians). To avoid conflicts of interest practitioners of the technique are best limited to supplying information rather than setting the standards which govern their own research. This is one of the major problems with the existing NIH Recombinant DNA Advisory Committee, and has tended to make the public ill at ease with the adequacy of the guidelines.

3. The NIH Recombinant DNA Molecule Program Advisory Committee must be reconstituted if it is to be involved in continued policy formation and standard-setting on this issue.

4. Any promulgation of new guidelines or revision of the existing guidelines must be open to wide public input. This could be accomplished by holding hearings not only in Washington, but regionally, preferably in areas such as Cambridge or San Diego. This will allow for new faces with potentially new information based on experience to have input into the process. The present attempt by the existing Recombinant DNA Advisory Committee to revise the guidelines without any provision for public input until after the fact, is an affront to the concept of public participation. This is a natural and logical place for the process to be opened up.

5. State and local governments should be encouraged to hold their own hearings and be allowed to promulgate regulations stricter but not more lax than federal law.

6. Creative and unprecedented use of the mass media should be encouraged. It is necessary for the public to be fully informed before they can decide.

The following comments will be addressed to the specific bills so far introduced.

S. 945 (Metzenbaum)

My remarks will be concerned only with the commission part of this bill since it is in principle the only part we could support. In general, the bill is well drawn; its mandate is broad. We would make the following suggestions:

Sec 202 (1) (A) The Commission should study the appropriateness and the ethical and scientific principles underlying both research and production using recombinant DNA technology. The industrial exploitation of this new technique poses special, perhaps more serious problems, which have yet to be adequately addressed.

Sec. 202 (1) (B) The Commission should also study mechanisms for public education and participation in the decision-making process. This seems the logical body to be charged with this responsibility.

The Commission could be empowered to educate the American public to the recombinant DNA issue through organizing and funding of regional and national forums, or by arranging prime time in-depth media coverage. Creative media strategies should be developed similar perhaps to the Advocate series jointly produced several years ago by WGBH in Boston and KCET in Los Angeles.

S. 621 (Bumpers)

While we wish to commend Senator Bumper's foresight and quick action on this important issue, we must reiterate that any regulatory bill is clearly premature, and based on insufficient information. This bill sets up a licensing procedure, but is extremely vague. There are no criteria, no guidelines, no provisions for public input, and no detailed enunciated goals of federal regulation. We could not support any guidelines promulgated without full public participation. Ninety

days is simply not long enough. Nevertheless, we strongly support the concept of full liability without regard to fault, and complete public disclosure. For further comments I will include my testimony before the House Health Subcommittee in which I critiqued more fully the House counterpart, H.R. 3592 (Ottinger).

S. 1217 (Kennedy)

Sec. 2 Findings - They appear to be more optimistic than the facts would suggest.

1) recombinant DNA may offer many potential benefits. This is very speculative now.

4) regulation has not always been a very effective way to minimize risk, particularly in numerous, decentralized, difficult to monitor sites, for example clean air and water provisions.

Sec. 3 (b) The Secretary should not be able to exempt with out provision for public review.

Section 4 Standards - Who will in actuality be setting these standards, with what mechanisms for public input?

1) There is no sound reason for Congress to exempt the NIH guidelines from the National Environmental Policy Act. These regulations will be in effect for at least a year and a half, and exemptions such as this set a dangerous precedent. Furthermore, recombinant DNA is precisely the type of potential danger that NEPA was designed to cover. In fact, this issue perhaps more than any other, requires extensive thought regarding environmental impact. What kind of testing will be required to determine whether these organisms will not disrupt the delicate ecological balance?

We need a NEPA review now, the NIH draft EIS is totally inadequate. If we don't know enough to assess the environmental impact of this technology, then perhaps we shouldn't be moving ahead so quickly.

Furthermore, there are no provisions for public participation in the standard setting. This is a logical point to open the process up, and there would

be time to implement these procedures.

Sec. 4 (b) This section needlessly limits judicial review. There should be no bar to standing for challenging any action of the Secretary under this section. Given the special nature of recombinant DNA, the wording "adversely affected" is restrictive when the language ought to be all encompassing. Furthermore, sixty days is totally inadequate in an area with such great uncertainties. It must be longer, and should accrue from when the person learns that he/she may be affected.

Sec. 10 (b) It should be made clear in this section that it is the Secretary's duty to permit stricter standards to be in force if a state sets them. His role should be non-discretionary, he must let stricter regulations stand, and not set up any procedures to discourage the states from exercising this prerogative. Furthermore, is this needed at all, or does it just set up another administrative hurdle for the states? Shouldn't any challenge under this section be in state court?

Sec. 12 This is a logical point to mandate an all citizen's advisory committee to consult with the Secretary. The public perspective is not otherwise adequately represented.

Sec. 13 (b) Are these administratively determined?

Sec. 16 Appropriate training in the safe handling of recombinant DNA must be mandated for all from project director to bottle washer.

This law effectively limits both the public and the Congress from having any input into the regulation of recombinant DNA research. It suggests that the Congress leave all discretion in standard-setting in the hands of the Secretary. There are no guidelines regarding what the Secretary should do under Sections 4, 5, & 6; ie. no goals he must meet, no minimum standards he must adhere to. Nothing in this act constrains him from promulgating guidelines weaker than what exists now. We feel that the lack of standards in this bill and all proposed so far support our position for immediate moratorium to avoid rushing into premature

and inadequate regulation.

Furthermore, to whom will the Secretary delegate his authority? While the NIH and its Recombinant DNA Advisory Committee may seem the logical place, we must not forget our recent experience with the Atomic Energy Commission. Congress must avoid repeating this serious error. One agency can not promote and adequately regulate.

The bill's apparent attitude towards the public increases our concern over its lack of standards. It has no provision permitting public participation. Its judicial review is limited to Section 4, and then only for 60 days to persons "adversely affected" without defining those words. What is the history of this phrase? There are no requirements for rulemaking, for notice-comment procedures, for hearings on the record. All that is left to the discretion of the Secretary, and again we do not know to whom the actual authority will fall. There is no requirement to follow the Administrative Procedures Act, no guarantee for public participation, no citizen suit provisions, no sunshine provisions, no intervenor funding.

In conclusion, we feel that the vagueness of these bills, and their lack of public participation mechanisms support our call for an immediate moratorium.

Many of the scientific proponents of the recombinant DNA technology complain about the misinformed public, but I would like to take this opportunity to address some of the misconceptions of the scientific community which appear to be hindering the beginning of a real dialogue.

The first and most prevalent is the myth of freedom of inquiry. Freedom of inquiry carried out through thought and speech is a protected right, but action is always subject to legal and moral restraints. Experimentation is action. Whatever impinges on the real world, and therefore on the lives of others, is subject to social control. This anachronistic distinction may have been valid in earlier times when the theoretical contemplative sphere was clearly separate from the active experimental sphere, when pure theory did not intervene in anyway with the real world, when knowledge was sought to comprehend and not to change the state of things. That time is long gone.

In the past, two conditions have allowed science to continue on in a relatively free manner; it has dealt with inanimate matter or non-human life, and generally on a small scale. But recombinant DNA cuts to the very basis of human existence, and in fact that of all life, and smallness of scale is something that present legislation will not ensure.

Many of the scientists insist to the public that they are doing pure research, that they are searching for knowledge, and that this is somehow separate from the application of this research. And yet it is difficult to think of any branch of science whose discoveries do not have some technological applicability and a random survey of any of the numerous high technology corporations will prove that the practical benefits of even the most abstruse scientific discoveries are quickly exploited for the market place. The recombinant DNA technology will be no different. As the prospectus of the Cetus Corporation, a company formed to commer-

cialize the "newest frontiers of genetics" points out:

> There remain many other fields to conquer, and Cetus intends to become a major factor in each ... In certain markets where marketing would not represent an overwhelming investment, Cetus might opt to "go it alone", for example, selling and licensing of microorganisms, processes, and/or products outright to various industries.
>
> A tremendous accumulation of scientific knowledge is waiting for the final touches needed for technological utility. The combination of people and processes that constitute Cetus will impel it to emerge as a dominant factor in the new industry of industrial biosynthesis.
>
> The Cetus team brings together experience and understanding of the fundamental problems of molecular biology, of medicine, of instrumentation, of computer technology, and of marketing and business management. 1

There is no such thing as free inquiry in our modern technological world, free, that is, of commericial and developmental pressures. As Dr. Hans Jonas, Professor of Philosophy at the New School for Social Research, has pointed out:

> First, much of science now lives on the intellectual feedback from precisely its technological application. Second, it receives its assignments from there; in what direction to search, what problems to solve. Third, for solving them, and generally for its own advance, it uses advanced technology itself: its physical tools become ever more demanding. In this sense even the purest science has a stake in technology, as technology has in science. Fourth, the cost of those physical tools and their staffing must be underwritten from the outside: the mere economics of the case calls on the public purse or other sponsorship; and this in funding the scientist's project (even with "no strings attached"), naturally does so in the expectation of some future return in the practical sphere. 2

The demands of the American people have not been unreasonable in return for their generous patronage. In fact Commerce Department figures illustrate that from 1970 to 1975, while 55% of all the scientific research and development in this country was payed for by federal funds, only 6% of that resulted in usable invention, and only 5% of that 6% actually got on to the market.[3] That is very little value for the money invested, and yet it is the Commerce Department and not the American people who are complaining about this poor return on investment. The concern over recombinant DNA is not a case of the public dictating immediate

But the most patronizing argument is that the public's concern is merely irrational fear of the unknown. We should not confuse caution with fear; it is rational to be wary of the unknown. The developing controversy over DNA research is an expression of a deep public concern over significant disagreement within the scientific community. We face an unprecedented potential danger, and an enormous amount of relevant data is simply not known. How quickly we forget our lack of foresight and understanding regarding nuclear energy, DDT, flourocarbons, PCBs, PBBs, asbestos, thalidomide, the list could go on.

If the debate is to begin in earnest, we must move beyond these diversionary arguments. Free inquiry is an outdated concept. The distinction between pure and applied research no longer clearly exists. The public has a right to exercise control and there is well founded disagreement on which to logically base their concern. After all, the scientists themselves declared a voluntary moratorium not so long ago, an action unprecedented in modern science.

The people will not be ignored on this issue, and many others in the coming years. Science and technology touch our lives in countless ways, many of which we are beginning to learn are harmful. It is these effects, and people's inability to protect themselves that are resulting in what is being decried as an anti-science backlash. It is important to note here that is is the scientists that are bringing this upon themselves. The self-serving insistence by many that their craft is inately right and good in the face of compelling evidence to the contrary does more disservice to the integrity of science than an army of public interest groups could ever do. Contrary to popular opinion, public interest advocates are not anti-science or even anti-technology. We favor science and technology that serve human needs. It is with this goal in mind that the debate should begin.

REFERENCES

1. Prospectus of the Cetus Corporation

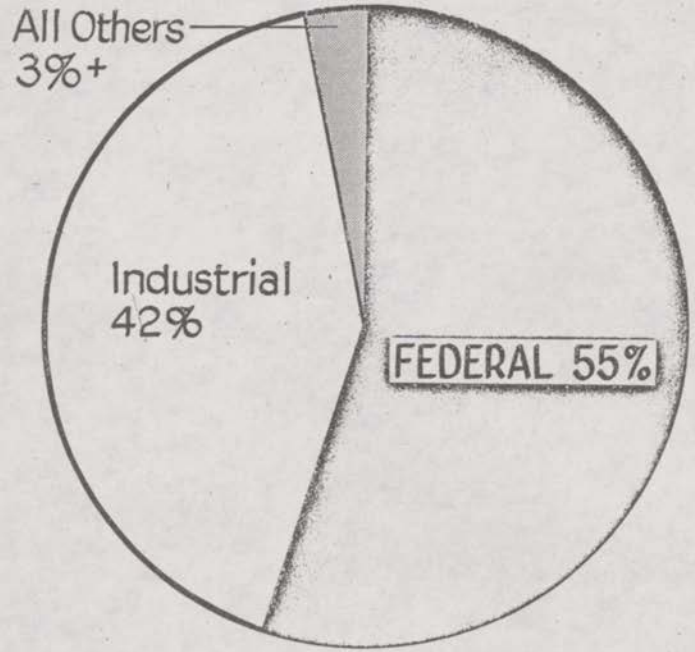
2. Jonas, Hans, The Accountability of Science As An Agent of Social Action, Freedom of Scientific Inquiry and the Public Interest, Hastings Center Report, Institute of Society, Ethics and Life Sciences, August 1976.

3. Commerce Department figures, graphs introduced into the record.

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RESEARCH & DEVELOPMENT EXPENDITURES

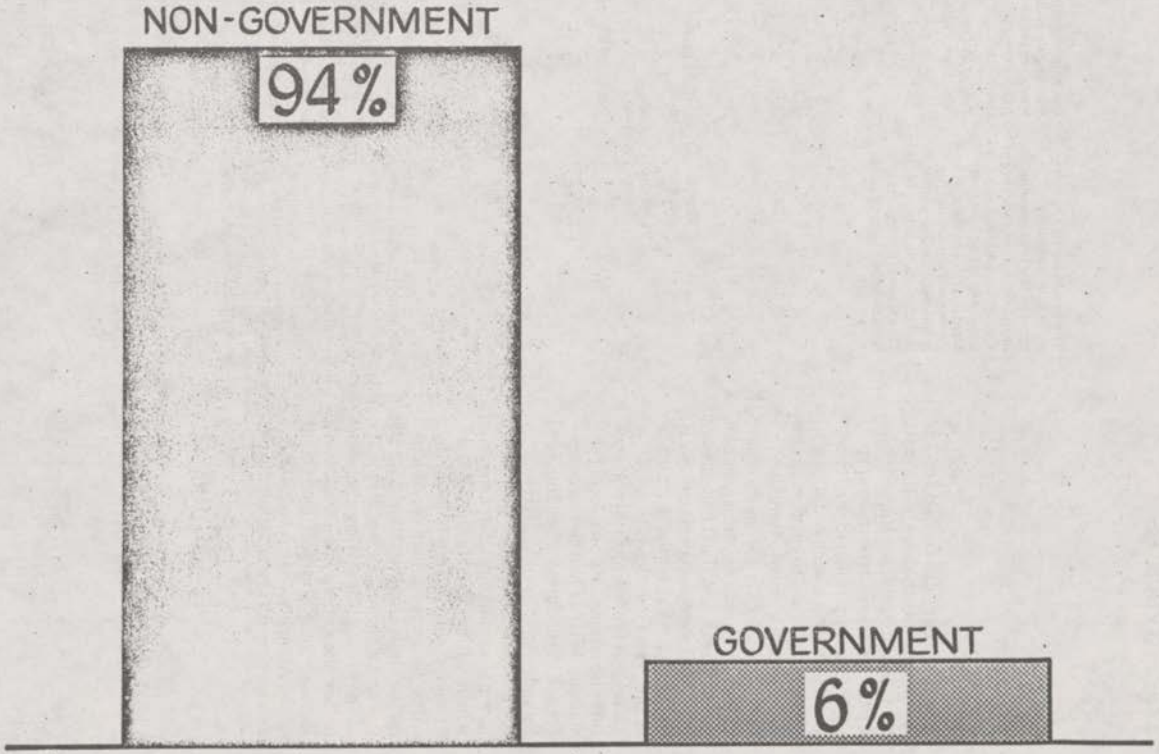
(Constant 1972 Dollars)



AVERAGE EXPENDITURES 1970-1975

YEAR	TOTAL $BILLIONS	FEDERAL $BILLIONS	FED'L%
1970	28.5	16.2	55.7
1971	27.9	15.6	55.9
1972	28.4	15.9	54.9
1973	28.7	15.5	54.0
1974	27.8	14.5	52.2
1975	27.9	14.7	52.6

RELATIVE DISTRIBUTION OF U.S. PATENTS DERIVED FROM GOVERNMENT & NON-GOVERNMENT EXPENDITURES 1970-75



The Advocates

The courtroom-like format for *The Advocates* was the idea of Harvard Law School Professor Roger Fisher: a pair of skilled advocates represented the opposing sides of the week's question; each could call expert witnesses to support his own position while cross-examining those called by his opponent. Months before there was any prospect of a national series, WGBH had already invited Fisher to experiment with the basic format notion in a number of programs broadcast locally. Thanks to the lessons learned by this allocation of its own local resources, WGBH had the basic approach in readiness when the Ford Foundation offered WGBH and KCET the grant support for a new national public affairs series – which, with a few months' further development, became *The Advocates*. Throughout its five seasons on the air, it was unmatched for its fair and rigorous presentation of the great public policy choices before the nation. The series won a Peabody, an Emmy, a Silver Gavel of the American Bar Association, and much critical respect.

The key value of *The Advocates* is in its ability to present the best possible arguments for either side of a question and then to have those arguments severely scrutinized. The dogmatic viewer is likely to find his opinions becoming a little less black, a little less white. This viewer, for one, has found himself tuning in on one side of a question and winding up either somewhere near the middle or flip-flopping to the other side completely.

John J. O'Connor
New York Times

The best public affairs program on television.

Clarence Peterson
Chicago Tribune

What makes *The Advocates* so appealing is the bloodlust. As delivered by the syndicated columnists in your evening newspaper, opinions just sit there solemnly, each inside its separate pillbox. As delivered on late-night talk shows, opinions are invariably disemboweled by the jokes or the obsequiousness of the host... As delivered on TV news specials, opinion is a veritable Vishnu of on-the-other-hands, all of them grasping after the FCC's fairness doctrine. *The Advocates* allows – indeed obliges – the opinionated to sink their fangs into each other's rhetorical fat.

Cyclops
Life

Taken from the WGBH Declaration of Independence
The Case for Programming Independence

TESTIMONY OF FRIENDS OF THE EARTH BEFORE THE SUBCOMMITTEE ON HEALTH AND ENVIRONMENT, COMMITTEE ON INTERSTATE AND FOREIGN COMMERCE HOUSE OF REPRESENTATIVES

MARCH 16, 1977

Mr. Chairman, my name is Pamela Lippe, and I am representing Friends of the Earth, a national environmental organization with 25,000 members and sister organizations in thirteen foreign nations. I want to thank you for the opportunity to testify here today and will open my remarks by reading the position statement endorsed by Friends of the Earth concerning recombinant DNA research.

Scientists, working toward the advancement of knowledge, have not concerned themselves in a substantial way with analyzing or evaluating the impact that their discoveries might have on society. Indeed, society as a whole has tended to acquiesce in the assumption that any advancement of scientific knowledge is a basis for progress, a potential source of future blessings of humanity. In spite of the pervasive effects of the atomic bomb and the increasing domination of so many aspects of life by technology, the assumption has been preserved by biologists who, to receive immediate public approval, have had to cite only the general relevance of their work to medicine.

For some years now, however, some scientific research has been creating products, information, and techniques having an impact that may be more dangerous than beneficial (fluorocarbon-induced ozone depletion, radioactivity, dioxin, DDT, PCB, vinyl chloride, photochemical smog, Mirex, etc.). In particular, genetic and molecular biological research is rapidly providing the basis for the practice of genetic engineering. The continuation of this research without public understanding and approval, and in fact without a full comprehension of its potential by most of the involved scientists, poses a worldwide danger which is intensified by the fact that industrial investment in the developing genetic technology has already begun.

Molecular biologists have now learned how fragments of DNA (the master hereditary chemical of all living cells) can be recombined or reassembled to produce new entities, "recombinant DNA's", which have the ability to enter cells and reproduce themselves therein. This type of agent has the theoretical capacity to alter fundamental biological processes in the biosphere. A primary

danger is that the deliberate production of "desirable" changes in certain organisms might result in the disruption of the infinitely complex and delicate balance among living things, a balance which has evolved slowly in nature. A sudden disturbance of natural relationships could precipitate a disastrous and irreversible breakdown of our ecosystem. More immediately, current research itself involves serious hazards which inadvertently could result in the spread of cancer or the creation of new diseases. In addition, the possibility of applying genetic engineering to the evolution of the human species raises fundamental ethical questions which need to be brought out into the open.

Scientists involved in this research are not unaware of its dangers, and indeed were the first to call attention to them. But, caught up in the intellectual fascination of their work, they have engaged in only a limited debate. A few ill-defined public benefits of questionable feasibility have been proffered to justify the hazards, and containment guidelines have been formulated in an effort to minimize accidents; but the more profound social questions have been ignored by all but a handful of scientists.

It is time to end the isolation of scientists from society, before the technique of genetic engineering has become a fait accompli. Scientists need to be made aware of what they are doing, in the broadest sense, and -- perhaps even more important -- the public must be awakened to the broad powers which have been left in the hands of scientists and industrialists who are unqualified or reluctant to judge their import, because of suspected impact upon investment (e.g. the SST, aerosol propellants, taconite tailings, Kepone, etc.). There is little or no precedent for an organized consideration of the ends of scientific research, of any type, with respect to social and ethical values.

Friends of the Earth calls for an immediate, moratorium on all research that would produce novel genetic combinations.

This pause should extend over industrial and military (biological warfare) research as well as academic research whether government-supported or privately-funded. Such a pause would allow time to pursue the following objectives:

1) ORGANIZED INTERNATIONAL CONSIDERATION OF THE ENDS OF THIS RESEARCH to examine carefully their profound ethical and social implications.
2) ASSESSMENT OF DANGERS before intellectual and economic investment in the development of this technology grows greater.
3) ESTABLISHMENT AND IMPLEMENTATION OF DEMOCRATIC PROCEDURES that will insure open discussion and public decision on the problems posed by gene implantation research.
4) DEVELOPMENT OF ALTERNATIVE TECHNOLOGIES for medical research that do not involve the manufacture of novel organisms.

The moratorium should continue until at least these four objectives have been met.

We have reviewed the three bills under consideration today and wish to praise the concern and foresight of Representatives Ottinger, Solarz, and Rogers and their quick action on this important issue. We agree that the problems presented by uncontrolled recombinant DNA research require immediate remedy, and are happy to see that the Congress has taken an early initiative to protect the health and safety of the American people.

The position of Friends of the Earth, as outlined above is a complete moratorium. While these bills are a commendable attempt to address some of the more disturbing aspects of the problem, their vagueness and imprecision, we believe, lend weight to our position. We simply do not know enough -- to assess the risks, to prepare for the benefits, or to regulate what promised to be the "new frontier" in bio-medical research and industrial application.

We need a moratorium to allow for calm and reasoned discussion and planning. This issue is one of enormous complexity and unprecedented importance. To make premature decisions based on insufficient information, without the full participation of all concerned and responsible parties will only lead to further controversy and increased polarization. We can avoid the mistakes of the past and set a clear precedent for the future through our handling of this issue. Recombinant DNA will certainly not be the last question of this magnitude that our institutions will have to deal with. Deliberate and long range planning seems a much more sensible approach than policy by patchwork. But this requires time and resources, and a firm decision now.

Why must we rush ahead? There are no immediate benefits to be lost. Deferring these experiments for a few more years can in no way be construed as a tragic mistake. Instead we should look at it as an unparalleled opportunity to develop mechanisms of negotiation between the scientific community and the society-at-large.

Mechanisms for arbitrating these conflicts of interest are sorely needed. The rapid growth of the public interest community in the last few years is one response to the problem. But no one, not even the public interest advocates themselves, are very satisfied with the courses of action available to them. It's always too little, too late. Providing for public education on the issue and then mechanisms for public input into the decison-making and policy formation will insure a well-informed public more amenable to whatever the final resolution may be.

Our recommendations in this regard would be:

1. Full open and public hearings held in Washington and around the country by the Secretary of HEW or whatever agency is given final authority to regulate, conducted <u>before</u> guidelines are promulgated, and open to all

who wish to testify.

2. That these hearings be publicized and carried by the mass media in their entirety. It is necessary for the public to be fully informed before they can decide.

3. That state and local governments be encouraged to hold their own hearings and be allowed to promulgate regulations stricter but not more lax than federal laws.

4. That any commission enjoined to study and evaluate the scope, consequences, or continuation of genetic research have over half its membership drawn from the non-scientific public.

Friends of the Earth can not support any of the bills so far introduced, but upon review we offer the following comments.

H.R. 4232 (Solarz)

In principle, we support the creation of a citizen commission to study the recombinant DNA issue, but with a much broader mandate than H.R. 4232. In particular, the commission should determine whether the research is needed at all, explore alternative means to the same ends, and consider the ethical, social, and political implications of going ahead with this new technology. We feel that a moratorium would be necessary to avoid prejudicial influences.

1. Members should be able to commit enough time to carry out their duties effectively. Government officials, such as senators and representatives would not have the time.

2. No money is authorized for the commission to operate.

3. There is no order to study alternative methods of research, regulation control, or oversight.

4. No provision for staff - only consultants.

5. The goal of the commission is not worked out. Why is it being set up? What does Congress want from it? Its mandate is much too vague.

6. No provision for public input.

7. Time limit of the Commission is too short.

8. There is no determination of how to proceed in the interim. What if the commission determines that the research should be stopped?

H.R. 3592 (Ottinger)

This bill sets up a licensing procedure, but also suffers from vagueness. There are no criteria, no guidelines, no detailed enunciated goals of federal regulation. We could not support any guidelines promulgated without full public participation. Ninety days is not long enough. We support the concept of full liability without regard to fault.

1. Sec. 4 - Will there be any Congressional oversight?

2. Sec. 5 - Why are there any patents allowed at all?

3. There is no guarantee of public procedure.

4. There are no enforcement provisions except threat of license suspension.

5. "Person" is not defined

6. The bill does not address steps beyond research.

7. Does this law impact the Federal role? How does this affect NIH?

8. "Significant hazard" is not defined.

9. If a significant hazard is found, why must activity be enjoined in court? Licenses could be administratively revoked, of course, judicial review must be guaranteed.

10. We strongly support full public disclosure.

H.R. 4759 (Rogers)

In general, this appears to be a better drafted bill. But again there are no provisions for public participation in the decision-making and regulation formation. We strongly support the concept of allowing state and local regulation stricter than federal guidelines, but do not feel that this right should be contingent on decision by the secretary.

In conclusion, while praising the attempts of Representatives Ottinger, Solarz, and Rogers to address the grave dangers posed by uncontrolled recombinant DNA research, we feel that it is clearly premature to attempt to regulate this new-born and ill-defined technology. We urge an immediate moratorium to allow for a slow and careful consideration of the problems posed by recombinant DNA research with full public input and participation. We would like to return to the original mandate to the NIH committee which was:

1. investigate the current state of knowledge and technology regarding DNA recombinants, their survival in nature, and transferability to other organisms;
2. to recommend programs of research to assess the possibility of spread of specific DNA recombinants and the possible hazards to public health and the environment;
3. to recommend guidelines on the basis of research results.

COMMENTS ON THE DRAFT ENVIRONMENTAL IMPACT STATEMENT FOR THE NIH GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT DNA MOLECULES

Submitted to the Director of NIH, 18 October, 1976

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The Draft Environmental Impact Statement for the NIH Guidelines for Research Involving Recombinant DNA Molecules discusses, among other topics, both the potential hazards and benefits of recombinant DNA research per se, the environmental impact of recombinant DNA research performed as specified in the Guidelines, and alternatives to the issuance of the Guidelines by NIH.

On page four of the introduction to the impact statement, it is stated, "In issuing the Guidelines, the NIH Director pointed out that they will be subject to continuous review and modification in the light of changing circumstances." The implication of this statement is that the Guidelines are the best we can do on the basis of existing knowledge, and that various parts of the Guidelines may be either overly strict or not sufficiently stringent to protect the public health and the environment from any untoward effects resulting from recombinant DNA research.

The impact statement then goes on to develop the argument that, in the absence of data, one must assume that recombinant DNA research poses a wide spectrum of potential risks to human health and the environment in general. It is further argued that if work is performed as described in the NIH Guidelines, there will be

little chance of a virus or organism being created which is rendered potentially harmful by the addition of recombinant DNA to its genome, and that if such a harmful agent should arise, the probability of the escape of such an organism or virus into the environment, either directly or by the infection of laboratory personnel, is extremely low. The statement on p. 64, "Any potential release of high-risk materials to the environment should be prevented by adherence to the NIH Guidelines," indeed suggests that the Guidelines have adequately taken into account all possible ways in which any potential biological hazard arising from recombinant DNA research could enter the environment, and that there is no further need to improve them. This conclusion would, thus, appear to be at odds with the statement in the introduction quoted above. It is possible that this apparent inconsistency reflects a measure of uncertainty on the part of the authors of the impact statement. Nevertheless, most of the impact statement expresses a great deal of confidence in the Guidelines and it is the presumed basis for this confidence that will be the subject of much of this discussion.

The conclusion of the impact statement, that the Guidelines, if observed, do indeed afford sufficient safety to the environment, is necessarily based upon three implicit assumptions. If any of these assumptions is not justified, then it follows that neither is the belief that the Guidelines will provide the measure of safety attributed to them in the impact statement. These assumptions are:

(1) That the Recombinant DNA Molecule Program Advisory Committee, which drafted the Guidelines, and the authors of the impact statement have accurately imagined or anticipated all possible kinds of potential hazardous situations resulting from recombinant DNA research or technology. While there is clearly insufficient data to accurately estimate the probability of occurrence of any untoward event, it is essential that the nature of the organisms or virus with a potential for environmental harm or disease be understood, in order that appropriate safety and containment procedures may be designed.

(2) All parties engaged in recombinant DNA research or technology will respect the NIH Guidelines and will comply with the safety precautions outlined therein, whether or not they are bound by law or regulation to do so.

(3) The safety procedures outlined in the Guidelines, including the use of biological containment, will always be faithfully executed. That is, it is assumed that there will be no serious human errors committed, equipment failures or accidents which would negate their presumed effectiveness.

I would dispute the validity of each of the above assumptions. It is, at best, naive and idealistic to assume that we are wise enough to be able to imagine all of the manifestations of the infinite variety of possible genetic chimeras, when we don't even understand much of the genetic function in existing organisms. At worst, it is preumptuous and arrogant. In no case, can it be justified. And it is equally naive and idealistic to assume that

all who contemplate doing recombinant DNA research will respect and comply with the Guidelines, as it is to assume that serious mistakes will never be made, or that accidents will not happen or that equipment will not fail.

In challenging the validity of these underlying assumptions, I do not wish to imply that the Guidelines, if strictly adhered to, do not afford a considerable degree of containment of agents containing recombinant DNA molecules. I would, however, conclude that the authors of the Draft Environmental Impact Statement, by not being sufficiently imaginative and by not showing sufficient respect for the unknown, have underestimated the nature and magnitude of some of the potential hazards. I would further conclude that through naivity and an idealism which assumes only the best in people, the effectiveness of the NIH Guidelines in reducing the potential risk to the public and the environment has been overestimated.

I would now like to analyze each of these assumptions in detail.

Assumption (1) Those drafting the guidelines and the impact statement were able to correctly imagine the nature and complete scope fo the potential hazards of recombinant DNA research.

The Guidelines consider all of the anticipated types of recombinant DNA experiments and assign a risk to each, dependent on the presence of "harmful genes" (never adequately defined in the Guidelines), the assumption that "the potential dangers are considered to increase as the organisms providing the source DNA approaches humans phylogenetically"although many have questioned

why this must be so, and the relative risk posed by either the host or the donor alone. A discussion of the principal fallacies of assumption (1) follows:

(a) One might well ask just what is meant by "harmful." The question was raised by commentators during and after the open meeting in February, 1976, of the Advisory Committee to the Director of NIH, at which time the working version of the Guidelines was made available to the public. A list of questions, based on public comment, was presented to the recombinant DNA Advisory Committee meeting on April 1 - 2, in which the Guidelines were given final revision. This list included consideration of the definition of "containing harmful genes." The Committee, however, spent much more time worrying about the definition of "contain" rather than "harmful" and the latter term remains in the Guidelines without a sufficiently broad definition.

It is clear that "harmful" cannot be restricted to the effect a gene may have on human health solely within the context of its original organism. The Guidelines mention specifically the obvious cases of genes coding for a toxin or a drug resistance factor, or perhaps even allow that some may be involved in the cause of virally induced cancer.

However, if expressed out of its normal context, one may argue that <u>any</u> gene has the potential for being "harmful." For example:

(1) If a gene, carried by a viral vector, coding for a normal metabolic enzyme, were to enter and be expressed in

human cells, there is a good chance that it would not be subject to the host's regulatory mechanism. Thus there is a real risk of completely disrupting some normal metabolic pathway, similar to instances of inborn metabolic diseases where the defect appears to be in a control gene (or enzyme, such as ATPase), resulting in a serious pathologic state.

(2) There is a chance that a piece of "foreign" DNA, even a relatively small piece, could exert a control function in the host cell, also leading to the disruption of a metabolic pathway.

(3) There is the risk that any foreign protein, even if it has no enzymatic function, could, if expressed in or on the surface of mammalian cells in sufficient quantity, elicit an immune response from the host, leading to the destruction of the cells, as in known auto immune diseases following certain bacterial infections.

(4) The genetic basis of virulence in viruses or bacteria is not understood. Why certain viruses infect some hosts readily, but only to a benign degree in closely related hosts is not known. There is not yet any evidence that SV 40 virus and polyoma virus produce any pathologic symptoms in humans, although they infect many animals and produce tumors in certain hosts. SV 40 virus has been found occasionally in patients suffering a variety of serious diseases, inluding cancer, but no causal relationship has been established. Papova virus is a human pathogen and is similar to SV 40 in many ways. Just how subtle a genetic alteration

would be required for SV 40 to acquire the capacity to produce cancer in humans? Perhaps this could occur by the acquisition of a piece of DNA not normally considered "harmful" in the donor organism, but specifying control signals common to many organisms. We have the example of influenza virus, which undergoes a natural reassortment of its RNA containing subunits[1/] so as to produce strains which can cause effects ranging from a case of the sniffles to a world-wide epidemic. The genetic determinants of why influenza virus covers such a wide spectrum of virulence are unknown. Thus we really have absolutely no information to enable us to predict the effect upon the infectivity, virulence or host specificity of adding any DNA to a viral genome.

(5) A similar argument can be extended to bacteria. The assumption is made in the Guidelines that the addition of almost any functional piece of "foreign" DNA will adversely affect its capacity to survive in its normal host. The extent to which that assumption is or is not valid is not known, with so little data to go on. Again, any gene in the context of a new organism must be considered to possess the potential for being "harmful," since the production of toxic or harmful gene products are by no means the only ways in which recombinant DNA containing bacteria could exert pathologic effects.

1/ B. D. Davis, et al., Microbiology, 1318 (2nd Ed., 1973).

It should be noted here that the above five examples of how a gene might be considered "harmful" were given little, if any, attention in either the Guidelines or the impact statement. It would seem that in so expanding the definition of "harmful gene," the probability of untoward effects occurring might be somewhat greater than that assumed by the Recombinant DNA Advisory Committee or the authors of the impact statement.

(b) The decision to go ahead with the approval of E. coli as the predominant host for recombinant DNA experiments on bacteria, ignores the fact that E. coli, in addition to being a common and possibly beneficial denizen of the human colon, is also a human pathogen. Although it is correctly asserted that E. coli K-12 does not normally colonize the human colon, it is incorrect to infer that this property renders it harmless to humans. The primary vectors for inserting "foreign" DNA into strains of E. coli K-12 are plasmid DNA's. The following points, most of which received little or no discussion in either the Guidelines or the impact statement would argue that the use of E. coli as a recipient for recombinant DNA is inherently riskier than either the NIH guidelines or the impact statement would lead us to believe:

(1) E. coli K-12 can colonize the bowels of a small fraction of individuals, usually already debilitated by some other pathological condition. In such individuals, recombinant DNA containing

bacteria could multiply in large numbers and exchange plasmids with other more ubiquitous strains of E. coli.

(2) Genetic exchange of plasmids in E. coli is well known, both between strains of E. coli as well as with Shigella and Salmonella. Thus, while a K-12 strain containing a recombinant DNA plasmid, even one constructed so as to greatly reduce as chances of survival in nature (EK2 or EK3), could donate its plasmid to one of the approximately 150 serotypes of E. coli found in the gastrointestinal tracts of most humans, most of which are capable of accepting plasmids. Survival per se is not the issue. Genetic exchange is.

(3) E. coli has, over the past eight years, emerged as one of the primary causes of gastroenteritis and diarrhea[1/2/3/] The disease can be produced by any of several strains of E. coli containing a plasmid which carries the genes for one of two toxins (or both), one of which resembles cholera toxin, plus the genes for a surface factor required for colonization of the small bowel, also carried by a plasmid.

(4) E. coli is responsible for nearly 100% of upper urinary tract infections, including severe cases where the kidneys are involved, and most cases of cystitis.[3/] This type of infection is independent of the capacity of E. coli to colonize the human gut.

1/ R. W. Ryder, et al., New England J. Med., 295, 859-853 (1976).

2/ R. B. Sack, New England J. Med., 295, 893-894 (1976).

3/ B. D. Davis, et al., Microbiology, 768 (2nd Ed., 1973).

(5) E. coli is the etiologic agent for 30 - 40% of the cases of sepsis[1/] (often fatal). This is also independent of the ability of E. Coli to colonize the gut.

It has been argued, in comments received by the Director of NIH during and after the February, 1976, meeting of the Advisory Committee to the Director of NIH, that E. coli, because it is ubiquitous in nature and resides in and infects humans in a variety of ways, is an inherently poor choice as a safe bacterial host for recombinant DNA research. The fact that it is already a human pathogen would argue that modifications which could possibly alter its infectivity or virulence should be avoided. Yet there is no guarantee that the recombinant DNA's which are now being added to E. coli via plasmids or bacteriophage will not alter the infectious properities of E. coli, the human pathogen. I consider this to be a much more serious potential threat to human health than the possibility of developing a strain of E. coli which produces a specific toxic gene product in quantity in the human colon.

The drafters of the Guidelines considered the latter possibility, and strict adherence to the Guidelines should make the possibility of E. coli producing a toxin from a foreign donor highly unlikely, simply by avoiding the use of DNA donors containing such genes. But E. coli as a human pathogen was not considered adequately, hence the impact statement underestimates the full spectrum of untoward events which could result from genetic modifications of E. coli.

1/ Dr. Halsted Holman-- Oral Testimony before a hearing of the Subcommittee on Health of the Senate Committee on Labor and Public Welfare, Sept. 22, 1976.

(c) The possibility of the totally unexpected was not considered. That an organism containing chimeric DNA could possess properties exhbited by neither the host or the organism providing the source of the recombinant DNA has not been considered. Yet, on page 27 of the impact statement, in discussing potential benefits of recombinant DNA research, it is stated, "It is important to stress that the most significant results of this work, as with any truly innovative endeavor, are likely to arise in unexpected ways and will almost certainly not follow a predictable path." This is indeed a fitting way to describe an endeavor as novel as recombinant DNA research -- one cannot predict what will happen. Why, then should not a nearly identical statement be made concerning the potential hazards? Are we so wise that we can accurately imagine all of the possible undesirable effects but cannot, at the same time, see the constructive uses of this area of research? I submit that the statement quoted from page 27 is one of the more insightful appearing in the impact statement and, because of the unknown nature of the range of properties possible in recombinant DNA containing organisms, we must be as agnostic about the hazards as we are about the benefits.

Our experience with atomic energy should give us an example of just how reliable our wisdom was in predicting the future in 1941. When the decision was made to proceed with the Manhattan project, very little was known about the biological

effects of radiation, particularly low level effects. DNA had just been discovered; the mechanism of mutation was unknown. The designers of The Bomb knew that $E = mc^2$, but did not anticipate radioactive fallout from the atmosphere as more than a trivial problem. "Allowable" exposures to radiation were many times higher than the current 5 rem/year, itself under much attack for being too high. No one imagined, nor cared, that plutonium would turn out to be a potent carcinogen. In short, all of the hazards, the undersirable effects and "environmental impacts" of the governmental action to develop atomic energy have been much worse than anyone imagined in 1941. The benefits (calling The Bomb a "benefit" is questionable), including nuclear power were fairly well anticipated in 1941. Thus this case seems to be the opposite of the sentiments expressed in the recombinant DNA impact statement. Just as the eminent authorities of antiquity insisted that the world was flat, the concept of a spherical earth being beyond their wildest dreams, we run the danger of being too smug, or too presumptuous. We must not be led into the trap of believing that we know more than we actually do about any manifestation of recombinant DNA research and technology. Yet the impact statement, while admitting that "neither the benefits or the risks can be precisely identified or assessed" implies that we know enough of the potential hazards so that "strict adherence" to the Guidelines should render "harmful effects from research with high risk recombinant DNA molecules . . . extremely unlikely." (page 63). I would contend that

in any situation as unprecedented and novel as this, one could not possibly justify a statement as strong as the preceding one. A number of important issues are discussed above, which received little or no attention in either the Guidelines or the impact statement, demonstrating that the assumption that the full range of possible hazards had been adequately considered is false.

Assumption (2). All parties engaged in Recombinant DNA research and technology will respect and comply with the NIH Guidelines.

Clearly, in order for the impact on the environment of recombinant DNA research conducted under the NIH Guidelines to be as it is stated in the impact statement, it is a minimum requirement that all such research be subject to the Guidelines. While Dr. Fredrickson, the Director of NIH, has called upon all government agencies, private institutions and industry to voluntarily adopt the Guidelines, only the National Science Foundation has thus far given any formal commitment. The pharmaceutical industry has, in principal, agreed that the safety measures outlined in the Guidelines are appropriate, but seemed unwilling to disclose all of its activities to the public -- or competitors -- in the interests of the presumed right to secrecy of private companies. The implications of this attitude would seem to be rather serious if one assumes, as do I and a large segment of the scientific community, that any form of research and technology should proceed only with the informed consent of the public. This is especially true if there exists even a remote possibility of undesirable

effects on even a small segment of the environment on the public, not to mention the potential for a global disaster. But the presumed need or right of private industry to protect its marketing and patent priorities by secrecy is in conflict with the spirit of the Guidelines, in which all projects, at the very least, require an impartial peer review as well as scrutiny by an institutional biohazards committee.

The hearings held by the Senate Subcommittee on Health of the Committee on Labor and Public Welfare, held on 22 Sept., 1973, illustrated, to the concern of many present, including the committee chairman, Senator Edward Kennedy, that willing compliance with the NIH Guidelines would probably not be forthcoming. The failure of General Electric to send a representative to the hearing was disturbing to many. General Electric is known to be using recombinant DNA techniques to develop petroleum degrading bacteria, and to add genes for cellulase to E. coli as a means of providing a new source of carbohydrate.

There is an irony here which disturbs those of us who have been contending with the questionable integrity and competence of private research and development in industry. The basic research on recombinant DNA supported by NIH and NSF is probably the most competent one will find in this country. The motivation and goals of such research are the acquisition

of knowledge, specifically, the understanding of genetic mechanisms. Although there are able scientists who have expressed disdain for the Guidelines, which they consider overly stringent, it is fairly certain that there will be sincere compliance with them by scientists supported by NIH or NSF.

However, it is my contention that the motives for undertaking a particular line of research and development in an industrial lab should properly be questioned by the public, for they may well have bearing on the way the work is to be carried out. In general, a drug company develops a pharmaceutical, or a product to sell it to the public and to make a profit. There is considerable pressure to get it to a marketable stage as soon as possible -- the fear that a competitor will get there first or the belief that the sooner a product is sold, the sooner will profits be realized. While someone engaged in basic research can afford to spend the extra time and effort necessary to conduct recombinant DNA experiments according to the Guidelines, there is little question that it would be much faster to conduct a particular set of experiments without having to worry about stringent safety precautions. It could be very tempting indeed for a company to be less careful than the Guidelines require, if it meant obtaining a patent potentially worth millions of dollars before a competitor.

It was also brought out before the Health Subcommittee that NIH did not even know all of the companies engaged in recombinant DNA technology. The Department of Agriculture had not responded to Fredrickson's query at that time. And is it not also possible that some of the many investigators supported by funds other than those from NIH or NSF at universities or other research institutions, including a small fraction supported from personal funds, will choose not to conform to the Guidelines? One scientist told me personally that he thought the Guidelines were ridiculous -- that he believed the unfettered freedom of inquiry and the acquisition knowledge far outweighed the importance of subjecting the public to even potentially serious risks to its collective health. It would be extraordinarily idealistic to assume that all will be as conscientious, responsible and respectful of the Guidelines as assumed by those within NIH, who believe so much in the inherent good in man to expect voluntary compliance with the Guidelines from all quarters.

This tone of optimistic idealism which runs through the impact statement was also expressed to me in a letter of 17 March, 1976, from Dr. Maxine Singer of NIH, who is of the opinion that I am incorrect in questioning the motives and the effect they may have upon the performance and integrity of those in private industry. However, the published record of the testing of prescription drugs for both

efficacy and safety is one of the worst examples of scientific research to be found anywhere. And there are volumes of testimony given in various governmental hearings which support the contention that lying, doctoring data, and a variety of unsavory practices have been common in the pharmaceutical industry. What is the basis for Dr. Singer's optimism? It would seem that if the potential risks are even no greater than those expressed in the Guidelines on the impact statement, one cannot rely simply on goodwill, the presumed pressure from one's peers, scientific journals or the concern over liability.

In order for the environmental impact of recombinant DNA research under the Guidelines to have been realistically assessed, it is at least necessary that all parties comply with the Guidelines. The Senate hearing on 22 September and experience with the drug companies over the years argues against that assumption. As Dr. Singer stated in her letter of 17 March, "However, the absence of sanctions will encourage sloppiness on the part of even well-meaning individuals and irresponsible behavior on the part of some others."

Thus, while the impact statement mentions extension of the Guidelines by law, it is implicit that voluntary compliance is to be expected. Yet, it would appear that at this time, some six weeks after the impact statement was released, that this likelihood is a very unrealistic expectation. Therefore,

since the estimated impact depends upon "strict adherence to the NIH guidelines" (page 63), it cannot be expected that the estimated environmental impact of recombinant DNA research performed with the guidelines in effect is at all realistic.

The foregoing analysis would argue that extension of the Guidelines to all parties by Federal regulation on law may be the only way maximum compliance may be expected. But Dr. Singer further points out "that neither a legislative nor non-legislative approach can assure absolute compliance from those who purposefully flout the Guidelines." A truly comprehensive impact statement should have indeed considered the environmental consequences of those who purposefully flout the Guidelines, as well as the possible deliberate misuse of recombinant DNA techniques. The impact statement on pages 31-32 only states that an analysis of such unknown quantities is "hypothetical and difficult." Yet the entire contents of the impact statement dealing with potential risks and benefits is equally speculative, a situation which did not prevent conclusions from being drawn concerning the effectiveness of the Guidelines. Not only is this inconsistent, but, at a time of considerable international tension, especially among smaller nations, it is not out of the question at all to suppose that the relatively easy and inexpensive development of possible biological weapons through recombinant DNA technology might well be preferred over the development of atomic weapons as a route to presumed power. The impact statement avoids consideration of such questions entirely.

Also essential for the effectiveness of the Guidelines is world-wide compliance. The more serious potential hazards of recombinant DNA research, if they become realities, will not recognize international boundaries. The environmental consequences of unregulated recombinant DNA research taking place outside the United States were not analyzed in the impact statement, as they properly should have been.

Assumption (3) The safety procedures outlined in the Guidelines, including the use of biological containment, will always be faithfully executed.

This important issue is to be distinguished from willful flouting of the Guidelines or either individual or institutional non-compliance. The question is whether or not the degree of error-free performance required for the Guidelines to be as effective as the impact statement implies is a realistic expectation.

There are many requirements which must be met, both by the personnel carrying out recombinant DNA research and the facilities in which such work is performed. From my own personal experience of fourteen years of laboratory research, it is apparent that human nature contains enough weaknesses to render meaningless any assumptions that errors will not be made, and not be made often. It is also well known that equipment failures are relatively common. When human or mechanical failure results only

in the loss of time or materials, or invalidates an experiment, the lack of perfection is tolerable. But when there are potentially grave global consequences of even one error, then there are grounds for concern. The impact statement makes no attempt to assess the "impact" of human and mechanical failing with respect to recombinant DNA research under the NIH Guidelines.

Among the difficulties is the fact that potentially hazardous biological materials or infectious agents are insidious. Their presence is known only by the proper label and a thorough knowledge of what has taken place in a laboratory. There is no impingement of any such agents upon any of the five senses. Hence, as with other invisible, silent and odorless hazards, it is easy for personnel to underestimate the importance of careful handling of such material.

Our experience with the use of radioactive materials in the laboratory, a situation supposedly under strict government regulation, shows us how many abuses occur due to the lack of thorough training in safety procedures or a lack of understanding of the nature of the hazard by personnel at all levels. Any realistic assessment of the causes of unnecessary radiation exposure in the laboratory must recognize the encouragement of carelessness because hazardous radioactive materials do not make their presence known.

But the situation is somewhat different in the case of organisms containing recombinant DNA. One error allowing the

creation of, infection of personnel by, or escape from the laboratory of a harmful organism containing recombinant DNA could have a world-wide and irreversible effect. Radioactivity is diluted in the environment; an organism capable of survival and reproduction could multiply and spread.

Is it to be assumed that a lab worker will never use a culture of an EK-1 strain of E. coli K-12, when the Guidelines specify an EK-2 strain? Or is it guaranteed that a culture flask of cells containing recombinant DNA will never be spilled or dropped? Will tubes break in the ultra-centrifuge? Or an autoclave fail and not kill potentially hazardous organisms before disposal? Similar errors and mishaps occur under the best of conditions with the most competent and best-trained personnel. There is no reason to assume that they will not happen with recombinant DNA experiments. The impact statement should properly have considered the consequences of human and mechanical failure. This it has not done. Instead, conclusions are drawn on the basis of qualified statements expressing the ideal: "...under standardized procedures employing stable, well-trained and well-disciplined personnel" (pg. 62). To assume this level of perfection in all cases is unrealistic. Therefore, to conclude, as the impact statement does, that adherence to the Guidelines will essentially guarantee public safety, must also be considered unrealistic.

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The foregoing discussion challenges the basic assumptions underlying the arguments and conclusions drawn in the impact statement, both regarding the nature and scope of potential hazards, and the effectiveness of the Guidelines in dealing with these possible risks. There are many grounds upon which one may assert that the analysis of environmental impacts set forth in the impact statement is incomplete, naive, idealistic, and overly optimistic. I would, therefore, recommend that the points raised above be thoroughly considered in preparing the final version of the environmental impact statement.

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COMMENTS OF THE

NATURAL RESOURCES DEFENSE COUNCIL, INC.

ON

THE NATIONAL INSTITUTES OF HEALTH GUIDELINES

FOR RESEARCH INVOLVING RECOMBINANT DNA MOLECULES

AND

THE DRAFT ENVIRONMENTAL IMPACT STATEMENT ON

RECOMBINANT DNA RESEARCH GUIDELINES

Dated: November 1, 1976

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I. INTRODUCTION

In these comments the Natural Resources Defense Council discusses both the National Institutes of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules (hereafter "the Guidelines") and the Draft Environmental Impact Statement on the Guidelines (hereafter the draft EIS). The comments on these two documents are combined because the issues raised overlap. Although the draft EIS and the Guidelines are discussed in separate sections the entire comment should be considered applicable to both.

The Natural Resources Defense Council, Inc. (NRDC) is a not-for-profit, tax-exempt corporation organized under the laws of the State of New York, with offices at 15 West 44th Street, New York, New York, 917 15th Street, N.W., Washington, D.C, and 2345 Yale Street, Palo Alto, California. NRDC is a national organization dedicated to protecting human health and the environment. Among the methods NRDC uses to achieve its objectives are: (1) monitoring the activities of federal and state governmental agencies to insure that health and environmental values are fully considered in decision-making; (2) improving agency decision-making which affects public health and the environment by submitting written comments on agency programs, participating in administrative proceedings, and filing lawsuits where legal duties are not being fulfilled; and

(3) providing information and assistance --both legal and technical -- to individuals and organizations interested in making governmental agencies more responsive to health and environmental values. NRDC has 24,000 members in the United States. These members and their families will be exposed to the hazards of recombinant DNA activities.

The NIH Guidelines grew out of the concern of scientists engaged in recombinant DNA research that their research might, if not properly controlled, cause irreversible harm to human health and the environment. In July of 1974, a committee of the National Academy of Scientists, chaired by Paul Berg of Stanford University,called for a voluntary moratorium on all recombinant activities. The committee also proposed an international meeting of scientists to develop guidelines for the safe conduct of such research. That meeting, known as the Asilomar Conference, was sponsored by the National Academy of Sciences with the support of NIH and was held in February of 1975. Immediately after the Asilomar Conference the NIH Advisory Committee on Recombinant DNA,which had been formed in October of 1974, met to develop guidelines based on the recommendations of the Asilomar Conference. After meeting three more times, this advisory committee submitted proposed Guidelines to the Director of NIH in December of 1975. In February of 1976, the Director called a special meeting of the Advisory Committee to the Director to review the proposed Guidelines. The public was allowed to comment and participate

at the meeting. After revisions in response to the comments and meeting, the final Guidelines were released on June 23, 1976 with the Director's opinion; and the voluntary moratorium on recombinant DNA research ended. The draft EIS was released September 2, 1976.

II. THE DRAFT ENVIRONMENTAL IMPACT STATEMENT

A. Summary of Comments and Recommendations on the Draft EIS

Nothing in this draft EIS explains the magnitude of the environmental issues raised by recombinant DNA research, which many scientists in the field have likened to the issues raised by nuclear technology. Both the scope and detail of this draft EIS are woefully inadequate. It falls far short of being a document on which NIH, other government agencies and society as a whole can make basic decisions on whether and under what conditions recombinant DNA technology should be developed and used.

The draft EIS as a whole conveys a false sense of security. By ignoring the impact of recombinant activities which are not supported by NIH and further ignoring the possibility that the Guidelines may not be adequate or followed in practice, the draft EIS gives the impression that the hazards of recombinant DNA are now under control and no further action is necessary. The environmental impacts of the Guidelines and

the alternatives must be discussed much more specifically. The hazards of experiments now being conducted or planned for the near future must be described in detail. The hazards foreseen by critics of the Guidelines should also be discussed. The draft EIS does not discuss the impacts of recombinant DNA as a major new technology which is just beginning. There is no analysis of the long range consequences of proceeding with recombinant research.

The draft EIS was issued after the final Guidelines were promulgated. It therefore does not accomplish the primary purpose of the impact statement process which is to assure that environmental values and alternatives are fully considered in the agency's decision-making. The discussion of alternatives is so inadequate it seems clear that the draft EIS is merely a post hoc justification of the Guidelines.

A new draft impact statement should be prepared and, when the impact statement process has been completed, the NIH Guidelines and alternatives to them should be considered de novo.

B. NIH Violated NEPA by Not Preparing a Final Impact Statement in Time to Accompany the Proposed Guidelines Through the Decision-Making Process.

Section 102(2)(C) of the National Environmental Policy Act (42 U.S.C. §4332(2)(C)) requires that an environmental impact

statement be prepared in time to accompany the proposal for federal action through the agency's review procedures. The U.S. Supreme Court in Aberdeen and Rockfish Railroad v. SCRAP, 422 U.S. 289(1975) interpreted this to mean that a final environmental impact statement must be prepared at the time the agency publishes a proposal on which it plans to hold hearings so that the impact statement can be considered at the hearing. According to this rule, a final EIS should have been prepared when the Director submitted the proposed Guidelines to his advisory committee for review at the February 1976 meeting. Contrary to what NEPA requires, NIH did not publish this draft EIS until two months after the final Guidelines were issued.

In its introductory statement concerning the draft EIS, NIH excuses this delay on the grounds that the Guidelines had to be promulgated before the draft EIS was ready because of the urgent need to protect the public from the hazards of recombinant research and because the decision-making process which preceded publication of the Guidelines was the functional equivalent of the environmental review required by NEPA. The claim that the Guidelines had to be published promptly to protect the public is not accurate. Until the Guidelines were published a voluntary moratorium was in effect and if anything the public was better protected then, than it is now. Publication of the Guidelines could have awaited completion of the impact statement process. Furthermore, NIH had ample time to prepare a draft and final

EIS while the Guidelines were being drafted; drafting began in February of 1975 and was completed in February of 1976.

NIH is not exempt from strict compliance with the procedural requirements of §102(2)(C) of the National Environmental Policy Act. The process of drafting and reviewing the Guidelines does not bring NIH within the narrow exception for agency actions based on review procedures which substantially comply with NEPA. This exception has been defined in a number of cases challenging actions taken by the Environmental Protection Administration under statutes designed to protect the environment. Environmental Defense Fund v. EPA, 489 F2d 427 (D.C. Cir. 1973); Portland Cement Ass'n v. Ruckleshaus, 486 F2d 375 (D.C. Cir. 1973). The exception has only been applied to EPA, because it is an environmental protection agency, when it is acting to protect the environment and its action was preceded by full environmental review including public participation equivalent to what is required under NEPA. NIH is not an environmental protection agency; its purpose is to promote scientific research. Whatever environmental review was conducted as the Guidelines were being drafted would have been colored by NIH's primary purpose of promoting research. Without the environmental impact statement, we cannot be sure that the environmental impacts of the Guidelines and alternatives were properly considered. Also NIH did not provide an adequate opportunity for public participation. Under the procedures followed by NIH, the general public had no opportunity to participate in the promulgation of the

Guidelines until the proposed Guidelines were released for comment. NEPA requires an opportunity for public and agency comment on a draft EIS before a formal proposal is made. Also the public has never had an opportunity to comment on alternatives to the Guidelines, because there was no EIS available when the proposed Guidelines were published.

C. The Draft EIS Does Not Adequately Describe The Proposed Action (Section V, Draft EIS)

The description of the federal action which is the subject of this draft impact statement is incomplete. Although section V describes the purpose and specific provisions of the Guidelines in detail, it does not describe the extent and nature of recombinant research being conducted now or planned for the near future. NIH should not limit the scope of the impact statement by defining its action narrowly. NIH's action on recombinant DNA activities includes awarding grants which promote the development of this new technology and the decision not to regulate non-NIH-supported research.

The award of grants for recombinant DNA research is clearly major federal action which must be discussed in an impact statement. The Guidelines have little meaning or impact by themselves. Their significance comes from the fact that they permit NIH-supported recombinant research to proceed if the Guidelines are followed. The description of the federal action must therefore describe the recombinant research now being conducted with NIH funds and research for which grant applications are now pending.

When NIH promulgated the Guidelines with the approval of the Secretary of Health, Education and Welfare, it decided not to regulate non-NIH-supported research, transportation or

commercial use of recombinant DNA techniques, even though the Secretary has the authority under section 361 of the Public Health Services Act (42 U.S.C. §264) to regulate all recombinant DNA activities. This decision not to regulate may well have more impact on the environment than issuance of the Guidelines because the environmental damage the Guidelines seek to prevent may well be caused by researchers at corporations, private-institutions or other federal agencies. Therefore the exclusion of non-NIH research from the Guidelines is a crucial part of the federal action which should be the subject of this impact statement.

NIH has or can gather the information it needs to describe the extent and nature of the recombinant DNA activities not covered by the Guidelines. In his opinion which accompanied the release of the Guidelines, Dr. Donald Frederickson stated that he had met with representatives of other federal agencies and industry to discuss their recombinant DNA activities. Partly as a result of these meetings, the Pharmaceutical Manufacturers Association has formed a committee to review the possibility of voluntary adoption of the Guidelines by the drug industry. At least two private corporations are known to be investigating commercial uses of recombinant techniques. General Electric is trying to develop a bacteria which could consume crude petroleum and be used to clean up oil spills. Imperial Chemical Industries (ICI) is investigating the use of recombinant techniques

to produce a bacteria which could produce insulin inexpensively (Janice Crossland, "Hands on the Code," Environment 18:6 (September, 1976)). NIH should also consult with international organizations to determine which recombinant DNA activities conducted by foreign countries may be subject to regulation by the United States.

D. The Adverse Environmental Impacts of Promulgating The Guidelines Are Not Adequately Described (Section VII, Draft EIS)

1. The Impact of Uncontrolled Recombinant DNA Activities

The impact of experiments which are not governed by the Guidelines are not discussed at all. Because these experiments are not subject to any safety procedures or prohibitions against especially dangerous experiments, they pose a much greater risk to the public health and environment than NIH-supported research. It is very likely that some of these uncontrolled experiments will result in the intentional or accidental release of organisms with recombinant DNA molecules into the environment. If those organisms survive and multiply, they could cause widespread disease and irreversible damage to the environment. Even one release of such organisms could have a substantial impact on the environment.

Section IV-C-1 of the draft EIS, which is entitled "Description of Issues Raised by Recombinant DNA Research:

Possible Hazardous Situations," provides some information about the possible hazards of recombinant research but is not the detailed discussion of impacts required by NEPA. This section describes in a theoretical way the effects that foreign genetic material may have on the host cell, or organisms the host cell comes in contact with, but does not describe specific adverse consequences. For example, this section states that foreign genetic material might improve or impair the survival ability of the host, but does not describe the kind of disease or environmental damage which might be caused by increasing the survival ability of micro-organisms which are commonly used as hosts in recombinant research.

The Final EIS should discuss in detail the biohazards which NIH foresaw and sought to guard against in promulgating the Guidelines. NIH considers some experiments so dangerous that they should not be conducted at all. The prohibited experiments are: 1) experiments with certain pathogenic organisms or oncogenic viruses; 2) deliberate creation of recombinant DNA containing genes for the biosynthesis of toxins; 3) experiments which increase the virulence or range of plant pathogens; 4) deliberate release of organisms with recombinant DNA into the environment; and 5) experiments which pass antibiotic resistence to non-resistent microorganisms. The danger of each of these experiments to humans and the environment should be fully explained.

The Guidelines also assume that experiments with Escherichia coli pose a particular hazard to humans because E.coli normally inhabits the human intestine. The Guidelines specify detailed biological containment requirements for experiments with E.coli host-vector systems. Although laboratory strains of E.coli differ from those found in humans, they may be able to colonize in the human gut or pass their genetic material to the strains which are found in humans. Also some strains of E.coli are pathogenic. Experiments with laboratory E.coli might create recombinant DNA which when passed to pathogenic strains increases their virulence or range. The specific hazards of E. coli, and other micro-organisms, which are commonly used as host-vector systems in recombinant DNA experiments must be discussed in the final EIS.

The Guidelines also assume that experiments which take genetic material from primates are more dangerous than those which take it from lower organisms. Generally, higher levels of biological containment are required for experiments with DNA from higher organisms. The final EIS must explain the reasons for this assumption and spell out the specific dangers NIH foresees from experiments with primate DNA.

The final EIS should also discuss the specific hazards foreseen by critics of the Guidelines. To assess the views of

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critics, NIH should consider both the comments that have been filed on the Guidelines and the published literature on the subject. NRDC attaches two articles which describe some of the risks foreseen by these critics.

Finally, in discussing the impact of uncontrolled recombinant DNA activities, the final EIS should discuss the extent to which the transportation of organisms with recombinant DNA molecules is currently regulated. Section II-C of the Guidelines states that transportation of recombinant materials is governed by regulations of the Public Health Service, the Department of Transportation, and the Civil Aeronautics Board. The final EIS should describe these regulations and point out the gaps in their coverage which might result in accidental release of these micro-organisms into the environment. For example, the PHS regulations (42 C.F.R. §72.25) apply only to etiologic agents listed in the regulation. If a micro-organism which is not listed were used as a host-vector in a recombinant experiment, it would not be subject to the transportation regulations. The hazards of transporting recombinant materials should be fully discussed.

2. Impact of the Guidelines and Experiments Conducted Under the Guidelines (Sections VII-A and VII-B Of the Draft EIS)

In considering the impact of the Guidelines and experiments conducted under the Guidelines, NIH makes two crucial

assumptions which must be discussed in the final EIS. Those assumptions are:

1. That all NIH-supported research will be conducted in accordance with NIH Guidelines.

2. That the Guidelines adequately protect the public from the hazards of recombinant research.

NRDC does not agree with either of these assumptions. It is unrealistic to expect that all NIH grantees will voluntarily comply with the Guidelines fully, all of the time. Furthermore, we do not believe that the enforcement mechanisms of the Guidelines are adequate to insure a high level of compliance. Experiments conducted in violation of the Guidelines will pose the same hazards as experiments which are not covered by the Guidelines. Even a few non-complying experiments pose a risk to public health and the environment, because even one release of an organism with recombinant DNA could, if it survives and multiplies, cause spreading and unstoppable harm. The final EIS must evaluate the likelihood that the Guidelines will be followed and the risk to the public from non-complying experiments.

NRDC does not believe that we know enough about the hazards of recombinant DNA to assume that the Guidelines, if followed, will adequately protect the public. These inadequacies of the Guidelines are discussed in detail in Parts III-B and III-C of these comments.

The draft EIS's discussion of impacts does not inform the public, in terms a layman can readily understand, of harm which may result from recombinant DNA research. The section on impacts merely describes the theoretical/mathematical bases for determining the probability that a particular hazard will occur. The discussion lists all of the events which must occur if an organism with recombinant DNA is to cause disease or environmental damage and states that the probability of harm occurring is the product of the probabilities of each listed event. But the draft EIS never goes on to describe specific damage which may be done to humans or the environment.

The discussion of impacts states repeatedly that all of the hazards of recombinant DNA research are very speculative. The mere fact that impacts are speculative does not excuse NIH from discussing them in detail. As outlined above NIH can describe the hazards it sought to guard against in promulgating the Guidelines, and discuss hazards foreseen by critics of the Guidelines, to give the public a clear picture of the real impact of this new technology.

E. The Discussion of Alternatives is Inadequate (Section VI, draft EIS)

The discussion of alternatives is very sketchy. It reads like an after-the-fact justification of the Guidelines.

So little detail is given about each alternative that it seems very unlikely that any alternatives to the Guidelines were seriously considered or will be considered in the future. The draft EIS makes no attempt to do cost/benefit analyses or weigh alternatives to determine which is best.

The alternatives themselves are barely described. For example, the description of federal regulation does not consider the types of regulation which could be adopted such as a total moratorium on research or different safety standards. Possible systems of enforcement are not discussed. The agencies which might be able to regulate recombinant research are listed, but the differences in their statutory authority are not discussed. For example, the Occupational Safety and Health Administration is mentioned as an agency which could regulate recombinant research, but the discussion does not mention that OSHA can regulate laboratory conditions but not transportation of recombinant materials. Regulation by each agency, the President, and Congress must be discussed separately so that the public and other government officials are fully informed of the options for regulating recombinant DNA.

The environmental impacts of the alternatives are not discussed in any detail. For example, the discussion of the alternative of providing no NIH support for recombinant DNA research, describes the impact this would have on American

research in this field,but doesn't mention the environment. There is no discussion of whether this would protect the public health and the environment better than the Guidelines.

Several reasonable and important alternatives are not discussed at all. The possibility of a moratorium on all recombinant research is not discussed. Such a moratorium could be passed by Congress or by executive order governing federal agencies and federal regulation governing industry and other private parties. The alternatives of promulgating the Guidelines or similar safety standards as regulations binding on all parties conducting recombinant research is not discussed,even though the Department of HEW has the authority to promulgate such regulations. Section 361 of the Public Health Services Act (42 U.S.C. §264) gives the Secretary of HEW the authority to promulgate regulations to protect the public from communicable diseases. The draft EIS repeatedly recognizes that recombinant DNA activities may create or increase the virulence of infectious agents, making regulation under §361 appropriate.

F. The Draft EIS Does Not Discuss or Evaluate Any of the Long-Range Impacts of Recombinant DNA Technology

By funding recombinant DNA research NIH is helping to launch a major new technology whose impact on man's environment

will be as widespread and profound as the impact of nuclear technology. Many scientists in the field have likened the splicing together of DNA from different organisms to the splitting of the atom. Yet the Draft EIS makes no attempt to discuss or analyze these impacts.

Section 102(2)(C) of NEPA explicitly requires the impact statement to discuss

> "(iv) the relationship between short-term uses of man's environment and the maintenance and enhancement of long-term productivity, and
>
> (v) any irreversible and irretrievable commitments of resources which would be involved in the proposed action should it be implemented."

The final EIS must contain a major section which discusses long-range impacts and conducts the analysis required by (iv) and (v) of §102(2)(C).

III. COMMENTS ON THE NIH GUIDELINES

A. Summary of Comments and Recommendations On The Guidelines

The Guidelines should be designed to prevent any release of recombinant organisms into the environment. All recombinant research should be conducted in P3 and P4 laboratories, since NIH recognizes that P1 and P2 laboratories are not designed to prevent accidental release. The Guidelines' system for classifying experiments on the basis of risk should be simplified. We do not now know enough about the hazards of recombinant DNA to determine which of thirteen containment systems a particular experiment should be assigned to. No more than three categories of hazard should be used. Finally, the enforcement provisions of the current Guidelines are weak. HEW should adopt revised Guidelines, which are regulations binding on all parties conducting recombinant research and which use a licensing system of enforcement.

B. The Guidelines Should Be Designed to Prevent Any Release of Organisms Containing Recombinant DNA Into the Environment (Section II of the Guidelines)

At present we do not know enough about the behavior of the novel organisms which will be created by recombinant DNA techniques to predict the effects they will have on human health or the environment. However, the scientists who first called for a moratorium, abided by it and participated in the Asilomar

Conference all assumed that some serious and widespread harm might result from the use of recombinant DNA technology. Since it is impossible to predict with any accuracy which organisms are hazardous, all organisms with recombinant DNA must be presumed to pose a risk to humans and the environment if they escape from the laboratory.

The role of the Guidelines must be to prevent any release of recombinant DNA. Although the Guidelines prohibit the intentional release of recombinant organisms, they assume that a certain amount of accidental release can be expected in P1 and P2 facilities. The draft EIS, in describing the protection provided by P1 and P2 facilities, states:

> "These measures do not provide absolute protection from exposures and the required primary barriers can be compromised by lack of attention to technique, poor placement of equipment and human error." 41 Fed. Reg. 38436.

The draft EIS goes on to explain that NIH assumes that such exposure will not harm the laboratory worker because only organisms which have been rated low or moderate-risk can be handled in P1 and P2 laboratories. There is no scientific basis for assuming that organisms which are rated low-risk by the NIH classification system will in fact prove harmless.

Since P1 and P2 facilities are not designed to prevent all accidental release of recombinant organisms, recombinant

research should be restricted to P3 and P4 facilities. If NIH believes that any recombinant organism is too hazardous to be released into the environment intentionally, then it should not permit research in facilities where accidental release is inevitable.

C. The System of Classifying Experiments On The Basis of Risk is Too Elaborate to be Workable (Section III of the Guidelines)

Under the NIH Guidelines, a proposed recombinant DNA experiment must be assigned to one of thirteen different containment levels depending on the hazard it poses to humans. It must be assigned one of four physical containment levels and one of three biological containment levels or it may be prohibited. We do not know enough about the hazards of recombinant DNA to make these finely tuned evaluations of risk. Since recombinant DNA molecules are placed in living host cells, which have the potential of surviving and multiplying in the environment, even one experiment, which is mistakenly considered harmless, could cause widespread, irreversible damage. Therefore, the classification system should be designed to protect us against our ignorance and recognize that our estimates of risk of different experiments are very crude.

The draft EIS repeatedly states that we cannot predict the effects of any particular genetic recombination.

> "Current knowledge does not permit accurate assessment of whether such changes will be advantageous, deterimental or neutral and to what degree, when considering a particular recombinant DNA. At present, it is only possible to speculate on ways in which the presense of recombinant DNA in a cell or virus could bring about these effects."
> 41 Fed. Reg. 38429

The draft EIS also lists thirteen events which must occur for a particular organism with recombinant DNA to cause harm. (41 Fed. Reg. 38438). The probability that such harm will occur cannot be calculated unless the probability of each of the thirteen events can be calculated. Such precise determinations cannot now be made, but a classification system with thirteen containment levels requires almost this level of precision if it is going to genuinely protect the environment from harmful organisms.

Since the likelihood of harm cannot be calculated with precision, the Guidelines provide for the ranking of experiments on the basis of untested theories. These theories may be inaccurate or not comprehensive enough to be used as the basis for predicting risk.

The central assumption underlying the classification system of the Guidelines is that host cells with foreign DNA from higher organisms are more hazardous than those with DNA from lower organisms. Therefore, more containment is required for experiments which take DNA from primates than those which

take DNA from birds or plants. But it is difficult to see how philogenic order or any other single factor can accurately predict risk when so many different factors (thirteen, according to the draft EIS) determine the likelihood that a particular organism will cause harm. It is possible to imagine an experiment with primate DNA which is less hazardous than one with prokaryote DNA. For example, an experiment which put primate DNA into E. coli might produce an organism with such a low probability of survival and such a low probability that the primate DNA would be fully expressed and create a primate protein, that it poses virtually no risk to humans. Another experiment in which DNA from a prokaryote was inserted in E. coli might improve the survival ability of E. coli, be transmitted to a pathogenic strain of E. coli, and make it more virulent, thereby substantially increasing the risk of disease in humans. Whatever single factor is picked to rank hazards, it will always be possible to make a credible argument that some experiment which is rated low-risk is in fact more risky than something which was rated higher-risk.

Some of the assumptions underlying the classification system are contradictory. In some situations higher containment levels are required because we know almost nothing about the hazards; in other situations, stricter containment is required because we have concrete information about the hazards. For

example, one of the reasons for requiring a high level of containment for experiments with primate DNA is that we know so little about such recombinations. On the other hand, the reason for prohibiting experiments which would transfer antibiotic resistance to non-resistent species is that we know such transfers will certainly impair our ability to cure human disease. Similarly, special provision is made in the Guidelines for experiments using E. coli host-vector systems because we know that E. coli colonizes in the human intestine. But other prokaryotic host-vectors, which do not colonize in humans, may prove equally harmful to the environment in ways we cannot now foresee.

The elaborate classification system of the current Guidelines should be abandoned in favor of a much simpler system. NRDC suggests that the Guidelines at most have three classifications for experiments: prohibited, very hazardous and hazardous. Such a classification system would provide some significant protection from all recombinant DNA experiments and thereby protect us from our ignorance about the consequences of this research. Using no more than three categories also frankly recognizes the imprecision of our judgments about risk.

D. The Enforcement Provisions of the Guidelines Are Inadequate (Section IV of the Guidelines)

The mechanisms for enforcing the Guidelines are not adequate to insure the high level of compliance which is necessary to protect the public from the hazards of recombinant DNA. The Guidelines rely primarily on the grantees to police themselves; this reliance is unrealistic.

Primary responsibility for enforcement rests with the principal investigator and the biohazards committees. The principal investigator is responsible for evaluating the biohazards of the experiments, training staff and insuring that safety procedures are followed. The biohazards committee of the institution must certify to the NIH staff that the experiment and facility comply with the Guidelines. The NIH staff determines the proper containment level for individual experiments and evaluates compliance with the Guidelines primarily on the basis of documents submitted by the grantee. The NIH staff is responsible for some on-site inspection of facilities and epidemiological monitoring of workers, but it is not clear how thoroughly it will be able to do either. The staff of NIH is also required to make a number of detailed factual determinations: assigning containment levels, approving applications for lower containment levels, approving host-vector systems for biological containment and approving large scale experiments. The only sanction for non-compliance is revocation or denial of a grant.

NIH grantees cannot be expected to police themselves effectively. They will always have a stong competitive interest in pushing research ahead and,when their desire to complete research conflicts with adherence to the Guidelines, safety procedures will be bent. The competitive pressures on NIH grantees will be particularly great if non-NIH researchers are not subject to any safety procedures.

Under the Guidelines the demands on the time of NIH staff will be so great that they will not be able to monitor effectively the compliance of facilities with physical containment requirements or monitor the health of laboratory workers,which unfortunately is the only available means of checking for "leaks" of recombinant DNA molecules. Because of the number of complex factual determinations the staff is required to make, virtually all of their time is likely to be spent reviewing and deciding applications. The task of monitoring is made more difficult because the Guidelines do not limit the number of facilities where recombinant research can be conducted.

NRDC recommends that the Guidelines be revised and promulgated by the Secretary of HEW as regulations binding on all parties conducting recombinant research. The revised Guidelines should contain enforcement provisions based on licensing of facilities to ensure compliance with physical

containment requirements and to limit the number of facilities where recombinant research can be conducted. Laboratory workers should be licensed to ensure that they are properly trained. The NIH grantee should be required to conduct and report regular epidemiological studies on its workers. The Guidelines should be revised to eliminate or simplify the factual determinations which have to be made by the staff. Finally, the regulations should include strong sanctions: criminal penalties for intentional or negligent release of recombinant organisms; license and grant revocation for lesser violations.

WHO SHOULD PLAY GOD ?

by

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WHO SHOULD PLAY GOD?

by Jeremy Rifkin
Peoples Business Commission

In the rather short period in which mankind has recorded its own history, there appear singular moments in time when one reality runs its course and comes face to face with an entirely new reality, unchartered and unfathomable by any standards of previous measurement. During these peculiar momentary transitions, humanity is thrust into a twilight zone of existence where everything that has come before is suddenly transformed from an objective truth to a subjective opinion; and everything that lies ahead is still totally formless and incomprehensible.

Such was the moment experienced between the 4th and 5th Century, A.D. with the fall of the Roman Empire and the beginning of the Dark Ages, and again in the 14th Century between the end of the Medieval Era and the birth of the Renaissance and the Industrial Age.

Mankind is experiencing just such a moment in history now, in the last quarter of the 20th Century, as 700 years of the Scientific Age - the age of technology and physics and the Industrial Revolution - are being challenged by a new reality with the dawn of the Organic Age and the prospect of post-man.

This moment in time, however, is qualitatively different from those in the past where waining and emerging realities converged. Before, man was always the undisputed central figure of history. He was the one that stepped from one reality into another and he was the one who defined what reality and history would be.

Now, for the first time, man's central role in the future and all future realities is itself in doubt.

With the discovery of DNA and the process of recombination, man has unlocked the mysteries of life itself. It is now only a matter of a handful of years before scientists will be able to irreversibly change the evolutionary wisdom of billions of years with the creation of new plants, new animals and new forms of human and post-human beings. Man is at the crossroads of his own history and is now faced with the task of choosing his successor in the evolutionary order or imposing a moratorium on the future itself . . .

* * * * * * * * * * *

The precedents are being set right now which could lead all of us inextricably into the kind of "Brave New World" of post-man that Huxley fantasized some 40 years ago. Although the biologists prefer not to talk about it, they know what their research can and will inevitably lead to, just as the physicists knew but refused to accept the knowledge of what their research into splitting the atom would inevitably lead to. Like the atomic physicists, the biologists are attempting to hide from the ultimate consequences of their individual experimentations. By reducing their work to specialization on this or that particular genetic process, they pretend not to see the contribution of each and every discovery to the overall process of redesigning the natural evolutionary order and eventually man as well.

Moreover, many of the scientists working in this field maintain that recombinant DNA experimentation and genetic engineering are two different and unrelated issues. This in nonsense. They are as related as the splitting of the atom and the development of the nuclear bomb. In fact, even the House Subcommittee on Science, Research and Technology has acknowledged that it is impossible to divorce recombinant DNA experimentation from genetic engineering. They are one and the same and for that reason the House report of December, 1976 combines both under one title, to wit: Genetic Engineering, Human Genetics and DNA Recombinant Research.

Recombinant DNA is a recently developed technique that recombines DNA segments (the basic material determining the hereditary characteristics of life) from two different organisms. Scientists became able to do this when they found that DNA segments had "sticky" ends that, under proper laboratory conditions, could be fastened to another organism's DNA segments; thus forming the genetic basis for new living and multiplying organisms that do not exist in nature.[1]

Although most scientists agree that recombinant DNA is one of the most important scientific breakthroughs of all time, they violently disagree as to whether the potential benefits of even the most restrictive experimentations outweigh the grave potential dangers to the environment and to life as we know it.

Paul Berg, a prominent recombinant DNA researcher at the Stanford University School of Medicine, believes experimentation in recombinants could result in the creation of major new food crops that can obtain nitrogen from the atmosphere rather than from fertilizer; a new form of medicine, gene therapy, to treat crippling diseases; and such things as cheap and efficient production of vitamins, antibiotics and hormones.[2]

I would like members of this subcommittee to know that there are scientists who seriously question whether recombinant DNA research should be pursued at all. Jon Beckwith and some of his colleagues at the Harvard Medical School have decided not to use this new technology under any circumstances. Their reasons go far beyond the current controversy over whether or not the laboratory procedures can be made safe enough to prevent deadly viruses from escaping into the environment causing illness and death. Beckwith, who has been doing research into bacterial genetics for the last 12 years, argues that recombinant DNA research is leading directly to genetic engineering and the modification and alteration of the human species. In a world riddled with strife, inequities and programmed institutional violence, he asks whether any reasonable person can really believe that human genetic engineering will not be used "to domesticate" entire classes, populations or races once it is available.[3]

Ethan Signer, a biologist at M.I.T., echoed Beckwith's concern at the recent National Academy of Sciences Forum on recombinant DNA. This "research is going to bring us one more step closer to genetic engineering of people. That's where they figure out how to have us produce children with ideal characteristics". "Last time around", says Signer, "the ideal children had blond hair, blue eyes and Aryan genes."[4]

Signer's concern that recombinant DNA research and genetic engineering could lead to a mass eugenics movement here in this country is based on the writings of many of the leading proponents of this research. The fact is that top geneticists working in the area of recombinant DNA research have warned that the human gene pool is deteriorating at such a rapid rate that an aggressive positive eugenics program must be initiated immediately if we are to upgrade the human race.

I suggest that members of this Senate subcommittee examine the hundreds of position papers, symposium transcripts and essays in which leading geneticists and molecular biologists have officially gone on record as favoring the kind of genetic alteration of the human species that Hitler envisioned and that Huxley warned of.

These documents can be found at the Kennedy Institute of Bio-Ethics at Georgetown University. I have personally looked thru the reams of documents in this library and I can assure members of this subcommittee that the American public has every reason to be alarmed about the implications of what some of America's leading geneticists are advocating.

Today, I would like to give the members of this subcommittee a sample of some of the philosophy behind this recombinant DNA research. Geneticist James F. Danielli, Director of the Center of Theoretical Biology, S.U.N.Y., Buffalo, argues that "from the point of view of genetics, man is a barbarian, clad in the trappings of a civilization in which he is ill at ease and barely able to contend. . ." Danielli goes on to say that "most men contribute little to civilization other than maintenance services (if that), and are consumers rather than originators of civilization." According to Danielli, with human genetics as it is. . .the possibilities of improving man's condition by improving human institutions and environments "is a dubious proposition." Danielli believes that it is now time to "consider other possibilities if civilization is to persist and advance to a modestly stable state." The other possibilities," says Danielli, "lie in genetic engineering."[6]

Nobel Prize-winning biologist, Joshua Lederberg, one of the undisputed leaders in the recombinant field, has gone even further. Lederberg, it seems, is quite at home with the Brave New World of "Alphas," "Betas," "Gammas," and "Deltas" Huxley wrote about. In discussing cloning (the artificial creation of genetically identical individuals with carefully chosen physical and mental qualities), Lederberg says: "If a superior individual is identified, why not copy it directly, rather than suffer all the risks of recombinational disruption, including those of sex? Leave sexual reproduction for experimental purposes," says Lederberg. "When a suitable type (person) is ascertained," Lederberg beleives that we should "take care to maintain it by clonal propagation."[7]

Even as many of the leading proponents in the recombinant DNA field continue to deny any intention of fundamentally altering human life through genetic engineering, it is clear by their own unpublicized remarks that they have given a great deal of attention to the long-range implications of their work. For example, in a lecture on the future of man that is reprinted by the Ames Aerospace Research Center, Dr. James Bonner of the California Institute of Technology lays out one possible scenario for post-man. "One suggestion," says Bonner, "has been to remove genetic material from each individual after birth and then promptly sterilize that individual. During the individual's lifetime, records would be kept of accomplishments and characteristics. After the individual's death," according to Bonner, " a committee decides if the accomplishments are worthy of procreation into other individuals. If so, genetic materials would be removed from the depository and stimulated to clone a new individual. If the committee decides the genetic material is unworthy of procreation, it is destroyed." Bonner concludes with an appeal that would make even Dr. Strangelove quiver. He says, "the question is indeed not a moral one, but a temporal one - when do we start?"[8]

How close are we, then, to this new post-man reality that Bonner, Lederberg, Danielli and other prominent scientists talk about? When, indeed, do we start? For those on this subcommittee who hope that such developments are far enough off in the future to delay any immediate consideration or concern, they should heed the prediction of the experts in the field.

We are now within a few years of mass gene surgery and gene upgrading techniques, according to Beckwith of Harvard. As to the spectre of Huxley's cloned humanoids, Nobel Prize-winning biologist, James Watson, (one of the pioneers in this field) predicts that if such technology "proceeds in its current nondirected fashion, a human being born of clonal reproduction will most likely appear within the next 20 to 50 years, and even sooner if some nation actively promotes the venture."[9] Lederberg says that clonal man is even closer to reality; he puts the scale at between 0 to 15 years from now. . .[10]

* * * * * * * * * * * * * *

The spectre of mass genetic engineering and post-man is about to leap out from the pages of science fiction and become a competing reality in this world. Nevertheless, proponents, as well as critics, continue to limit the recombinant DNA debate to questions of proper safety procedures in the laboratories and what kind of Federal guidelines and regulations should be applied to the research. Not one member of Congress has even questioned whether the research should go on in the first place, and no one on Capitol Hill has even mentioned the social, moral and theological implications of artifically creating new forms of life and genetically altering the human species.

If Congress refuses to address the more overiding ethical and social questions involved in recombinant DNA experimentation, then it will, by ommission, be giving its consent to a course of scientific exploration that will, in the words of The New York Times editorial, "be carried to its ultimate conclusion."[11]

And like its stand on the Gulf of Tonkin resolution over 12 years ago, Congress, each and every member, will come to regret that they refused to recognize the issue of recombinant DNA for what it truly represents. Because, when the American public begins to understand the implications of this research, it is likely to result in a malestrom of protest that will make issues like abortion and right-to-life appear as tiny brush fires in comparison.

Artifically creating new life forms and genetic engineering challenge many of the basic tenents of 2000 years of Judao-Christian thought. For this reason, pulpits and parishes from one end of the country to the other are going to erupt in controversy over the technological forces that these biologists have unleashed.

Members of Congress had better be ready for this upheaval, because it's going to come; and each and every elected representative better be able to defend his or her indifference in these hearings to the profound moral and theological questions raised by recombinant DNA experimentation.

The creation of new forms of life, the redesigning of billions of years of evolution and the alteration of the human species are not technical issues that can be decided with a few Congressional hearings and a neat package of legislative guidelines.

The entire basis of Western values - our religious and cultural legacy - are challenged as never before by this experimentation and this new reality. We are dealing with the most important question ever to face the human family.

For this reason, the first question on the public agenda is not how to proceed, but whether we should proceed at all.

To begin answering that question we should look at what recombinant DNA and genetic engineering might be used for over the next 25 years.

1.) THE ENVIRONMENT

It is estimated that research laboratories in the U.S. will produce 10^{15} - 10^{16} recombinant organisms per year. Some of these organisms will be commercially applied and introduced into the environment.[12]

Liebe Cavalieri, of the Sloan Kettering Institute, and many of his colleagues are deeply concerned over the possibility that a new Andromeda-type virus, for which there is no known immunization, might be developed which would spread a deadly epidemic across the planet, killing hundreds of millions of people. They also fear that a new, highly resistant plant might be developed that could wipe out all other vegetation and animal life in its path.[13] Dr. Robert Sinsheimer, who chairs the Biology Division of the California Institute of Technology, warns, "the intervention and introduction of new self-producing living forms may well be irreversible." Sinsheimer asks, "How do we prevent grievous missteps, inherently unretraceable?"[14]

2.) WHO WILL DETERMINE WHAT ARE NORMAL GENES AND WHAT ARE DEFECTIVE?

Isn't it possible, given what we know of past history, that genetic upgrading might be used selectively against certain racial, ethnic or economic groups in the population? Or that genetic surgery might even be applied to those who disagree with the established order, such as activists and dissenters? Is there anyone here who can say that this is not a possibility in light of past abuses perpetrated by those in positions of power?[15]

3.) INDUSTRIAL SUSCEPTIBILITY SCREENING

There are those within the scientific and medical community who believe that particular types of individual are more genetically susceptable to certain kinds of pollutants than others. Might not this argument be taken up by the major corporations both as an excuse for not dealing with the adverse effects of industrial pollution and as a weapon in hiring different types or groups of workers? Isn't it also possible that such reasoning might lead to genetic breeding or alteration of certain groups in the population in order to fit them into a particular industrial niche? Might this not also be seen as the first precursory step toward the "Alphas," "Betas," "Gammas," and "Deltas" of Brave New World?[16]

4.) CORPORATE PATENTS ON NEW FORMS OF LIFE

Pharmaceutical, chemical and agricultural corporations are asserting a right to patent processes for new forms of life and to profit from such new life forms in the marketplace. If this claim to patents is upheld, what will be the implications 10 or 15 or 25 years from now when it will be possible to genetically alter and even create new types of human life? Should private corporations be granted the ultimate authority to control the application of genetic engineering?[17]

5.) CROSSING THE THRESHOLD BETWEEN HUMAN AND POST-HUMAN LIFE

With each successive step in recombinant DNA experimentation and genetic engineering, biologists come a bit closer to the threshold where the homo sapien species becomes an artificial humaniod or post-homo sapien strain. As this experimental process unfolds, who will make the basic decisions as to what the genetically perfect homo sapien or post-man should look like and how it should behave?

At what point with gentic engineering do we redefine or discard terms like mother, father, family, soul, conscience, God?

Given the above scenarios, all of which are within the realm of possibility in our and our children's lifetimes, are there any institutions that presently can be trusted with the authority and responsibility of making the basic decisions concerning recombinant DNA research and application over the next quarter century? The Government? The Corporations? Academia? The Churches? . . .

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The proponents of recombinant DNA research argue that experiments into the creation of new forms of life and genetic engineering are very much a part of the natural evolutionary process. Their reasoning is that since man is part of that evolutionary order and has succeeded in developing the technology to extend the evolutionary boundaries beyond their present confines, that, in itself is the most persuasive argument that the whole thing is part of a "natural" progression.

However, it is also true, as Ted Howard of the Peoples Business Commission pointed out in his remarks at the N.A.S. Forum, that like all other species in the evolutionary chain, man is likely to fiercely resist his own extinction. Comparing man to the other endangered species, Howard told the assembly of biologists that, "we humans will not go gently into that Brave New World." "We will fight back," he said, "in order to insure our survival."[18]

Fighting back, in this case, will be no small task. Placing a moratorium on further research into what one Nobel Prize-winning biologist refers to as the most important scientific discovery of all time, will require saying NO to 700 years of the technological imperative: to wit, "what can be done, will be done." This technological imperative has been the measuring stick of our reality for hundreds of years; it is the primary means by which we have judged our own progress. Now, the technological imperative threatens to become an end in and of itself; an end so powerful that it can eventually remake and even dispose of mankind if we allow it.

It took 3 billion years of evolution to evolve to where we are now. This is the progress of life itself; all of life. Measured against this backdrop, are we denying progress by temporarily holding back on a new technology that has it within its powers to radically alter and potentially destroy everything that has come before? Is progress to be measured solely by doing everything that can be done, regardless of the consequences? Or should new ideas and new realities be explored more selectively and with greater concern for all of the prior occurances that effect and will be effected by it?

Perhaps the best argument in favor of a moratorium on this technology is made, inadvertantly, by the very scientists, public officials, and corporations who advocate an immediate go-ahead.

None of these three groupings are willing to engage in a dialogue over the possible consequences of this research 20, 50 or 100 years from now. They dismiss such appeals as irrelevant and immaterial. Yet, at the same time, they are more than willing to assume the authority for changing billions of years of evolutionary development virtually overnight without any thought to its long-term effects. This blatant display of hubris is unconscionable and unacceptable.

Mankind needs to impose a temporary moratorium on this new technology so that it can decide on what course of action to follow. During that time, humanity might

well decide that a longer moratorium of 25 or 50 years or more are required for the societies of this earth to catch up culturally and institutionally with the technological reality of an emerging Organic Age.

We are caught in a gap between two historical realities, the greatest mankind has ever had to cross. History has shown us that man, like all other species, needs time to adjust to radically altered environments. When environments change too rapidly, all living things are threatened and become a potentially endangered or even extinct species. If we refuse to give ourselves enough time to prepare ourselves for the journey into this new reality, we homo sapiens might not even reach the other side. If, on the other hand, we are able to use a moratorium period to close the gap between our social infancy and our incredible technological maturity, then, perhaps, we can move more confidently on to the next great period, the Organic Age, conscious of the implications and options before us.

The Peoples Business Commission recommends a 4-year moratorium on recombinant DNA research.

During that time a National Commission would be set up composed of a cross-section of Americans selected by the same techniques used in administrating national public opinion surveys. This scientifically selected crossection of the American public would be empowered to collect information, hold hearings and present findings and options to the American public.

To insure that the American public is fully informed of all aspects of the recombinant DNA issue, the Commission would be granted 4 seven-day segments of prime time T.V. (one each year) to present their findings to the American people for deliberation.

At the end of this 4-year period a National Referendum would be drawn up by the Commission listing all the possible options available, and the American public would vote their preferences, at the ballot box during the Presidential election of 1980.

I know that some members of Congress and the press will scoff at this proposal as unrealistic. On the contrary, we are dealing with the most important social issue ever to face the human family and it requires that everyone be personally informed and involved in the decisions that are made.

FOOTNOTES

1. New Strains of Life - or Death. Liebe F. Cavalieri. New York Times Magazine. August 22, 1976

2. Ibid.

3. Recombinant DNA: Does the Fault Lie with the Genes? Jonathan Beckwith. Harvard Medical School. Written remarks presented at the National Academy of Science Forum on Recombinant DNA, March 7, 8 and 9, 1977.

4. Recombinant DNA: It's Not What We Need. Ethan Signer, Department of Biology, Massachusetts Institute of Technology. Written remarks presented at the NAS Forum March 7, 8, and 9, 1977.

5. A Few Remarks Regarding Research on Recombinant DNA. Erwin Chargaff. Professor Emeritus of Biochemistry, Colombia University. Oral remarks presented at the NAS Forum March 7, 8, and 9, 1977.

6. The Politics of Genetic Engineering: Who Decides Who's Defective. Psychology Today. June, 1974.

7. The Human Agenda. Dr. R. Gorney. Simon & Schuster. p. 222. (1968)

8. The Next Billion Years. Ames Research Center. NASA p. 64. (1974)

9. The Politics of Genetic Engineering. Psychology Today. June, 1974.

10. Future Shock. Alvin Toffler. Bantam. (1971)

11. New York Times Editorial. March 11, 1977.

12. Written Remarks. R. L. Sinsheimer. NAS Forum March 7, 8, and 9, 1977.

13. New Strains of Life - or Death. New York Times Magazine. August 22, 1976.

14. DNA: Have the Corporations Already Grabbed Control of New Life Forms? J. Rifkin. Mother Jones Magazine. February/March 1977.

15. Beckwith. Written remarks. NAS Forum. March 7, 8, and 9, 1977.

16. Beckwith.

17. One Small Step Beyond Mankind. J. Rifkin. The Progressive. March, 1977.

18. Oral remarks. T. Howard. NAS Forum. March 7, 8, and 9, 1977.

April 2, 1977
Washington, D.C.

Dr. Neil Solomon
Secretary of Health
State of Maryland
Baltimore, Md.

RE: DNA, Research - a Challenge and Decision.

Dear Dr. Solomon,

Referring to our pleasant conversation of March 10, 1977, regarding scientific genetic research and development at which time I promised to follow-up the salient factors of our discussion in a letter to you, outlining an expression on the diversified subject in a non-technical treatise form and the underlying principles involved therein, on man's ageless struggle to unlock the secrets of the universe.

The basis for the discussion was the fact that the prestigious National Academy of Sciences has just completed a 3-day academic Forum embodying scientific research pertaining to the mixing of recombinant DNA-DeoxyriboNucleic Acid, the chemical substance which contains all genetic information, (National Academy of Sciences, Forum Program, Exh. "A").

Briefly stated: The Forum on the subject matter was in a series of complex experiments involving a process of recombinant DNA scientific research. The implication of a comprehensive reaction is only an opening skirmish in a battle that is quickly becoming the most important social and moral controversy this nation has ever faced, and furthermore, it does raise many complex issues--wherein, a whole new kind of daily life may begin to unfold. We are now entering a new highly sophisticated scientific age area where we will have the ultimate power to create life, and genetically alter the human species. Unfortunately, to express the belief, there are virtually nevertheless serious and far-reaching problems posed by such experimentations with new form of life, (Diagrammatic sketches of the aftermath of alleged altered human life, Exh. "B" and "C").

Being so stated, it is indeed a controversial subject of a universal nature of paramount importance as there are remaining questions to be answered: Nevertheless, the scientists in good faith claim the unlocking of genetic secrets may be a fine bridge between the ancient and modern day medicine. Therefore, I for my part, am convinced there is some margin of truth to the procedure, for it may be said, the frontier of genetic secrets is the basic studies which, now are extending our understanding of the nature of matter. This could benefit mankind in its fight against disease. For we must understand matter if we are going to be able

to use it successfully. Therefore, the scientists are necessarily looking for evidence to support or contradict any theories of creation, or promising hopes of a superior way to treat many health problems that plague the human race. While they are not a panacea, moreover the antibiotics and vaccines have materialistically reduced many an ancient malady to little more than a memory, and otherwise, what might prove to be beneficial results now held in store, for man and history in the future.

One might be safe in saying this recombinant DNA scientific research is quietly spreading from academia, into the research and development laboratories of America's business corporation, (Pharmaceutical Firms, Exh. "D").

I recently spoke (on March 18, 1977), to some members comprising the professional staff, whom I happen to know from past dealings, associated with the "Subcommittee on Health of the United States Senate," the Chairman is Edward M. Kennedy, (D-Mass). I questioned them about the proposed DNA Congressional Hearing to form a basis for "legislative history," and was informed the date has not as yet been scheduled, but could be in April or May, 1977.

For your information regarding the future use of Fort Detrick facility at Frederick, Maryland, to conduct recombinant DNA, genes as a research center; a $3 million National Center for controversial experiments in new or radically different forms of life, including plant, animal and human life, will be built at Fort Detrick, by the National Institutes of Health.

Historically speaking, the old Fort Detrick buildings were used by the United States Army from 1939 to 1972. I can say this for my part because during World War II,I served as the Staff Officer for the U.S. Army for 5½ years, in charge of the Safety and Health Section, during which period it was the secret medical experimental United States Army Biological Warfare Headquarters.

Furthermore, traveling down memory lane, regarding Fort Detrick based upon my connections with the Installation Safety and Health Section factors, I remember, I was questioned about any knowledge I might have or could offer regarding an episode by the "United States Senate Select Committee to study Governmental Operations with respect to intelligence activities," (Excerpts of Select Committee Intelligence statement, Exh. "E").

This involved "plague cases" about soldiers stationed on guard duty at the sprawling installation. In the above paragraph reference is made to a xerox copy excerpt from an October 22, 1975 statement of mine under questioning from a 3-page report, (Aftermath-Plague epidemics in England--year 1348, Exh. "F").

No doubt about it, we had various serious safety factors, accidents and health problems at Fort Detrick, with the biological agents which caused diseases such as anthrax, plague, and encephalitis-- you name it. The State of Maryland may be in due course confronted with one of a similar nature, when the DNA experimentation activities get under way.

In the new National Institutes of Health safety guidelines they established four physical containment levels--categorized from P-1 (normal laboratory precautions) to the P-4 level that requires sealed rooms, double doors, protective clothes, handling of organism through sealed glove boxes, in which experiments are on the other side of a protective window, and chemical treatment and the sterilization of any wastes.

Furthermore, to elucidate the category P-1 level is reserved for experiments in the modification of simple species, like some bacteria, that naturally interact genetically.

Regarding category P-2 level allows scientists to work with all nonpoisonous snakes, lizards, frogs or fish genes.

Experiments requiring category P-3 level precautions involve modification of mammalian genes.

Further, to conduct experiments with new biology--mixing the DNA or deoxyribonucleic acid, (that is the stuff of genes) of primates--namely human being or monkeys--or with an animal tumor filtrable virus, that retains its virulence after filtration, this is categorized P-4 level and a license is required. The proposed Fort Detrick, National Institutes of Health high-containment laboratories will be the country's first, certified category P-4 level facilities and will permit by far the most hazardous scientific research known to man.

Regarding current events I understand the forecast genetic research at Fort Detrick raises few hackles in the town of Frederick, Maryland. No doubt, they have become accustomed to the idea of the nature of exotic research going on over the last three decades in their own backyards.

Furthermore, word of the planned experiments did not make the community's papers until the afternoon of March 9, 1977, when the banner headline "Genetic Research Planned at Fort Detrick--front page! Briefly, it stated that the National Institutes of Health plan to install a $3-million DNA facility, construction of which is set to begin later this (1977) year.

In general one might say that the community has accepted the recombinant DNA proposed scientific research with a sort of science-fiction feeling. With the belief that the geneticists will be able, in the not-too-distant future, to join genetic material from different organism in combination unknown to us today.

The equanimity with which residents such as Frederick, Maryland, greeted the genetic research experiments contrasts sharply with the hostile reception last summer when the City Council of Cambridge, Massachusetts, voted to prohibit scientific research on recombinant DNA, at Harvard Medical School and Massachusetts Institutes of Technology pending further public investigation.

Like a devastating surprise bombshell, this unparalled public restriction of scientific research focused, for the first time, the attention of the national media on the question of the creation of a new DNA, life forms, of multiplying organisms that do not exist in the natural evolutionary order today. Novus ordo seclorum. "The new age now begins," a conglomeration of a near fever pitch, because of a national hysteria which now seems to exist, fomented by the media.

If in the twilight land of dreams DNA materializes the creation of monsters for the future, we may have go back and study the New Jersey Karen Anne Quinlan case, the "Right to Live" or the "Right to Die," again without a respirator to deal with the evolutionary by-product, bizarre events.

Mention should be made that according to Liebe Cavalieri and other scientists of the Sloan-Kettering Institute for Cancer Research argue for a complete moratorium on recombinant DNA research until the long-range implications are fully discussed. The scientist Cavalieri points out that such research "involves many unknown factors beyond the control of the scientists." Further, according to Cavalieri, "it is necessary to create vast number of cells with unknown genetic alterations in order to obtain a cell containing a specific recombinant DNA."

Cavalieri continues: Therefore, "the probability of creating a dangerous genetic agent in the process is real, and there is no way to test for danger. Likewise, the scientist does not know what he has done until he has analyzed the newly created cell--at which point it may be too late." At that point science fiction's most horrible scenarios become facts. It is my belief that man's inability to deal with disease delayed, the onset of the enlightenment.

Cavalieri and his colleagues are deeply concerned over the possibility that a new Andromedia-type virus, for which there is no known immunization, might accidentally be developed in a laboratory somewhere and spread a deadly epidemic across the planet, killing hundreds of millions of people. They also fear that a new, highly resistant plant might be developed that could wipe out other vegetation and animal life in its path.

Another scientist's view point likewise is equally concerned, Dr. Robert Sinsheimer, who chairs the biology division of the California Institute of Technology, warns in a similar vein, "the invention and introduction of new self-producing living form may well be irreversible." The unanswered question is: how do we prevent grievous missteps inherently unretraceable? In the light of this Sinsheimer wrote to focus attention to the National Institutes of Health the consequences, he says, "are highly predictable and likely, highly dangerous."

In the Fall of 1976 the Federal Government assembled without fanfare or publicity a small "Interagency Committee" to review the concept of Federal policy on the conduct of research involving the creation of new forms of life, and submission of report through the National Institutes of Health direct to the President concerning the commercial development of new forms of life.

Likewise, in January 1977 the Patent Office announced that it is according "special" status to patent applications involving recombinant DNA, Although further such applications should serve notice therein a statement that National Institutes of Health guidelines are being followed." As an alternative "the patent application may, instead, include explanations of "deviations considered to avoid disclosure of proprietary information or loss of patent rights."

The thought before us should be a more immediate question as we enter the Organic Age: Should our present corporate system be used as the developing and marketing process when life itself is the product? (Putting a Patent on Life, Exh. "G").

Legalistically speaking, you can only patent the process and not by any means,the resulting product, under existing patent law procedure (Certificates U.S. Patent Office, Exh. "H" and Patent Appeals Court, Exh. "I").

I might mention at this point, on Thursday, March 31, 1977, a medical Doctor connected with Johns Hopkins University Hospital, and also a full-time representative of the United States Environmental Protective Agency, paid me a visit. He informed me that his agency was engaged in assembling material relative to the preparation of a report covering recombinant DNA scientific research, for the National Institutes of Health, Director Donald S. Fredrickson to discuss with the President. Furthermore, it was stated that if by chance certain aspects of the material to be considered was completed by next week he would bring it by for me to examine and make a comment.

Any response will no doubt be inadequate, but through shared perspectives something of the "wisdom of Solomon" can emerge! Likewise, the following question is: "Who will be Judas?"

In closing I might say regarding the subject matter of our discussion "Re: DNA, Research - a Challenge and Decision," presents a magnitude of reliance, it spells out an end to "make believe" a new product of hope and aspiration. Furthermore, science has a determination to compete with the laws of Nature herself to win over the limitations of matter and an often expressed biblical term offering to us seemingly "an everlasting life."

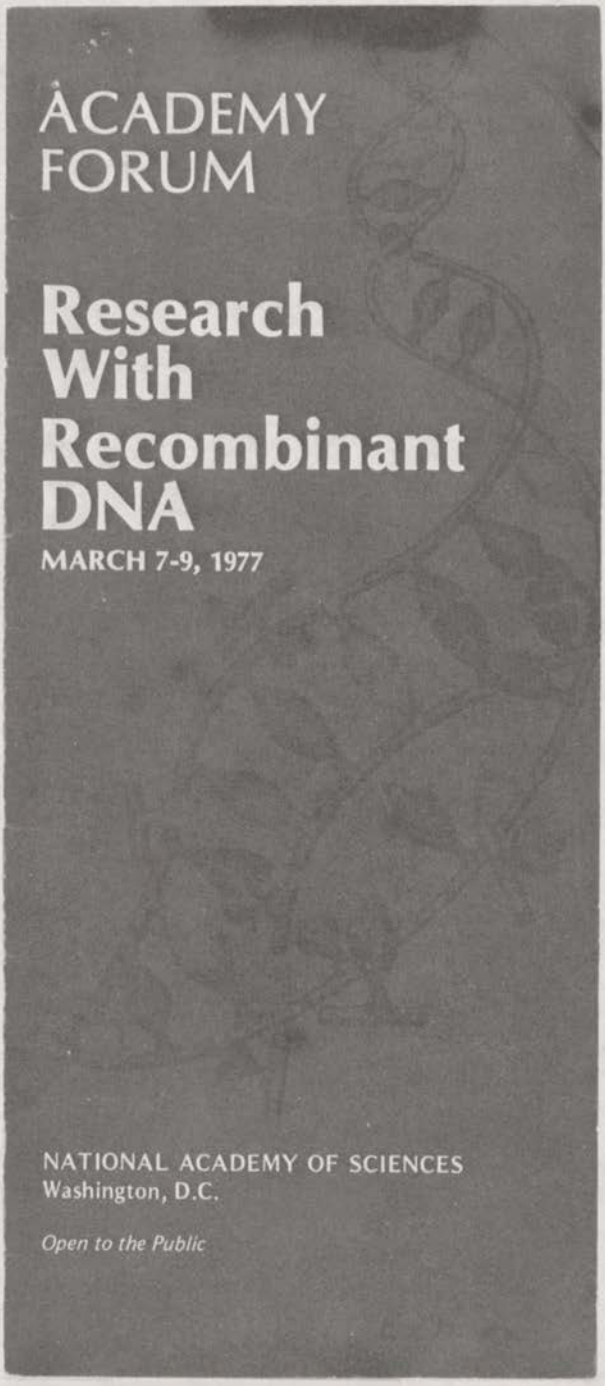
Respectfully submitted

Robert W. Hicks, Ph.D.
Biomedical Engineer.

Address:
1920-Eye Street,N.W.
Washington, D.C. 20006

Enclosures: Exhibits, "A" thru "I" Incl.

EXHIBIT A


ACADEMY
FORUM
Research
With
Recombinant
DNA
MARCH 7-9, 1977
NATIONAL ACADEMY OF SCIENCES
Washington, D.C.
Open to the Public

This Academy Forum
has received support from:

Abbott Laboratories
Ford Foundation
Hoffmann - La Roche Inc.
Merck Sharp & Dohme Research Laboratories
National Academy of Sciences
National Institutes of Health
Pfizer Inc.
Smith Kline & French Laboratories
The Upjohn Company

Cover
Painting by Davis Meltzer, (c) National Geographic Society.

The Forum of the National Academy of Sciences is designed to aid in the resolution of dilemmas arising from the discoveries of science. We hope, at the minimum, to clarify the issues and to illuminate the facts. Beyond that, we hope to develop a consensus for the formulation of sound science policy or to identify the new information needed to generate such a consensus.

Research with recombinant DNA, a subject of great importance and considerable controversy, is particularly suited to the Forum process, which brings together scientists, officials of government, and numerous categories of citizens with wide ranges of expertise and viewpoints. As many individual research institutions, both educational and industrial, and government bodies at all levels now are poised to determine courses of conduct in relation to this research, the timing for such a Forum is appropriate. Inquiries over the past months indicate that the ideas to emerge from this Forum will have great weight in the design of both public and private policy.

The success of the Forum depends primarily on the individuals who participate and their ability to develop constructive and imaginative ideas around questions that emerge from the topic and the known facts. We welcome your participation in this Forum, your evaluation of it, and your suggestions for future Forum topics.

Sincerely yours,

Daniel E. Koshland Jr.

Daniel E. Koshland, Jr.
Chairman
General Advisory Committee
The Academy Forum

1

91-939 O - 77 - 21

ACADEMY FORUM

Research With Recombinant DNA

March 7-9, 1977

NAS Auditorium
2100 Block, C Street N.W.

REGISTRATION

A registration and information desk will be stationed in the lobby of the C Street entrance to the Academy. There also will be a bulletin board for messages at this location. The schedule for these facilities is:

Monday, March 7: 7–10 p.m.

Tuesday, March 8: 8 a.m. to 10 p.m.

Wednesday, March 9: 8 a.m. to 5 p.m.

The telephone number for the message center and registration desk is 389-6824.

The Forum of the National Academy of Sciences is open to the public. There is no registration fee. Parking is not available.

COCHAIRMEN:
David A. Hamburg, President, Institute of Medicine
Alexander Rich, Sedgwick Professor of Biophysics, Massachusetts Institute of Technology

MARCH 7

7:00 p.m.
REGISTRATION, C Street Entrance

7:30 p.m.
WELCOME AND INTRODUCTION TO THE FORUM
Daniel E. Koshland, Jr., Chairman of the General Advisory Committee of the Academy Forum; Professor and Chairman, Department of Biochemistry, University of California, Berkeley

INTRODUCTION TO THE EVENING
David A. Hamburg, Cochairman

How Scientists Interact with the Public: Historical Perspective

Scientist Involvement *Maxine Singer*, Head, Nucleic Acid Enzymology Section, Laboratory of Biochemistry, National Cancer Institute

Lay Involvement *Daniel Callahan*, Director, Institute of Society, Ethics and the Life Sciences

INQUIRY AND DISCUSSION FROM THE FORUM

Overview of the Research With Recombinant DNA

Potential Benefits *Daniel Nathans*, Boury Professor and Director, Department of Microbiology, The Johns Hopkins University School of Medicine

Potential Risks *Erwin Chargaff*, Professor Emeritus of Biochemistry, Columbia University

INQUIRY AND DISCUSSION FROM THE FORUM

10:00 p.m.
ADJOURNMENT

MARCH 8

8:00 a.m.
REGISTRATION, C Street Entrance
Coffee, Great Hall

9:00 a.m.
PRIORITIES FOR THIS SESSION
Alexander Rich, Cochairman

Case Analyses

I MAPPING THE MAMMALIAN GENOME

Potential Benefits *Paul Berg*, Willson Professor of Biochemistry, Stanford University Medical Center

Potential Risks *Robert L. Sinsheimer*, Chairman, Division of Biology, California Institute of Technology

INQUIRY AND DISCUSSION FROM THE FORUM

Coffee

Three Commentaries

The Stability of Biological Species *Francisco José Ayala*, Professor of Genetics, University of California, Davis

Research with Recombinant DNA In Europe *Sir John Kendrew*, Director General, European Molecular Biology Laboratory, Heidelberg

The Public Interest: A Point of View *Stephen E. Toulmin*, Professor, Committee on Social Thought and the Department of Philosophy, University of Chicago

INQUIRY AND DISCUSSION FROM THE FORUM

Lunch

2:00 p.m.

II THE DANGERS OF PLANNED OR INADVERTENT LABORATORY INFECTIONS AND EPIDEMICS

The Epidemiology of Enteric Infections and Laboratory Infections *H. Bruce Dull*, Assistant Director for Program, Center for Disease Control

Natural Selection, Virulence and Communicability: The Handling of Dangerous Organisms *Bernard D. Davis*, Adele Lehman Professor of Bacterial Physiology, Harvard Medical School

INQUIRY AND DISCUSSION FROM THE FORUM

Health Hazards to Labor *Anthony Mazzocchi*, Director, Citizenship-Legislative Department, Oil, Chemical & Atomic Workers International Union

An Environmental Overview of Research with Recombinant DNA *Wilson K. Talley*, Assistant Administrator for Research and Development, Environmental Protection Agency

INQUIRY AND DISCUSSION FROM THE FORUM

Coffee

III PHARMACEUTICAL APPLICATIONS: MICROBIAL PRODUCTION OF INSULIN

Potential Benefits *Cornelius W. Pettinga*, Executive Vice President, Eli Lilly and Company

Potential Risks *Ruth Hubbard*, Professor of Biology, Harvard University

INQUIRY AND DISCUSSION FROM THE FORUM

INTRODUCTION TO THE WORKSHOPS
David A. Hamburg, Cochairman

5:00 p.m.
ADJOURNMENT

7:00 p.m.
Workshops Convene

Workshops

1 Is it likely that *E. coli* can become a pathogen?

Richard Goldstein, Assistant Professor, Department of Microbiology and Molecular Genetics, Harvard Medical School, Chairman

2 How can we assess the benefits and risks of this research?

William W. Lowrance, Special Assistant for Science and Technology to the Deputy Under Secretary of State, Department of State, Chairman

3 In what way should laymen be involved in decisions concerning the conduct of research with recombinant DNA?

Stanley B. Jones, Staff Director, Subcommittee on Health, Committee on Labor and Public Welfare, United States Senate, Chairman

4 National and international efforts to develop guidelines: Should there be voluntary or enforced rules of conduct?

Charles Weissmann, Professor of Molecular Biology and Director, Institute for Molecular Biology, University of Zurich, Cochairman
Harold P. Green, Professor of Law, National Law Center, The George Washington University, Cochairman

5 What is the appropriate role of sponsoring institutions and of federal, state, and local governments in relation to recombinant DNA research?

Clifford Grobstein, Professor of Biology and Vice Chancellor for University Relations, University of California, San Diego, Chairman

6 The use of recombinant DNA research in biological warfare is ruled out for nations by the Biological War Convention of 1972 and the Geneva Protocol of 1925. Is it conceivable that terrorists or nations may try to use this technology to develop weapons?

Matthew S. Meselson, Thomas Dudley Cabot Professor of the Natural Sciences, Chairman, Department of Biochemistry and Molecular Biology, Harvard University, Chairman

7 How effective is physical and biological containment in recombinant DNA research?
Roy Curtiss III, Professor of Microbiology, University of Alabama Medical Center, Chairman

8 Can the results of basic research with recombinant DNA be transferred to industrial applications?
A. M. Chakrabarty, Staff Microbiologist, Physical Chemistry Laboratory, General Electric Research & Development Center, Chairman

9 How natural is the exchange of genes between "unrelated" bacteria? The benefits and risks of prokaryote-prokaryote gene exchange.
Stanley N. Cohen, Professor of Medicine, Stanford University Medical Center, Chairman

MARCH 9

8:00 a.m.
REGISTRATION, C Street Entrance
Coffee, Great Hall

9:00 a.m.
PRIORITIES FOR THIS SESSION
Alexander Rich, Cochairman

Workshop Reports
David A. Hamburg, Cochairman

INQUIRY AND DISCUSSION FROM THE FORUM

IV FOOD PRODUCTION

Potential Benefits *Raymond Valentine*, Associate Professor of Agronomy, Plant Growth Laboratory, Department of Agronomy and Range Science, University of California, Davis

Potential Risks *Ethan R. Signer*, Professor of Biology, Massachusetts Institute of Technology

Coffee

V GENETIC ENGINEERING: THE FUTURE

David Baltimore, American Cancer Society Professor of Microbiology, Center for Cancer Research, Massachusetts Institute of Technology

Jonathan Beckwith, Professor of Microbiology and Molecular Genetics, Harvard Medical School

INQUIRY AND DISCUSSION FROM THE FORUM

Lunch

2:00 p.m.

Problems of Control & Regulation

An Overview of the Role of NIH and Other Federal Agencies in the Conduct of Research with Recombinant DNA *Donald S. Fredrickson*, Director, National Institutes of Health

The Economic Implications of Regulation by Expertise *Roger G. Noll*, Professor of Economics, California Institute of Technology; Visiting Professor, Stanford University

Reports from Workshops Dealing with Control and Regulation

Coffee

INQUIRY AND DISCUSSION FROM THE FORUM

Summary

Tracy M. Sonneborn, Distinguished Professor Emeritus of Zoology, Indiana University

Donald Kennedy, Benjamin Scott Crocker Professor of Human Biology, Chairman, Program in Human Biology, Stanford University; and Senior Consultant to the Office of Science and Technology Policy

5:00 p.m.
ADJOURNMENT

PANEL FOR INQUIRY
ACADEMY FORUM ON RESEARCH
WITH RECOMBINANT DNA

Herbert W. Boyer, Professor of Biochemistry, University of California Medical School, San Francisco
Donald D. Brown, Director, Department of Embryology, Carnegie Institution of Washington
Liebe F. Cavalieri, Member, Sloan-Kettering Institute for Cancer Research; Professor of Biochemistry, Graduate School of Medicine, Cornell University
Stanley N. Cohen, Professor of Medicine, Stanford University Medical Center
Freeman J. Dyson, Professor of Physics, School of Natural Sciences, The Institute for Advanced Study
Daniel J. Hayes, Jr., Chairman, Cambridge Experimentation Review Board
Sheldon Krimsky, Associate Director, Program in Urban, Social and Environmental Policy, Tufts University
Kurt Mislow, Hugh Stott Taylor Professor of Chemistry, Princeton University
Robert S. Morison, Class of 1949 Visiting Professor, Massachusetts Institute of Technology
Robert R. Murray, Jr., Professor of Pediatrics and Medicine, Chief, Division of Medical Genetics, College of Medicine, Howard University
Talbot Page, Senior Research Associate, Resources for the Future
Judith E. Randal, Science Correspondent, Washington Bureau, *New York Daily News*; Science Policy Editor, *Change* Magazine
DeWitt Stetten, Jr., Deputy Director for Science, National Institutes of Health
John Tooze, Executive Secretary, European Molecular Biology Organization, Heidelberg
George Wald, Higgins Professor of Biology, Harvard University
Milton R. Wessel, Attorney and Adjunct Professor, New York University School of Law
Luther S. Williams, Associate Professor of Biology, Department of Biological Sciences, Purdue University
Norton D. Zinder, Professor, Rockefeller University

PROGRAM COMMITTEE
ACADEMY FORUM ON
RECOMBINANT DNA RESEARCH

Lawrence Altman, Medical Writer, *New York Times*
David Baltimore, American Cancer Society Professor of Microbiology, Center for Cancer Research, Massachusetts Institute of Technology
Paul Berg, Willson Professor of Biochemistry, Stanford University Medical Center
Daniel Callahan, Director, Institute of Society, Ethics and the Life Sciences
Freeman J. Dyson, Professor of Physics, School of Natural Sciences, The Institute for Advanced Study
Richard Goldstein, Assistant Professor, Department of Microbiology and Molecular Genetics, Harvard Medical School
Harold P. Green, Professor of Law, National Law Center, The George Washington University
Clifford Grobstein, Professor of Biology and Vice Chancellor for University Relations, University of California, San Diego
David A. Hamburg, President, Institute of Medicine, *Cochairman*
Howard H. Hiatt, Dean, Harvard School of Public Health
Stanley B. Jones, Staff Director, Subcommittee on Health, Committee on Labor and Public Welfare, U.S. Senate
Daniel E. Koshland, Jr., Professor and Chairman, Department of Biochemistry, University of California, Berkeley
Cornelius W. Pettinga, Executive Vice President, Eli Lilly and Company
Alexander Rich, Sedgwick Professor of Biophysics, Massachusetts Institute of Technology, *Cochairman*
Maxine Singer, Head, Nucleic Acid Enzymology Section, Laboratory of Biochemistry, National Cancer Institute
Robert L. Sinsheimer, Chairman, Division of Biology, California Institute of Technology

GENERAL ADVISORY COMMITTEE

Robert McC. Adams, Professor, The Oriental Institute
Kenneth J. Arrow, James Bryant Conant University Professor, Project on Efficiency of Decision Making in Economic Systems, Harvard University
David Baltimore, American Cancer Society Professor of Microbiology, Center for Cancer Research, Massachusetts Institute of Technology
Arthur M. Bueche, Vice President, Corporate Research and Development, General Electric Company
Freeman J. Dyson, Professor of Physics, School of Natural Sciences, The Insitute for Advanced Study
Donald S. Fredrickson, Director, National Institutes of Health
Gertrude S. Goldhaber, Senior Physicist, Brookhaven National Laboratory
Michael Kasha, Director, Institute of Molecular Biophysics, Florida State University
Daniel E. Koshland, Jr., Professor and Chairman, Department of Biochemistry, University of California, Berkeley, *Chairman*
Philip Morrison, Institute Professor, Department of Physics, Massachusetts Institute of Technology
John R. Pierce, Professor of Engineering, Steele Laboratory, California Institute of Technology
Alexander Rich, Sedgwick Professor of Biophysics, Massachusetts Institute of Technology
Frederick C. Robbins, Dean, School of Medicine, Case Western Reserve University
Lewis Thomas, President, Memorial Sloan-Kettering Cancer Center
Alvin M. Weinberg, Director, Institute for Energy Analysis, Oak Ridge
David A. Hamburg, President, Institute of Medicine, *ex officio*
Courtland D. Perkins, President, National Academy of Engineering, *ex officio*

STAFF

Robert R. White	*Director*
M. Virginia Davis	*Staff Associate*
Betsy S. Turvene	*Editor*
Marcie S. Lofgren	*Administrative Secretary*

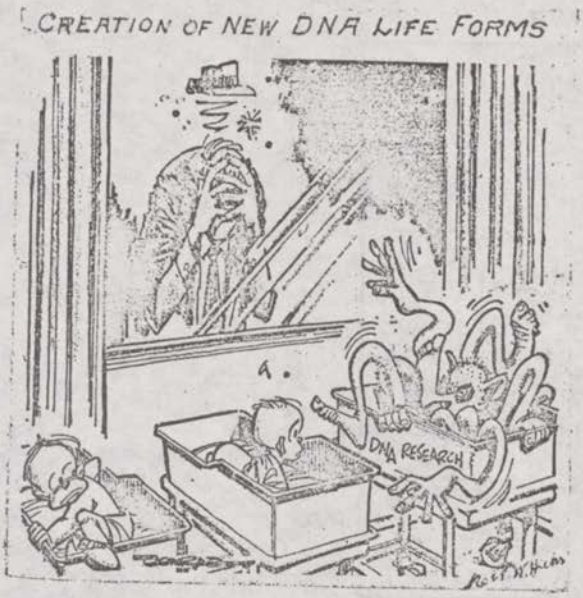
THE NATIONAL ACADEMY OF SCIENCES is a private, co-optative society of distinguished scholars in scientific and engineering research, dedicated to the furtherance of science and its use for the general welfare. Its charter, an Act of Incorporation passed by the U.S. Congress and signed by Abraham Lincoln on March 3, 1863, called upon it to serve as an official adviser, upon request and without fee, to the federal government on any question of science or technology.

THE NATIONAL ACADEMY OF ENGINEERING was established in December 1964, under the charter of the National Academy of Sciences, as a parallel organization of distinguished engineers, autonomous in its administration and membership, and sharing with the NAS its responsibility for advising the federal government.

THE NATIONAL RESEARCH COUNCIL was organized by the National Academy of Sciences in 1916 at the request of President Woodrow Wilson. Most of the activities undertaken by the two Academies are carried out through the NRC, which is representative of the major scientific and technical societies, and whose members are derived from universities, industry, and the scientific agencies of the federal government.

THE INSTITUTE OF MEDICINE was chartered by the National Academy of Sciences in the summer of 1970 in recognition of the important and complex problems posed in the provision of adequate health services to all sectors of society. Its members are selected from all fields of health and medicine as well as from related activities in the natural, social and behavioral sciences, law, administration, government service, and engineering.

EXHIBIT B





"He isn't really reading. He's just looking at the pictures."

EXHIBIT C



"—And stay out of our gene pool!"



"What's all the big fuss about genetic research?"

EXHIBIT D

CORPORATIONS IN DNA RESEARCH

Company	Location of Laboratory	State of Research
Miles Laboratories (Dr. Robert Erickson, Dept. of Science Information and Communication Services)	Rochester, NY, and South Bend, IN (Research under contract to universities)	Ongoing
Eli Lilly & Company (Dr. Cornelius W. Pettinga, Executive Vice President)	Indianapolis, IN	Ongoing
Hoffman-LaRoche (Dr. Sidney Udenfriend, Director, Roche Institute)	Nutley, NJ	Ongoing
The Upjohn Company (Dr. Joe Grady, Section Head, Infectious Diseases)	Kalamazoo, MI	Ongoing
Merck, Sharpe & Dohme Research Laboratories (Dr. Jerome Birnbaum, Executive Director, Basic Biological Sciences)	Rahway, NJ	Tooling up
Pfizer, Inc. (Dr. John DeZeeuew, Research Scientist)	Groton, CT	Tooling up
Abbott Laboratories (Dr. Lacy Overby, Director, Experimental Biology)	North Chicago, IL	Tooling up

EXHIBIT E

Note: Attached Xeroxed copy of excerpts from Questionnaire Form 1010.4, dated November 15, 1943 (better known as Job Sheet), together with an affidavit duly executed on February 2, 1965, by Mr. Sacket T. Duryee, Administrative Assistant, War Department; also Circular No. 10 dated November 12, 1942, which lists the location of the various stations (installations) under my jurisdiction, at which I conducted monthly periodic safety and health field inspections.

Four other Federal installations are not listed thereon because they were highly secret establishments. Nevertheless, at this point I may say Fort Detrick was one of those such stations. The Fort Detrick secret experimental projects (which had a code name) we never talked about nor reduced anything to writing--complete silence was the key word at all times.

Furthermore, regarding the facts that have now been revealed to a degree, in the fall of 1959 during the Summit Conference between Premier Nikita Khrushchev and President Eisenhower, held at Camp David, Maryland, but less than nine miles away, it was discovered that Ralph T. Powell, a young soldier stationed at Fort Detrick, the Army Biological Warfare Headquarters, located at Frederick, Maryland, had contracted a plague. This soldier was released from the base hospital in about a month after being stricken.

The plague case was quickly diagnosed as of a pneumonic type rather than bubonic, often referred to in biblical times as the "Black Plague," which in times past almost wiped out whole nations. Only the highest levels of the Army knew anything about this outbreak of the plague, and it was, as I understand, very successfully covered up for fear of the magnitude of any international ramifications with Russia.

If need be, no doubt I could round up several former O.S.S. agents to reveal background information on their O.S.S. Agency's operation, as well as to point out the center of responsibility before it became the Central Intelligence Agency.

EXHIBIT F

SKELETONS DO A DANCE OF DEATH OVER ENGLISH CORPSES FELLED BY PLAGUE IN 1348



EXHIBIT G

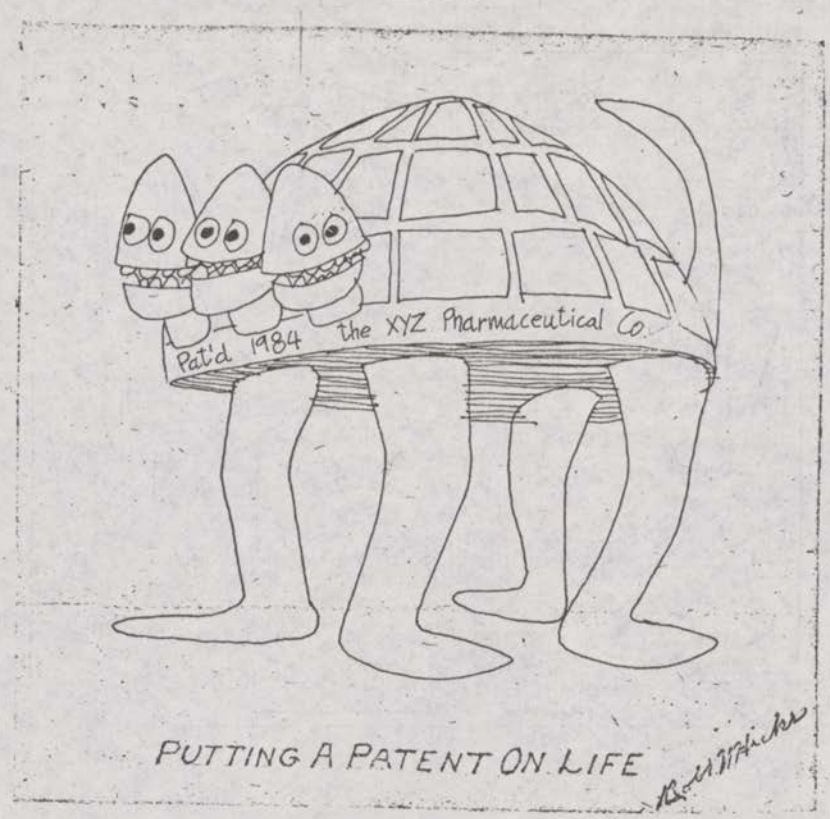

Pat'd 1984 the XYZ Pharmaceutical Co.
PUTTING A PATENT ON LIFE

EXHIBIT H

U.S. DEPARTMENT OF COMMERCE
Patent Office

Address Only: COMMISSIONER OF PATENTS
Washington, D.C. 20231

This is to certify that

ROBERT W. HICKS

Whose address is 1920 Eye St., N.W.,
Washington, D. C. 20006

was on the 17th day of July 1952 registered to practice before the United States Patent Office as an attorney (Registration No. 17,204) and is now in good standing.

In Testimony Whereof, I have hereunto set my hand and caused the seal of The United States Patent Office to be hereunto affixed, at the City of Washington, this fourth day of November, in the year of Our Lord nineteen hundred and seventy-five.

Mariana E. Ford
Clerk, Committee on Enrollment

FORM PO-20815-701

USCOMM-DC 61[illegible]-P70

EXHIBIT I

UNITED STATES COURT OF CUSTOMS AND PATENT APPEALS

-oOo-

Certificate of Membership

-o-

I, CABELL N. PRYOR, Assistant Clerk of the United States Court of Customs and Patent Appeals, do hereby certify that ROBERT W. HICKS of Washington 6, D. C., was duly admitted to practice in this court on March 3, 1947, and that he has been continuously since that date an attorney of this court in good standing.

Witness my hand and the seal of this court this fifth day of June, A. D., 1947.

Cabell N. Pryor
Assistant Clerk

INTERIM REPORT OF THE
FEDERAL INTERAGENCY COMMITTEE ON RECOMBINANT DNA RESEARCH:
SUGGESTED ELEMENTS FOR LEGISLATION
March 15, 1977

I. Introduction

Recent scientific developments in genetics, particularly in the last four years, have culminated in the ability to join together genetic material from different sources in cell-free systems to form recombinant deoxyribonucleic acid (DNA) molecules. DNA is the material that determines hereditary characteristics of all known cells. Recombinant DNA research offers great promise for better understanding and improved treatment of human diseases. Medical advances through use of this technology include the opportunity to explore complicated diseases and the functioning of cells, to better understand a variety of hereditary defects, and possibly in the future, to create microorganisms useful in producing medically important compounds for the treatment and control of disease. Aside from the potential medical benefits, a variety of other applications in science and technology are envisioned. An example is the large-scale production of enzymes for industrial use. Potential benefits in agriculture include the enhancement of nitrogen fixation in certain plants and the biological control of pests, permitting increased food production.

There are risks in this new research area as well as anticipated benefits. A potential hazard, for example, is that the foreign DNA in a microorganism may alter it in unpredictable and undesirable ways. Should the altered microorganism escape from containment, it might infect human beings, animals, or plants, causing disease or modifying the environment.

Or the altered bacteria might have a competitive advantage, enhancing their survival in some niche within the ecosystem.

Until the potential risks are better delineated and evaluated in light of developing scientific knowledge, the public should expect such research to be conducted under strict conditions ensuring safety. This was the fundamental principle that guided the Federal Interagency Committee on Recombinant DNA Research in its deliberations--that is, the desire to allow this significant research to continue while simultaneously protecting, as much as humanly possible, man and the environment from effects of potential hazards whose nature is as yet unknown.

The Committee formally adopted this interim report by unanimous consent, save for abstentions by the representatives from the Council on Environmental Quality and the Department of Justice.

II. Development of the NIH Guidelines on Recombinant DNA Research

Approximately three years ago, because of the perceived potential hazards, scientists engaged in this research voluntarily called for a moratorium on certain experiments pending an assessment of risk and the development of appropriate guidelines. These scientists called upon the National Institutes of Health (NIH), of the Department of Health, Education, and Welfare, to create an advisory committee to develop such guidelines. After what NIH considered to be extensive scientific and public review, it released guidelines on June 23, 1976, which established strict conditions for the conduct of NIH-supported research in this area. The NIH Guidelines prohibit certain types of experiments and require special safety conditions for other types. The provisions

are designed to afford protection with a wide margin of safety to workers and the environment. The NIH Guidelines were published in the Federal Register on July 7, 1976, for public comment.

The NIH also prepared and filed in the Federal Register on September 9, 1976, a Draft Environmental Impact Statement on the Guidelines for public comment. The final NIH Environmental Impact Statement will be published shortly. In August 1976 the NIH published a volume containing the transcript of a public hearing held on the Guidelines as well as the correspondence received by the NIH Director on this matter prior to the release of the Guidelines in June.

III. Federal Interagency Committee on Recombinant DNA Research

The Interagency Committee on Recombinant DNA Research was created to address extension of the NIH Guidelines beyond the NIH to the public and private sectors. The Committee was convened by the Secretary of Health, Education, and Welfare with the approval of the President. Dr. Donald S. Fredrickson, Director of NIH, serves as chairman at the Secretary's request. The Interagency Committee is composed of representatives of Federal Departments and agencies that support or conduct recombinant DNA research, or that may do so in the future, and representatives of Federal Departments and agencies that have present or potential regulatory authority in this area. (The membership of the Committee is included in Appendix I.) The mandate of the Committee is to

(1) review the nature and scope of Federal- and private-sector activities relating to recombinant DNA research;

(2) determine the extent to which the NIH Guidelines may currently be applied to research in the public and private sectors;

(3) recommend, if appropriate, legislative or executive actions necessary to ensure compliance with the standards set for this research; and

(4) provide for the full communication and necessary exchange of information on recombinant-DNA-research programs and activities throughout the Federal sector.

Two meetings of the Committee were held in November 1976. The first of these, on November 4, was devoted to a review of the development of the NIH Guidelines for Research Involving Recombinant DNA Molecules. The Committee also reviewed activities in other countries on the development of guidelines for this research. Recombinant DNA research is being conducted in a number of countries, including Canada, the United Kingdom, most of Western Europe, the Scandinavian countries, Eastern Europe, the Soviet Union, and Japan.

In many countries appropriate governmental or scientific bodies have reviewed the research and have agreed that it should proceed. Several of the countries have acted to establish guidelines to govern the conduct of this research, including the United Kingdom and Canada. In the United Kingdom a parliamentary committee addressed the issue and indicated that work in this area should continue under appropriate safety conditions. Scientific advisory committees of international organizations, such as the World Health Organization, the International Councils of Scientific

Unions, and the European Molecular Biology Organization, have made similar recommendations.

The European Science Foundation, representing member nations from Western Europe and Scandinavia, has recommended to its members that they follow the guidelines of the United Kingdom. These guidelines are, in intent and substance, very similar to those of the National Institutes of Health. The NIH is currently working closely with the United Kingdom and the European Science Foundation to ensure a commonality of standards in the conduct of this research. Thus far, there has been very close cooperation and coordination among the various international and national scientific bodies, with a view to reaching a consensus on safety practices, programs, and procedures.

At the meeting of the Committee held on November 23, the Federal research agencies discussed their activities and possible roles in the implementation of the NIH Guidelines. All Federal research agencies endorsed the Guidelines to govern recombinant DNA research. At present, the NIH, the National Science Foundation, the Veterans Administration, and the U.S. Department of Agriculture are supporting or conducting such research. The NIH has 123 grants in which recombinant DNA research is involved. The National Science Foundation has 52 grants supporting such research in whole or in part. The Veterans Administration has eight projects. The Department of Agriculture and Agricultural Experiment Stations will soon have an estimate of the number of projects in their area. The Department of Defense, the National Aeronautics and Space Administration, and the Energy Research and Development Administration do not at present conduct such research, but all have endorsed the NIH Guidelines to govern future research should it be undertaken.

IV. Subcommittee Review of Existing Legislation

At the November 23 meeting of the Interagency Committee, the Federal regulatory agencies also reported on their regulatory functions. Following that review, a special Subcommittee was formed to analyze the relevant statutory authorities for the possible regulation of recombinant DNA research. All regulatory agencies were represented on the Subcommittee, assisted by attorneys from their offices of general counsel. (See Appendix II for the membership of the Subcommittee.) The Subcommittee held meetings on December 13, 1976, and on January 11 and February 8, 1977.

The Subcommittee was charged to determine whether existing legislative authority would permit the regulation of all recombinant DNA research in the United States (whether or not Federally funded) and would include at least the following regulatory requirements:

(1) review of such research by an institutional biohazards committee before it is undertaken,

(2) compliance with physical and biological containment standards and prohibitions in the NIH Guidelines,

(3) registration of such research with a national registry at the time the research is undertaken (subject to appropriate safeguards to protect proprietary interests), and

(4) enforcement of the above requirements through monitoring, inspection, and sanctions.

It was the conclusion of the Subcommittee that present law could permit imposition of some of the above requirements on much recombinant DNA laboratory research, but that no single legal authority or combination of

authorities currently exists that would clearly reach all research and other uses of recombinant DNA techniques and meet all the requirements. The complete Subcommittee analysis is included in Appendix III. The Subcommittee, in reaching this conclusion, reviewed the following laws that were deemed most deserving of detailed consideration:

(1) the Occupational Safety and Health Act of 1970 (Public Law 91-596),

(2) the Toxic Substances Control Act (Public Law 94-469),

(3) the Hazardous Materials Transportation Act (Public Law 93-633),

(4) Section 361 of the Public Health Service Act (42 U.S.C. Sec. 264).

The Occupational Safety and Health Act gives the Occupational Safety and Health Administration (OSHA) broad powers to require employers to provide a safe workplace for their employees. The term "employer" in the Act, however, is defined in such a way as to exclude States and their political subdivisions unless the OSHA standards are voluntarily adopted. Twenty-four States have adopted the standards, but twenty-six states are not subject to them. Further, the OSHA standards do not cover self-employed persons. For these reasons it was determined that OSHA at present could not regulate all recombinant DNA research.

The Environmental Protection Agency, under the Toxic Substances Control Act, is directed to control chemicals that may present an "unreasonable risk of injury to the health or the environment." The Subcommittee determined that the materials used in recombinant DNA research

would appear to be covered in most cases by the Act's definition of "chemical substance." Section 5 of the Act, however, explicitly exempts registration of chemical substances used in small quantities for the purposes of scientific experimentation or analysis. This represents a most serious deficiency, as the registration of activities was thought to be an essential element of any regulatory effort. Also, in order to meet the specifications of the Act, recombinant DNA research would have to be found to present "an unreasonable risk of injury to health or the environment."

The Hazardous Materials Transportation Act (HMTA) and Section 361 of the Public Health Service (PHS) Act give the Department of Transportation (DOT) and the Center for Disease Control (CDC), respectively, authority to regulate the shipment of hazardous materials in interstate commerce. Both the DOT and the CDC, in implementing these acts with respect to biological products, have essentially aimed at imposing labeling, packaging, and shipping requirements, and were found to be wanting for regulation of all recombinant DNA research.

The Environmental Defense Fund, in November 1976, petitioned the DHEW to regulate recombinant DNA research under Section 361 of the PHS Act. (The petition is included in Appendix IV.) The Subcommittee carefully reviewed this section, which is directed to organisms that are communicable and cause human disease. Thus, under this section, there would have to be a reasonable basis for concluding that the products of all recombinant DNA research may cause human disease and are communicable. Further, Section

361 does not apply to plants, animals, or the general environment. It was the conclusion of the Subcommittee that Section 361 lacked the requisite authority to meet all of the requirements set for the regulation of this research.

The Subcommittee also considered the authority of the CDC to license and control the operation of clinical laboratories under Section 353 of the PHS Act, but this provision was not considered to be applicable to research laboratories.

Other authorities of EPA under the Clean Air Act, the Federal Water Pollution Control Act, and the Resource Conservation and Recovery Act of 1976 were considered briefly and thought only to apply, if at all, to isolated aspects of recombinant DNA research. The authorities of the Food and Drug Administration (FDA) were also reviewed, but it was concluded that recombinant DNA research has not yet reached the stage of commercial application that comes under the FDA's jurisdiction. The regulatory powers of the U.S. Department of Agriculture (USDA) were also reviewed and found applicable solely to nonhuman animals and plants.

In summary, the group concluded that no single legal authority, or combination of authorities, currently exists which would clearly reach all recombinant DNA research in a manner deemed necessary by the Committee. Although there is existing authority that might be broadly interpreted to cover most of the research at issue, it was generally agreed that regulatory actions taken on the basis of any such interpretation would probably be subject to legal challenge.

After completing an analysis of existing legislation, the Subcommittee on February 8, 1977, considered elements which might be

included in legislation to regulate recombinant DNA research. The Subcommittee referred the analysis of existing legislation and elements for new legislation to the full Committee at a meeting held on February 25, 1977. The full Committee adopted the report of the Subcommittee on existing legislation and agreed that new legislation was required.

V. Suggested Elements for Legislation

In considering the elements for legislation, the Committee reviewed Federal, State, and local activities bearing on the regulation of recombinant DNA research. Among congressional proposals reviewed were Senate Bill 621, "The DNA Research Act of 1977," introduced by Senator Dale Bumpers, and the companion measure introduced by Representative Richard L. Ottinger in the House (H.R. 3591). The Committee also noted the resolution (H. Res. 131) introduced by Representative Ottinger on January 19, 1977, requesting DHEW to regulate recombinant DNA research under Section 361 of the PHS Act.

Hearings held by State and local governments, including State legislatures, were among State and local activities reviewed. Recommendations for State regulation by the New York State Attorney General's Environmental Health Bureau, and for city regulation by the Cambridge (Massachusetts) City Council, were also considered.

Several committee representatives also reported on meetings with other interested parties whose views had been solicited on legislation to regulate recombinant DNA research. Those who were contacted include agricultural scientists, biomedical scientists, environmentalists, labor

unions, and private industry. At the request of the Chairman of the Committee, the Industrial Research Institute and the Pharmaceutical Manufacturers Association are surveying their member firms to determine the scope of the research efforts in the private sector. The Pharmaceutical Manufacturers Association has adopted the NIH Guidelines for safe conduct of this research.

In light of this review, the full Committee recommends that the following elements should be included in proposed legislation for the regulation of recombinant DNA research:

(1) Definitions:

"Recombinant DNA molecules" should be defined in a manner consistent with the NIH Guidelines.

Through an appropriate definition of the term "person," the legislation should cover any individual, corporation, association, Federal, State, or local institution or agency, or other legal entity.

"Secretary" should mean the Secretary of Health, Education, and Welfare.

(2) General requirements:

The legislation should bar any person from engaging in the production or use of recombinant DNA molecules in a State of the United States, in the District of Columbia, the Commonwealth of Puerto Rico, the Virgin Islands, American Samoa, Guam, the Trust Territory of the Pacific Islands, Wake Island, Outer Continental Shelf Lands as defined in the Outer Continental Shelf Lands Act, Johnston Island, or the Canal Zone, unless (a) such production or use is permissible under standards promulgated by the Secretary, (b) such production or use is in compliance with any such

standards, (c) the licensing requirements prescribed in the legislation have been satisfied, and (d) the registration requirements prescribed in the legislation have been satisfied.

The legislation should permit the Secretary to exempt activities from these requirements (a) where the activity is for specific commercial purposes found by the Secretary, after consultation with the regulating agency, to be regulated under other Federal law, or (b) where the Secretary determines that the activity poses no unreasonable risk to health or the environment.

(3) Standards:

The Secretary should be directed, as soon as practicable after passage of the legislation, to promulgate the NIH Guidelines for Research Involving Recombinant DNA Molecules as initial standards, with such clarifications and modifications as the Secretary determines to be necessary. Standards should assure, on the basis of the best currently available evidence, that no employee will suffer material impairment of health or functional capacity even if such employee engages in the production or use of recombinant DNA molecules for an entire working lifetime.

The legislation should authorize the Secretary to modify and revoke any of these initial standards and to promulgate new standards.

The legislation should include an appropriate provision for judicial review.

(4) Licensure of laboratories:

The legislation should bar any person from engaging in the production or use of recombinant DNA molecules except at a facility licensed by the Secretary. A license should not be issued unless the Secretary determines that the facility will be operated in accordance with standards promulgated under the legislation and such other conditions as the Secretary deems appropriate.

The Secretary should have authority to exempt from the licensure requirement categories of activity which he determines pose no unreasonable risk to health or the environment. He should also, at his discretion, be able to utilize qualified accreditation or licensing bodies to assist him in carrying out this licensing function.

The legislation should have appropriate provisions for revocation, suspension, and limitation of licenses and for judicial review.

(5) Registration:

The legislation should bar any person from engaging in the production or use of recombinant DNA molecules unless the activity has been registered with the Secretary, provided that the Secretary should be able to exempt from the provisions of this section categories of production or use which he determines pose no unreasonable risk to health or the environment.

(6) Imminent hazards:

The Secretary should have authority to sue to enjoin the production or use of recombinant DNA molecules where he believes the activity would constitute an imminent hazard to health or the environment.

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(7) Inspections, subpoenas, record-keeping, and reports:

The Secretary, in carrying out the legislation, should have authority to inspect facilities, make environmental measurements, conduct medical investigations, inspect medical records, issue subpoenas and citations, and require record-keeping and reports.

(8) Disclosure of information:

The legislation should provide that all records submitted to, or otherwise obtained by, the Secretary or his representatives under the legislation shall be available to the public upon request, except (a) information now exempt from disclosure under the Freedom of Information Act, and (b) other information the disclosure of which would cause the loss of proprietary rights.

At the time of request, persons who have submitted records should be given an opportunity to identify those portions which they believe to be excepted from disclosure under the preceding paragraph. The Secretary should not release such portions unless (a) he has found the portions so identified not to be excepted and has given the submitter advance notice of this finding and an opportunity to rebut it, or (b) the public need to know so outweighs the interest of the submitter as to require release. Where the Secretary releases records or portions thereof because of the public need to know, he should notify the submitter, setting forth the urgent health or environmental needs which serve as the basis for his action.

(9) Coordination:

The legislation should provide specifically for interagency coordination in setting standards and avoiding duplicative requirements.

(10) Preemption:

The legislation should specifically preempt all State and local laws regulating the production or use of recombinant DNA molecules; except that where a State passes a law imposing requirements identical to those contained in the Federal legislation, the Secretary should have discretion to enter into an agreement with the State to carry out the Secretary's responsibilities under the legislation.

(11) Enforcement:

The legislation should contain provisions for enforcement and sanctions.

(12) Employee rights:

The legislation should contain protections for employees who cooperate in the enforcement of these provisions.

(13) Sunset:

The legislation should remain in effect for a period of five years from the date of enactment, unless further action is taken by Congress.

VI. Suggested Elements for Legislation: Committee Analysis

In considering these elements for proposed legislation, a number of issues were raised and discussed by the Committee. The issues that the Committee considered of importance are described below.

(1) Definition of the Term "Secretary":

The Committee considered the appropriate locus in the Government for the regulation of the use and production of recombinant DNA molecules. It determined that the Department of Health, Education, and Welfare

is the appropriate locus in light of

(a) NIH's role as a lead agency in setting the standards,

(b) the petition by the Environmental Defense Fund to DHEW to issue regulations in this area,

(c) the congressional proposals that placed regulatory responsibility in DHEW, and

(d) the experiences of DHEW's Center for Disease Control in regulating infectious agents, and of its Bureau of Biologics (FDA) in licensing the production of biological products, in close cooperation with other Federal Departments and agencies.

This recommendation was formally approved by all members of the Committee. The Committee also urges close cooperation and coordination in DHEW between the NIH and regulatory agencies to ensure effective implementation of the standards set for this research.

(2) The Scope of Regulation:

The Committee reviewed at great length the nature and scope of regulation. Consideration was given to regulation of all laboratory research where hazardous or potentially hazardous substances were employed. Dr. Fredrickson reviewed the activities of committees at the NIH other than the Recombinant DNA Molecule Program Advisory Committee which have been created to study and recommend, if necessary, safety standards for other research involving actual or potential biohazards.

There was general Committee agreement that, for the present, legislation should be restricted to recombinant DNA techniques, allowing for sound administrative and scientific expertise in developing safety standards

and regulation in other areas. The Committee considered whether, in the proposed legislation, the regulations should be limited to research. As noted above in the analysis of existing legislation, no current single, legal authority reaches all research under requirements set for regulation by the Committee. However, the Occupational Safety and Health Administration and the Environmental Protection Agency do have authority for regulation of commercial applications of recombinant DNA molecules.

Regulation of research alone presents a problem because of the difficulty in determining the border between research and pilot production. Therefore, the Committee recommends that regulation cover the production or use of recombinant DNA molecules. Such language would include research activity, and makes immaterial any consideration of whether a given activity constitutes research, pilot production, or manufacture. The Committee recommends that the Secretary, in consultation with appropriate regulatory agencies, be allowed to determine the nature of the activity and should defer to a regulatory body he determines is better empowered and equipped to deal with it.

The Committee also recommends as a suggested element for legislation a "sunset provision" for the regulatory authority. This provision is intented to mandate a review of regulation in light of accumulated scientific and safety information. This provision, the Committee wishes to emphasize, does not refer to records and other data relevant, for example, to medical, occupational, or environmental surveillance.

(3) Registration:

There was general agreement by the Committee that registration of projects and other activities involving the use or production of recombinant

DNA molecules was an important element of regulation. It was the consensus of the Committee that registration should occur prior to the initiation of the project, but that approval before commencing the project should not be required. Further, the Committee recommends that the Secretary have the authority to exempt certain classes of projects from this requirement.

(4) Licensure of Facilities:

It was the consensus of the Committee that the licensure provision should apply only to facilities, and that the facility would, under the terms of its license, accept responsibility for the particular activities and individuals at the facility. The Committee concluded that licensure of the facility and registration of projects would meet the needs for safety monitoring without extension of licensure to the projects themselves. The Committee discussed the possibility of revoking a license for serious and willful violations of the regulations. There was concern expressed that revocation was a very punitive measure, but it was agreed that the Secretary may wish to consider it for serious violations of the standards.

(5) Disclosure of Information:

It was the scientific community that brought to public attention potential hazards of recombinant DNA research, and the NIH Guidelines, in that spirit, promote disclosure and dissemination of scientific and safety information. The Committee urges full disclosure to the appropriate regulatory body of all relevant safety and scientific information on the use or production of recombinant DNA molecules. However, the Committee

recognizes the important world-wide commercial potential of recombinant DNA molecules in medicine, agriculture, and other areas of science and technology. It believes that the potential commercial uses of recombinant DNA techniques require that information of a proprietary nature and patent rights be given appropriate protection from disclosure by the regulatory agency receiving such information. Some Committee members expressed concern that universities and inventors with limited resources may be unable to adequately protect data of a proprietary nature if the regulatory agency acts to disclose such information. The regulatory agency should consider the burden of its action on these inventors.

(6) Preemption of State/Local Laws:

The potential hazards posed by the use of recombinant DNA techniques extend beyond the local to the national and international levels. Therefore, the Committee recommends that a single set of national standards must govern and that, accordingly, local law should be preempted to ensure national standards and regulations. The Committee, however, took into account the activities at the State and local levels on regulation of recombinant DNA research. It was agreed that if a State passes a law imposing requirements identical to those contained in the Federal legislation, then the Secretary may enter into an agreement with the State to utilize its resources to assist the Secretary in carrying out his duties.

(7) Inspection and Enforcement:

The Committee proposes that there be inspection and enforcement requirements to ensure that standards are being met. In order to protect

the public health from an imminent potential hazard, the Committee also recommends that the Secretary have authority to enjoin the use or production of recombinant DNA molecules when he deems it necessary.

The Committee also reviewed the question of civil liability in the event of injury to humans or the environment. It believes that actions for damages should be left to State and local law. It is concerned that the inclusion of standards for strict liability as proposed in S. 621 could place a severe constraint on the ability of an institution to obtain liability insurance. It was predicted that, without insurance, institutions might have to terminate their research efforts unless national legislation were passed to indemnify them against adverse judgments.

(8) Interagency Cooperation:

Because of the wide potential use and production of recombinant DNA molecules and the need for uniform development and implementation of standards, the Committee recommends that mechanisms be established by the Secretary to ensure cooperation and coordination among appropriate Federal Departments and agencies. The National Institutes of Health is developing appropriate liaison between its Recombinant DNA Molecule Program Advisory Committee and relevant Federal research agencies, such as the Department of Agriculture, the National Science Foundation, and the Energy Research and Development Administration.

VII. Future Agenda

Pending action on possible legislation, the Committee stands ready to assist DHEW or whatever agency is made responsible for regulation of activities involving the use or production of recombinant DNA molecules.

For example, research agencies on the Committee are working in coordination with the National Institutes of Health and its Recombinant DNA Molecule Program Advisory Committee on setting standards and certifying new host-vector systems. The research agencies have also been developing a registry of projects supported by Federal funds. The survey being taken in the private sector by the Pharmaceutical Manufacturers Association and the Industrial Research Institute will provide data on the industry, in anticipation of registration under a new law.

The Committee will consider suggestions by the representatives from the State Department concerning further means to ensure international control in the use and production of recombinant DNA molecules. At present, there is voluntary coordination and cooperation among national scientific bodies. The Biological Weapons Convention is considered by the State Department to prohibit development, production, or stockpiling of recombinant DNA molecules for purposes of biological warfare. The Committee will review whether other measures need to be considered for international control.

The Committee will also be reviewing current Federal policies on the matter of patenting recombinant DNA inventions and other matters of concern that may need to be addressed before the Committee concludes its business and files a final report.

January 14, 1977

APPENDIX I

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APPENDIX III
REGULATION OF RECOMBINANT DNA
RESEARCH IN LABORATORIES

BACKGROUND

On December 20, 1976 a meeting was held at NIH of attorneys 1/ from the Departments of Justice, Agriculture, HEW, Labor, and Transportation and the Environmental Protection Agency, for the purpose of assessing whether legislative authority currently exists for imposing at least the following regulatory requirements on all recombinant DNA laboratory research in the United States (whether or not Federally funded):

1. Review and approval of such research before it is undertaken by a local biohazards committee.

2. Compliance with the physical and biological containment standards and prohibitions in the NIH Guidelines.

3. Registration of such research with a national registry at the time the research is undertaken (subject to appropriate safeguards to protect proprietary interests).

4. Enforcement of the above requirements through monitoring, inspection, and sanctions.

It was generally conceded for purposes of the discussion that these requirements could be imposed by funding agencies on Federally conducted or supported research, and primary attention was therefore directed to whether authority now exists to mandate these requirements

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1/ A list of attendees is attached.

for all recombinant DNA laboratory research without regard to the source of funding.

SUMMARY CONCLUSION

In summary, the group concluded that, while present law would permit imposition of some of the above requirements on much recombinant DNA laboratory research, no single legal authority or combination of authorities currently exists which would clearly reach all such research and all requirements. Although there is existing authority which could be interpreted to cover most of the research at issue, it was generally agreed that regulatory actions taken on the basis of any such interpretation would probably be subject to legal challenge.

LAWS CONSIDERED

In reaching this concensus, discussion centered on the following laws:

1. The Occupational Safety and Health Act of 1970 (Public Law 91-596).
2. The Toxic Substances Control Act (Public Law 94-469).
3. The Hazardous Materials Transportation Act (Public Law 93-633).
4. Section 361 of the Public Health Service Act (42 U.S.C. §264).

Authorities of EPA under the Clean Air Act, the Federal Water Pollution Control Act, and the new Resource Conservation and Recovery Act of 1976, were mentioned in passing, but it was felt they would apply, if at all, only to isolated aspects of recombinant DNA research carried out in the laboratory. The FDA was also discussed briefly. However, inasmuch as recombinant DNA research has not yet reached the stage where it has practical applications in fields regulated by FDA, it was agreed that FDA probably does not have authority to impose requirements on such research. The Department of Agriculture's regulatory powers were also touched upon, but not considered in depth because they relate solely to certain forms of non-human animal life and plants.

DEPARTMENT OF LABOR

Of the four statutes on which discussion centered, primary attention was directed to the Occupational Safety and Health Act and the Toxic Substances Control Act, because each on its face would give broad powers to the administering agencies.

The Occupational Safety and Health Act, administered by the Occupational Safety and Health Administration (OSHA) in the Department of Labor, requires every employer to: (1) furnish to each of its employees ". . .employment and a place of employment which are free from recognized hazards that are causing or are likely to cause death

or serious physical harm. . ." to the employees, and (2) ". . .comply with occupational safety and health standards promulgated under this Act." The aforesaid Act gives OSHA broad power to enforce compliance with the Act, including a right of entry, authority to require record-keeping and reports, and sanctions. In addition, the Act specifically directs that trade secret information shall be treated by OSHA as confidential.

The term "employer" is defined in such a way, however, as to exclude States and their political subdivisions, as well as the United States. The Act contains a separate provision requiring Federal agencies to follow OSHA standards, but States and their subdivisions are subject to OSHA requirements only by voluntary agreement on the part of each State. Only 24 States have so agreed and there is no immediate expectation that this number will increase. Hence, such organizations as State universities in 26 States are not subject to OSHA requirements.

In addition, OSHA has authority only in cases where an employment relationship exists. Hence, it could not prevent a self-employed person from conducting recombinant DNA research as long as no employees would thereby be affected.

Turning to the requirements themselves, since recombinant DNA research does not necessarily present a "recognized hazard" with respect to all areas of that research, and because of possible litigation problems in proving a recognized hazard, the imposition of all NIH Guidelines on employers can best be achieved by adopting them

as standards, in accordance with detailed rulemaking procedures spelled out in the Act. Among the policy questions which would have to be resolved in contemplating this step are: (1) whether OSHA would agree to an outright ban of some activities since it has never in the past actually prohibited a total activity, and (2) whether OSHA should give priority to establishment of these standards over others that have been awaiting promulgation, taking into account the statutory test of ". . . urgency of the need for mandatory safety and health standards for particular industries, trades, crafts, occupations, businesses, workplaces or work environments."

ENVIRONMENTAL PROTECTION AGENCY

The Toxic Substances Control Act (TSCA), primarily administered by EPA, was enacted in October 11, 1976, effective January 1, 1977. Section 6 of the TSCA states in part that:

> "If the Administrator [of EPA] finds that there is a reasonable basis to conclude that the manufacture, processing, distribution in commerce, use, or disposal of a chemical substance or mixture, or that any combination of such activities, presents or will present an unreasonable risk of injury to health or the environment, the Administrator shall by rule apply one or more of the following requirements to such substance or mixture to the extent necessary to protect adequately against such risk using the least burdensome requirements:
>
> (1) A requirement (A) prohibiting the manufacturing, processing, or distribution in commerce of such substance or mixture, or (B) limiting the amount of such substance or mixture which may be manufactured, processed, or distributed in commerce.

> (2) A requirement--
>
> (A) prohibiting the manufacture, processing, or distribution in commerce of such substance or mixture for (i) a particular use or (ii) a particular use in a concentration in excess of a level specified by the Administrator in the rule imposing the requirement, or
>
> (B) limiting the amount of such substance or mixture which may be manufactured, processed, or distributed in commerce for (i) a particular use or (ii) a particular use in a concentration in excess of a level specified by the Administrator in the rule imposing the requirement."

The TSCA contains inspection and penalty provisions, and a section limiting disclosure of data.

NIH scientists agree that materials used in recombinant DNA research in the laboratory, and the immediate products of such research, would appear to be covered in most cases by the definition of "chemical substance" in the TSCA. The term "manufacture" is defined as meaning ". . .to import. . .produce, or manufacture." The term "manufacture" does not normally connote scientific experimentation in the laboratory. Thus, some question could be raised as to whether section 6 has any applicability to such research. However, another section of the TSCA (section 5), which requires manufacturers to give EPA advance notice of plans to manufacture a new chemical substance, contains an exemption from the notice requirement for ". . .manufacturing or processing of any chemical substance. . .only in small quantities. . .solely for purposes of. . .scientific experimentation or analysis. . . ." The wording of this provision would seem to indicate that scientific

experimentation constitutes manufacturing under the TSCA, 2/ and also the absence of a similar provision in section 6 creates a negative implication that section 6 applies to such experimentation. Nevertheless, this is an area of some controversy that could well lead to future litigation in the event EPA attempts to regulate laboratory research.

In the event EPA can regulate recombinant DNA laboratory research under the TSCA, it can do so only if it finds such research presents an "unreasonable risk of injury to health or the environment." This offers another area of potential controversy should EPA attempt to regulate all such research through this mechanism. 3/

The most serious deficiency in the TSCA, as a vehicle for regulation of recombinant DNA laboratory research, is presented by section 5, noted above, which requires manufacturers to notify EPA when they intend to manufacture a new chemical substance. As has already been indicated, scientific experimentation is specifically exempted from this requirement. Since section 5 deals directly with notice, and in effect registration,

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2/ A point supported by the fact that section 5 has a separate definition of "manufacture" solely for purposes of that section, which is limited to manufacture "for commercial purposes." The quoted phrase does not appear in the more general definition applicable to section 6.

3/ On the other hand, the TSCA authorizes any person to commence a civil action to compel EPA to perform any act or duty under the TSCA which is not discretionary. In some cases, this could lead to litigation regardless of what course of action EPA adopts.

and provides an exemption for such experimentation, it is doubtful that section 6 could be utilized to require registration. Otherwise, the intent of Congress in enacting the exemption would be undermined.

DEPARTMENT OF TRANSPORATION
AND
CENTER FOR DISEASE CONTROL, HEW

The Hazardous Materials Transportation Act (HMTA) and section 361 of the PHS Act give the Department of Transportation and the Center for Disease Control, HEW, respectively, authority to regulate shipment of hazardous materials in interstate commerce. 4/ The HMTA authorizes the Secretary of Transportation to issue and enforce regulations governing any safety aspect of the transportation of hazardous materials, including but not limited to packing, repacking, handling, labeling, mailing, placarding, and routing, and the manufacture, fabrication, marking, maintenance, reconditioning, repair, or testing of packages or containers represented, marked, certified, or sold by certain persons for use in the transportation of certain hazardous materials. 5/

Section 361 authorizes the Secretary of HEW to ". . .make and

---

4/ Including intrastate commerce that affects interstate commerce.

5/ In the Federal Register for November 26, 1976, at page 52086, the Department of Transportation has asked for public comment as to whether it should expand the definition of "etiologic agents" in DOT regulations". . .to include biological materials (such as recombinant DNA) used in or derived from genetic studies."

enforce. . .such regulations as in his [the Secretary's] judgment are necessary to prevent the introduction, transmission, or spread of communicable diseases. . .from one State. . .into any other State. . . ."

Both DOT and CDC, in implementing the HMTA and section 361 with respect to biological products, have essentially aimed just at imposing labeling, packaging, and shipping requirements. This approach is in line with the statutory language which emphasizes movement. Section 361 could perhaps be interpreted more broadly to serve as legal support for more comprehensive regulation. However, in order to do so there would presumably have to be a reasonable basis for concluding that the products of all recombinant DNA research cause or may cause human disease. Such a conclusion would undoubtedly be tenuous at best, and it is unlikely that resulting requirements could be effectively imposed and enforced.

Under section 353 of the PHS Act, however, CDC does have general authority to license and control the operation of clinical laboratories. While this authority would not in general have applicability to research laboratories, CDC's experience in implementing this legislation, which imposes comprehensive requirements on clinical laboratories, could be of value in the implementation of any new legislation needed to regulate laboratories conducting recombinant DNA research.

OTHER ISSUES CONSIDERED

1. In the event new legislation is sought, a model for the

registration requirement may be found in the Federal Insecticide, Fungicide, and Rodenticide Act (Public Law 92-516), which sets forth a detailed procedure for registration of pesticides.

2. On the issue of protection of proprietary information submitted to the Government as part of the registration process, while the Freedom of Information Act (FOIA) provides in general that records in the possession of Government agencies are available to the public upon request, the FOIA does not apply to, among other things, ". . .trade secrets and commercial or financial information obtained from a person and privileged or confidential. . ." (exemption 4). Moreover, 18 U.S.C. §1905, part of the Federal criminal code, makes it illegal for a Government employee to disclose ". . .to any extent not authorized by law any information coming to him in the course of his employment. . . which information concerns or relates to the trade secrets, processes, operations, style of work, or apparatus. . .of any person, firm, partnership, corporation, or association. . . ." In Charles River Park "A," Inc., et al. v. The Department of Housing and Urban Development, et al., a 1975 decision, the United States Court of Appeals for the District of Columbia held that, where an agency record is exempt from FOIA disclosure by virtue of exemption 4 and the record contains information covered by section 1905, the record would be subject to the prohibition against disclosure in section 1905.

In Washington Research Project, Inc., v. Department of Health, Education, and Welfare, et al., the same court had ruled in 1974 that research designs submitted in certain NIMH grant applications are not "trade secrets" within the meaning of exemption 4. However, in that case the court noted". . .the burden of showing the trade or commercial character of the research design information was on the agency, and. . .it did not introduce a single fact relating to the commercial character of any specific research project. . . ." Thus, Washington Research Project would not appear to govern situations in which the agency could show that patentable information or similar proprietary matter was involved.

3. While it would be desirable from a scientific standpoint to retain the flexibility to modify at least some parts of the Guidelines without the delays attendant to the rulemaking process, most regulatory legislation must be implemented by regulations promulgated in accordance with the Administrative Procedures Act (APA) or similar rulemaking procedures. One approach which might overcome this problem would be to publish regulations which set forth general standards but rely on cross references to the Guidelines with respect to specific details. However, this could present enforcement problems because any enforcement action based on a cross reference could be challenged for noncompliance with the APA. For this reason, a regulatory agency would probably insist upon specificity in its regulations.

4. It was the general concensus of all attorneys present that, to the extent no statutory basis existed for regulating non-Federally funded recombinant DNA laboratory research, this could not be achieved by Executive Order of the President. 6/

5. There was a brief discussion of whether, if agency X could regulate one type of recombinant DNA research and agency Y could regulate another type, agency Y could delegate its authority to agency X so that there could be comprehensive regulation by one agency. No conclusion was reached as to whether such an arrangement was legally barred. However, the only instance of this which any attorney could recall took place in the context of a specific statutory provision allowing the agency (the Customs Service) to do so.

6/ Particularly insofar as the entity conducting the research received no Federal funds for other recombinant DNA research.

Mr. Anthony C. Liotta
Mr. Martin Green
Department of Justice

Mr. Alexander W. Samofal
Office of the General Counsel
Department of Agriculture

Mr. Charles I. Hadden
Ms. Joan Hollenbach
Office of the Solicitor
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Department of Health, Education, and Welfare

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Animal and Plant Health Inspection Service
Department of Agriculture

APPENDIX IV



Environmental
Defense
Fund

1525 18th Street, NW, Washington, D.C. 20036 • 202/833-1484

November 11, 1976

The Honorable David Mathews
Secretary
Department of Health, Education & Welfare
South Portal Building, Room 615 F
200 Independence Avenue, S. W.
Washington, D. C. 20201

Dear Dr. Mathews:

The Environmental Defense Fund and the Natural Resources Defense Council hereby submit to you a petition concerning the regulation of recombinant DNA research and technology. We would very much appreciate your giving this matter prompt attention.

Enclosed also are copies of letters from Dr. Robert L. Sinsheimer of the California Institute of Technology and Mr. Alan McGowan, President, Scientists' Institute for Public Information.

Sincerely yours,

Burke K. Zimmerman

Burke K. Zimmerman, Ph.D.
Staff Scientist
Environmental Defense Fund

CALIFORNIA INSTITUTE OF TECHNOLOGY

PASADENA, CALIFORNIA 91125

DIVISION OF BIOLOGY 156-29

October 28, 1976

Dr. A. Karim Ahmed
Natural Resources Defense Council, Inc.
15 West 44th Street
New York, New York 10036

Dear Dr. Ahmed:

I am pleased to support the petition of the Environmental Defense Fund and the National Resources Defense Council to the Secretary of Health, Education and Welfare concerning recombinant DNA activities. This petition has two components: the first requests the Secretary to promulgate interim regulations to make the present NIH Guidelines concerning recombinant DNA research binding on all parties engaged in recombinant DNA research in the United States. The second requests the Secretary to conduct a "legislative-type" hearing to obtain very broadly based testimony which might guide a reformulation of the present recombinant DNA Guidelines, taking into consideration issues not addressed and points of view not presented during their development.

The Guidelines have been developed out of the concept that there is a potential hazard to public health in certain forms of recombinant DNA research. It is evident that this hazard is not restricted to recombinant DNA research conducted with the aid of NIH (or other Federal) funds. I therefore support their extension to cover all research activity in this field, however supported and wherever performed. This research does not require elaborate facilities and large capital investment. There is, therefore, no reason to believe that it will be limited to large institutions or industrial concerns with proven records of responsibility. Further, the virtual certainty of the development of new techniques and of the extension of these techniques to additional organisms and higher life forms will require a free flow of information, a continuing updating of guidelines, and the continuing scrutiny of this field of research by a body which will endeavor to reflect the public interest.

The need to consider the reformulation of the Guidelines derives from the perception that they were developed from too narrow a perspective. In my opinion the Guidelines were developed to address solely the immediate medical

hazards that might arise in the conduct of such research. The Guidelines do not address what I perceive as the larger, potential ecological and evolutionary hazards implicit in this research. Nor do the Guidelines address the potential significance of the availability of this new technology - developed by scientists to solve their own scientific problems - to other diverse sectors of our society, which may wish to use it for their own ends.

I believe the Guidelines do not provide sufficient recognition of the fact that we are here creating novel living organisms - unprecedented in the evolutionary order. As living organisms they are self-perpetuating and destined to their own individual evolution. I do not believe we can predict the properties of these organisms - created by the fusion of genes from disparate species - or their subsequent evolution, or their impact, present and future, on the existent biosphere. We do not know that there is a hazard here but neither do we know there is not. If such hazard exists or develops it will be in this instance uniquely irreversible. I believe a thoughtful reformulation of the Guidelines to take these circumstances into account would be most appropriate.

Sincerely yours,

Robert L. Sinsheimer
Chairman

SCIENTISTS' INSTITUTE FOR PUBLIC INFORMATION
49 East 53 Street, New York, NY 10022 212/688-4050

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Official Publication:
Environment
Julian McCaull,
Publisher

November 5, 1976

Dr. Burke Zimmerman
Staff Scientist
Environmental Defense Fund
1525 18th Street N.W.
Washington, D. C. 20036

Dear Dr. Zimmerman:

The controversy over recombinant DNA research has brought one of the most important facets of biomedical research out into the open. Although there are substantial benefits that may accrue from the research, there is also the possibility of enormous costs, both short and long term.

The public is being asked to support this research, both with its tax dollars and by being in the physical vicinity of the recombinant DNA research laboratories. Fortunately, some public inquiry has begun in the form of open hearings on the subject. These public hearings have been held in Cambridge, New York City and San Diego, and have expressed deep concern over how and whether this research should be continued.

The public at large, however, is still in the dark concerning the relevant issues in the debate. The scientific jargon that accompanies the discussion within the scientific community is, at best, confusing to non-scientists. There is an overwhelming need for accurate, up-to-date information, with the issues clearly presented in terms understandable to all of us. The public, government officials, and members of the Legislature are in need of this information. Only with substantantive understanding of all the issues will effective programs and regulations be promulgated.

Public hearings are absolutely essential in this process of discussion and debate. The Scientists' Institute for Public Information wholeheartedly supports the petition of the Environmental Defense Fund and the Natural Resources Defense Council for the conduct of public hearings on recombinant DNA research.

Sincerely yours,

Alan McGowan
President

AM:m
cc: Dr. Karim Ahmed
Natural Resources Defense Council
15 W. 44th St.
New York, New York 10036

UNITED STATES OF AMERICA

BEFORE THE

DEPARTMENT OF HEALTH, EDUCATION AND WELFARE

PETITION OF ENVIRONMENTAL DEFENSE FUND, INC.
AND NATURAL RESOURCES DEFENSE COUNCIL, INC.
TO THE SECRETARY OF HEALTH, EDUCATION AND WELFARE
TO HOLD HEARINGS AND PROMULGATE REGULATIONS UNDER
THE PUBLIC HEALTH SERVICE ACT GOVERNING RECOMBINANT
DNA ACTIVITIES

The Environmental Defense Fund (EDF) and the Natural Resources Defense Council (NRDC) hereby petition the Secretary of Health, Education and Welfare (hereafter "the Secretary") under the authority granted him by §361 of the Public Health Services Act (42 U.S.C. §264) to hold public hearings and promulgate regulations governing recombinant DNA[1/] research and technology in which fragments of DNA from different organisms, cells or viruses are combined in novel ways and introduced into a living host organism or cell.

1/ DNA - deoxyribonucleic acid, the chemical substance which contains all genetic information.

Recombinant DNA technology permits the creation of organisms or viruses with an unprecedented genetic make-up which may have the potential of causing grave and irreversible harm to humans and the environment. The extent of our current knowledge does not allow us to predict all of the possible results of experiments involving the manipulation of genes. Because most of the present and proposed recombinant DNA research and technology involves the genetic modification of bacteria or viruses, there exists the potential danger of creating a highly deleterious communicable infectious agent that could be introduced into and spread among laboratory workers and/or the general population (see infra, pp. 9 - 12).

Recognizing the potential hazards inherent in recombinant DNA research, the National Institutes of Health (hereinafter "NIH") on 23 June, 1976 promulgated guidelines [1/] which prohibit certain experiments where the potential risks to human health are deemed to be particularly high, and require a graded set of safety procedures for all other experiments (see 41 Fed. Reg. No. 131, part II, pp. 27902-27943, July 7, 1976). NIH also filed a draft environmental impact statement (hereinafter the "impact statement") on 1 September, 1976, which sets forth some of the possible dangers of recombinant DNA research and technology (see 41 Fed. Reg. No. 176, pp. 38425-44, Sept. 9, 1976). NIH indicated that the guidelines are not a final statement of public policy on

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1/ The petitioners take no position at this time concerning the adequacy of the safety standards set forth in these guidelines.

recombinant DNA research and technology but rather the beginning of full public consideration of all relevant issues.

The guidelines apply only to recombinant DNA research supported by the NIH. While Dr. Donald Fredrickson, the director of NIH, has called on all government agencies and "all who support or conduct such research throughout the United States" (41 Fed. Reg. No. 131, p. 27906, July 7, 1976) to voluntarily adopt the NIH guidelines, only the National Science Foundation, Department of Defense, and the Energy Research and Development Administration haye formally done so.* Therefore, a significant portion of recombinant DNA research and technology is not covered by any mandatory set of safety procedures, leaving the public unprotected from its potential hazards. Furthermore, it is the position of the petitioners that the public did not have an adequate opportunity to participate in the basic policy decisions underlying the NIH Guidelines.

For these reasons, EDF and NRDC request that: (1) a public hearing of broader scope than those held this year at NIH be held on the questions of to what extent and under what conditions recombinant DNA research and technology should be allowed to proceed; (2) final regulations be promulgated based on the record of that hearing which would apply to all recombinant DNA research and technology in the

---

* Dr. Joe Perpich, National Institutes of Health, personal communication.

United States; and (3) the present NIH guidelines be promulgated immediately as interim relief regulations governing all parties conducting or supporting such research.

This document includes:

I. A description of the scope of this petition (p. 4);

II. A description of the petitioners (p. 6);

III. A discussion of the need to control recombinant DNA research and technology in the interest of public health (p. 7);

IV. A discussion of the legal basis for the regulation of recombinant DNA research and technology by the Secretary of HEW (p. 13); and

V. A description of proposed relief (p. 15).

I. Scope of the Petition

By this petition EDF and NRDC seek interim and final regulations which will protect the public from the potential hazards of uncontrolled recombinant DNA research and technology.

In this petition the term "recombinant DNA research and technology" means all procedures in which DNA fragments from two or more different organisms or viruses which do not normally recombine in nature are recombined in the laboratory and inserted into a living host cell or organism in such a way as to alter its genetic make-up. This includes, but is not limited to, any experiments involving transportation of or commercial use of recombinant

DNA molecules or the products derived therefrom. NRDC and EDF seek regulations governing all recombinant DNA research and technology including, but not limited to:

(a) All experiments discussed in the "Guidelines for Research Involving Recombinant DNA Molecules" issued by the National Institutes of Health on June 23, 1976 and published in the Federal Register Part II on July 7, 1976;

(b) All experiments in which chemically or enzymatically synthesized DNA is inserted into a living host, plasmid or virus; and

(c) All other procedures in which DNA from any two sources which do not normally exchange genetic information may function within the same cell.

NRDC and EDF seek regulations which would cover all persons and organizations conducting or supporting recombinant DNA research including, but not limited to:

1. Recipients of Research grants awarded by any agency within the Department of Health, Education and Welfare;
2. Private corporations;
3. Private and public universities; and
4. Other departments and agencies of the Federal Government.

II. Petitioners

Petitioner Environmental Defense Fund, Inc., is a not-for-profit public-benefit membership corporation organized and existing under the laws of the State of New York. Its principal office and place of business is located at 162 Old Town Road, East Setauket, New York. It also maintains offices in Washington, D.C.; New York, New York; Denver, Colorado; and Berkeley, California. EDF has a nationwide membership of over 40,000 persons, composed of scientists, educators, lawyers, and other citizens dedicated to the protection of the environment and the wise use of natural resources. Many of these persons and their children will be subjected to the increased risk of adverse health effects discussed in at pp. 9 - 12, infra, if the Secretary does not adopt effective regulations controlling the relevant procedures. By its activities, EDF seeks the preservation and restoration of environmental quality and the protection of the country's natural resources on behalf of the general public. Its objectives include combining "the best scientific findings with the most appropriate social action discovered by the social sciences and legal theory in order that practical decisions shall be made which shall best promote a quality environment." (EDF By-laws, Art. 1:2(d)).

Petitioner Natural Resources Defense Council, Inc., is a not-for-profit, tax-exempt corporation organized under the laws of the State of New York, with offices at 15 West 44th Street, New York, New York; 917 15th Street, N.W., Washington, D.C.; and 2345 Yale Street, Palo Alto, California. NRDC is a national organization dedicated to environmental protection, including

protection of the human environment. NRDC has 24,000 members and contributors in the United States. Many of these persons and their children will be subjected to the increased risk of adverse health effects discussed in pp. 9 - 12, infra, if the Secretary does not adopt effective regulations controlling the relevant procedures. Among the methods NRDC uses to achieve its objectives are: (1) improving federal agency decision-making which affects the environment by commenting, furnishing information, participating in administrative proceedings, and bringing lawsuits where legal duties are not being fulfilled; and (2) improving federal agency decision-making which affects the environment by encouraging agencies to solicit and utilize the views, knowledge, and expertise of members of the general public.

III. The Need to Control Recombinant DNA Research and Technology in the Interest of Public Health

The techniques defined above enable scientists to recombine the DNA from two unrelated species and, thus, construct organisms which may express genes from biologically unrelated sources. Because the properties of such deliberately or accidentally constructed organisms are unknown and may represent hitherto nonexistent hazards both to human health and the ecology, members of the scientific community have raised the questions of whether or not proceeding with this type of research at this time is prudent, and, if so, whether or not the public and the environment can be adequately protected

from potentially hazardous novel organisms which might arise from such research.

Addressing these questions, NIH formed a committee (the Recombinant DNA Molecule Program Advisory Committee) composed of scientists, many of whom were directly involved in recombinant DNA research, to draft guidelines governing the conduct of recombinant DNA research and establish safeguards to protect the public and the environment from potential hazards. The guidelines, applying only to NIH supported research, were made public June 23, 1976. Recognizing the far-reaching environmental consequences which could result if infectious or otherwise dangerous organisms able to compete successfully with existing organisms were to be produced by recombinant DNA research, and in response to requests from the public, NIH prepared a Draft Environmental Impact Statement which was released September 1, 1976.

The Impact Statement, in discussing the alternative of "no action," unambiguously concludes that regulation of recombinant DNA research and technology is essential for the protection of the public:

> "the 'no action' alternative would greatly increase the probability that possible hazardous organisms would be released into the environment. . . . It is concluded that the 'no action' alternative would not afford adequate protection of laboratory workers, the general public, and the environment from the possible hazards described in section IV-C-1." (at p. 48).

Some of the possible hazards which could arise either directly or as an inadvertent result of recombinant DNA research are discussed in Section IV-C of the Impact Statement. One may

expand this list to include additional untoward health effects. The following are examples of potential threats to human health which could result from recombinant DNA research and technology:

1. Most of the proposed and ongoing recombinant DNA research involves strains of the bacterium Escherichia coli (E. coli) as a host for plasmids containing DNA from other sources. E. coli is a common resident of the human colon, is responsible for nearly 100% of human upper urinary tract infections[1] and for approximately 30-40% of the cases of sepsis[2] (infection of the human bloodstream), which is often fatal. While the strains of E. coli used in recombinant DNA research (variants of strain K-12) do not normally colonize the human colon, they can under unusual conditions, particularly in patients weakened by another disease state. Perhaps more serious, however, is the capacity of K-12 strains of E. coli to exchange DNA with other similar or related organisms.[3] Genetic exchange between E. coli and strains of Salmonella,[4] a human pathogen, is well documented. Since the genetic determinants in infectivity and virulence of bacteria are not understood, one must consider the possibility that even a seemingly trivial modification of the E. coli genome might greatly alter its capacity for infection and propagation within humans.

1/ B. D. Davis, et al., Microbiology 768 (2nd ed. 1973).

2/ Dr. Halsted Holman - Oral testimony before a hearing of the Subcommittee on Health of the Senate Committee on Labor and Public Welfare, Sept. 22, 1976

3/ Davis, et al., supra at 182-200.

4/ Id. at 194.

In view of the ubiquitous nature of E. coli, the fact that all strains including K-12 already have the capacity for human infection, and E. coli's ability to exchange genetic material with other bacteria, the deliberate genetic modification of even "weakened" strains of E. coli poses a potentially serious threat to human health.

2. DNA can be taken from organisms that produce toxins (e.g. botulinum) creating the possibility that the host organism, which occupies a different ecological niche, will acquire the ability to produce the toxin. This would be particularly serious if such genes were expressed in strains of E. coli capable of colonizing the human colon.

3. Genes which code for resistance to antibiotics are transferred by some recombinant DNA experiments to strains of bacteria that were not previously resistant.

4. The animal virus on which the most genetic information is available is simian virus 40 (SV-40), which produces tumors in some animals and infects humans, although apparently with no pathological symptoms. However, the genetic basis for the virus causing tumors in monkeys but not humans is not understood. Therefore, the possibility exists that even an apparently innocuous modification of SV-40 DNA could render the virus tumorigenic or otherwise pathogenic to humans, thus creating a serious hazard to human health. Yet it is SV-40, and polyoma

virus, which also produces tumors in animals, which are the primary objects of recombinant DNA research in animal viruses.

5. The virulence of influenza virus, and the spontaneous occurrence in nature at certain times of devastating flu epidemics (such as the one of 1918) is apparently controlled by the reassortment in nature of the 12 subunits of the viral RNA[1/]. Yet the genetic basis and the mechanism by which these viruses are rendered highly virulent is not understood. Again, therefore, any recombinant DNA procedure involving any animal virus or cells containing such a virus must be considered to pose the risk of creating highly virulent or infectious strains.

6. The expression of <u>any</u> foreign gene, however seemingly innocuous it may be in the cells of a human or other mammal, whether inserted by viral infection or some other mechanism, poses the risk that a protein will be produced in the infected cells which has never been seen by the host's immune system. Thus the possibility of an auto immune disease exists (as in rheumatic fever or degenerative kidney disease) in which the body produces antibodies against proteins within or produced by its own cells, ultimately destroying the cells themselves. The NIH guidelines discuss "harmful" genes in the sense of DNA specifying antibiotic resistance factors or protein toxins.

1/ Davis, et al., supra at 1318. RNA = ribonucleic acid. Some viruses contain RNA rather than DNA.

In the context of auto-immune disease, however, the gene specifying any foreign protein must be considered potentially harmful.

7. The expression of even a "normal" metabolic enzyme in human, animal or plant cells which was not under the control of the cell's normal complex regulatory mechanism, could lead to severe metabolic disruptions and an ensuing disease state, similar to existing cases of metabolic disease where the defect is in a regulatory gene, rather than once coding for a specific enzyme.

Both the NIH guidelines and the Impact Statement recognize that humans harboring or infected by bacteria or viruses containing recombinant DNA may, under certain conditions, suffer a variety of serious adverse health effects. If such modified bacterial or viral agents can survive and propagate outside the laboratory and thus produce new identical organisms capable of producing infection and/or toxic effects on human beings, there exists the potential for a "communicable disease" within the meaning of Section 361 of the Public Health Service Act (42 U.S.C. §264). (see Section II above). Because some of the organisms created by recombinant DNA research have never existed before, the health and environmental effects of such novel microorganisms are inherently unpredictable. Nevertheless, the danger of the creation of a potentially serious communicable disease organism makes it incumbent upon the Department of Health, Education and Welfare to exercise its statutory authority and take whatever regulatory measures are necessary to protect the public health.

While EDF and NRDC commend the monumental effort made by NIH to regulate this potentially hazardous branch of research within its own jurisdiction, we are disturbed by the fact that the guidelines cover only NIH supported research, leaving large segments of the scientific and industrial communities subject to no required safety procedures. Recombinant DNA research and technology is now being pursued and supported by private corporations, agencies of the Federal government, as well as scientists at universities and private institutions.
General Electric is trying to develop a bacteria which can degrade petroleum and could be used to consume oil spills. Imperial Chemical Industries Ltd. (ICI) of Britain is trying to develop a virus which produces insulin. (Janice Crossland, "Hands on the Code", Environment 18:6, September 1976). The drug industry in the United States has also expressed interest in the commercial use of recombinant DNA techniques. Federal agencies such as the Department of Defense may contemplate conducting experiments. Scientists at universities whether they receive government grants or not are conducting recombinant DNA research. Therefore, we consider a uniform set of regulations covering all parties engaging in recombinant DNA research to be absolutely necessary.

IV. The Secretary of HEW Has the Authority To Regulate All Recombinant DNA Activities

Section 361 of the Public Health Services Act (42 U.S.C. §264) gives the Secretary of Health, Education and Welfare the authority to regulate all recombinant DNA research and technology. The Section empowers the Secretary to:

> ". . . make and enforce such regulations as in his judgement are necessary to prevent the introduction, transmission, or spread of communicable

> diseases from foreign countries into the States or possessions, or from one State or possession into any other State or possession . . ."

It further provides that:

> for purposes of carrying out and enforcing such regulations, the [Secretary] may provide for such inspection, . . . disinfection . . . and other measures, as in his judgment may be necessary.

Recombinant DNA research and technology could create novel infectious agents or increase the virulence and range of existing infectious agents. The Draft Environmental Impact Statement recognizes that recombinant DNA activities could produce microorganisms that cause disease in laboratory workers and the general public. In describing the Guidelines the Draft EIS states:

> "The emphasis on protection of laboratory workers from infection reflects the fact that laboratory workers are the persons at the greatest risk of infection and that the most likely route of escape of possibly hazardous agents from the laboratory is the laboratory worker." (41 Fed. Reg. 38432)

In describing the highest level of physical containment required by the Guidelines to the Draft EIS states that such facilities are:

> "designed to contain microorganisms that are extremely hazardous to man or may cause serious epidemic disease."

The kinds of disease which may be caused by recombinant DNA activities are described in Section III of this petition (infra at pp. 9 - 12).

The Secretary has defined "communicable disease" in regulations promulgated under Section 361 to govern the importation of animals and establish drinking water standards. For the purposes of both these sets of regulations a communicable disease is "An illness due to an infectious agent or its toxic product . . ." transmitted by persons, animals, plants or the inanimate environment. (42 C.F.R. §§71.1(b), 72.1(b)). These regulatory definitions of communicable

disease illustrate that the Secretary has the authority under §361 to regulate infectious agents from any source, transmitted by any means.

Because microorganisms produced by recombinant DNA activities may spread disease among humans, it has already been recognized that regulations promulgated pursuant to authority under §361 control transportation of DNA materials. Section II-C of the NIH Guidelines (41 Fed. Reg. 27914) states that the shipment of recombinant DNA materials is governed by 42 C.F.R. §72.25 which specifies safety requirements for the transportation of etiologic agents.[1/] An "etiologic agent" is defined as ". . . a viable microorganism or its toxin which causes, or may cause, human disease." (42 C.F.R. §72.25(a)(1)) Recombinant research and the commercial use of recombinant technology pose an even greater risk that the public will be exposed to infectious agents than does transportation. The same risk of communicable disease which gives the Secretary the authority to regulate the transportation of recombinant materials under §361 gives him the authority to regulate all recombinant DNA activities.

V. Relief

By this petition EDF and NRDC seek the following relief:

1. A legislative-type hearing to develop a policy on recombinant DNA research and technology.

2. Regulations binding on all parties conducting recombinant DNA research or otherwise engaged in recombinant DNA technology.

1/ §72.25 applies to microorganisms listed in subsection (C) which includes most microorganisms used in recombinant DNA research such as E. coli, Simian Viruses, Salmonella.

3. As interim relief, regulations which make the NIH guidelines binding on all parties engaged in recombinant DNA research and technology.

This relief is necessary to insure that the public has an adequate opportunity to participate in the decision of whether and under what conditions recombinant DNA research and technology should be permitted and to insure that the protection provided the public by the NIH guidelines is immediately extended through the application of the NIH guidelines to all recombinant DNA research and technology.

A. The Need for a Legislative-Type Hearing

The NIH guidelines, which at present are the only statement of government policy on recombinant DNA research and technology, are the product of the deliberations of scientists who are now conducting recombinant DNA research. The NIH guidelines had their origin in the Asilomar Conference held in Pacific Grove, California in February 1975. Many of the participants at that conference were the foremost molecular biologists from all over the world. The NIH Recombinant DNA Molecule Program Advisory Committee translated the recommendations of that conference into concrete proposals which became the NIH guidelines. The first opportunity the public had to participate in the regulation of recombinant research was in February of 1976 when the draft guidelines were released for public comment, and the Advisory Committee to the Director of NIH[1/] held an open meeting.

1/This committee should not be confused with the NIH Recombinant DNA Molecule Program Advisory Committee, which drafted the guidelines, but is one assembled early in 1976 from representatives of science, law, teaching, public interest groups, students, etc. to advise the director of NIH on the correctness or shortcomings of its efforts to regulate recombinant DNA research.

Although this meeting was not well publicized, many scientists, public interest groups and laymen were invited to attend and to comment on the guidelines.[2/] Additional input was sought from these same individuals during the two-month period following this meeting. A considerable body of material was received by commentators by the office of the Director of NIH, and is summarized, in part, in the Decision of the Director, NIH, to Release Guidelines for Research on Recombinant DNA Molecules (see 41 Fed. Reg. No. 131, pp. 27902-27911, July 7, 1976).

Little discussion was devoted to whether or not these experiments ought to be performed at all, even though the question was raised both by concerned laymen and by prominent scientists. That there is an intrinsic and even necessary good in recombinant DNA research has been a tacit assumption on the part of the NIH advisory committee which drafted the guidelines from the onset of its deliberations. We believe that this is, at least in part, a reflection of the fact that many of the committee members are now doing recombinant DNA research and have a vested interest in its future. In the public meeting held on February 9-10, 1976, the request was made that such potentially hazardous research should at least await the development of a strain of bacteria which is not a ubiquitous inhabitant of the human colon. E. coli is the current organism of choice simply because a large body of genetic information exists concerning this bacterium. This

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2/ A copy of the comments submitted by EDF at that time are attached as Appendix 1.

request was denied in an administrative decision by the director of NIH and not even submitted to the advisory committee for further debate in its April 1-2, 1976 meeting in which final revisions of the guidelines were made. At this meeting, all of the outside comments had been distilled down to ten typewritten pages of questions for the consideration of the recombinant DNA advisory committee, the same committee which had drafted the working version prepared early in 1976. Except for relatively minor changes in wording, the committee dealt summarily with the questions from the public, and the final version of the guidelines did not differ significantly from the version prepared prior to public input.

The legislative-type hearing should consider the following issues which were not adequately considered in the NIH proceedings which led to the promulgation of the guidelines:

(a) Whether or not recombinant DNA research on <u>any</u> level should be permitted at this time in view of our present state of knowledge.

(b) If some areas are to be permitted, what are they and what precautions are necessary to adequately protect the public and the environment? For example, what degree of physical containment should be considered adequate in light of human fallibility?

(c) Whether or not a strain of bacteria should be sought and studied to replace E. coli as the subject of most recombinant DNA experiments before this work be allowed to proceed.

(d) Whether or not an "ordinary" or normal, non-hazardous gene from one organism might become dangerous if expressed in the wrong place and wrong time in the wrong organism (this important question was virtually ignored by the advisory committee).

A legislative-type hearing conducted by HEW is the best forum for full consideration of the issues raised by recombinant DNA research and technology. In effect, such a hearing would amount to a broad-based public review of the existing NIH guidelines and would permit open debate on issues given little or no attention by the NIH Drafting Committee or the office of the director. Whether the activity is transportation of recombinant DNA materials, research, commercial production or use in the environment, HEW has the authority to regulate corporations and scientists whether or not they receive federal research support. Therefore, it is highly appropriate for HEW to hold such a hearing.

B. Final Regulations Governing All Parties Engaged

Promulgation of the NIH guidelines reflects a consensus that recombinant DNA research and technology pose a sufficient hazard to the public health and the environment to require the prohibition of some experiments and the imposition of safety

procedures for others. The hazards of recombinant DNA research and technology are no different if the research is being conducted by scientists employed by private corporations rather than the NIH. The risk that necessitated regulation of NIH grantees necessitates regulation of other research and technology. The need for regulation of all parties conducting recombinant DNA research is particularly great because even one release of a hazardous genetically altered bacterium, virus or plasmid could cause widespread illness or disruption of the environment.

C. Interim Relief

During the period before the hearing is held and final regulations are promulgated the public will be exposed to the potential hazards of recombinant DNA research and technology not now subject to NIH guidelines. Individuals who do not receive NIH grants or work for NIH are not effectively restrained from conducting any of the experiments which NIH deemed so dangerous that they should not be conducted at all. Nor are scientists not now covered by the guidelines required to practice physical and biological containment of organisms with recombinant DNA molecules. To protect the public until final regulations are promulgated, EDF and NRDC request that the Secretary immediately promulgate regulations which make the NIH guidelines binding on all parties engaged in recombinant DNA research and technology.

Respectfully submitted,

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November 11, 1976

GENERAL ELECTRIC

GENERAL ELECTRIC STATEMENT

For:

The Senate Subcommittee on Health (Committee on Labor & Public Welfare)

Honorable Edward M. Kennedy, Chairman

HEARINGS ON RECOMBINANT DNA TECHNOLOGY

General Electric appreciates the opportunity to participate in your Subcommittee's investigations and deliberations on recombinant DNA research.

General Electric has traditionally undertaken basic research in areas of potential importance where people's needs might be met and our own commercial interests served. In this tradition, we have engaged in a limited program of recombinant DNA research.

Our research team is small, consisting of one Ph. D. scientist and two technicians. They have been engaged in the area of genetic engineering research on microorganisms for about six years and in recombinant DNA research for about two years.

The team functions under National Institutes of Health guidelines. These have been established to carefully control conditions for this work. General Electric does this voluntarily, and we have notified the Director of NIH of our willingness and intent to continue this voluntary compliance.

We are interested in recombinant DNA research because it holds promise of great long-range benefits for mankind. While some of the specific payoffs may not occur for years, it is clear that the potential benefits from recombinant DNA research are great. Here are some examples:

- This work holds promise for a better scientific understanding of the molecular basis of life.
- New ways to mass-produce drugs that are now scarce and expensive may be developed.
- Cost reductions in agriculture could be achieved by enabling certain plants to make their own fertilizer from nitrogen in the surrounding air.
- We may gain an increased understanding of the causes of certain types of cancer and other diseases.
- The extraction of scarce minerals from seawater and "played-out" mines might become economical.
- Research might develop ways to convert waste materials into chemical feedstocks, animal feeds, and liquid and gaseous fuels, such as methane and ethanol.

And those are just a few of the possibilities.

But, as with basic research in any branch of science, the work needs to be conducted with due regard for the protection of health and safety. This is why we are voluntarily complying with the NIH guidelines. Under the guidelines, for example, the regular clean-up and minor maintenance work in our DNA research facility is performed by the research team itself. Other maintenance or cleaning workers are admitted to the facility only when specific services are necessary and even then not before specific approvals have been obtained from our independent Recombinant DNA Safety Committee and not without special supervision and monitoring.

As a further comment on safety, it might be noted that much of the world's recombinant DNA research is being carried out with a strain of the bacterium E. coli, which has been genetically weakened so that it has a very low probability of survival outside the laboratory.

At General Electric, much of our recombinant DNA work is focused on a search for an even safer system. We are now in the early stages of work with a microbe called Pseudomonas putida.

This microbe is found in soil and water and is non-pathogenic to humans. It requires oxygen for growth and functions best at relatively low temperatures; it simply does not survive well when subjected to the temperatures and oxygen-deficient environment encountered in the digestive tracts of humans.

From a safety standpoint, Pseudomonas putida may afford us with an opportunity to reduce significantly the potential hazards of recombinant DNA experimentation.

Genetic engineering is not a brand new science. Mankind has been engaging in it for decades, as exemplified by the cross-breeding of plants, animals and bacteria. Recombinant DNA technology is only the latest -- and the most flexible -- tool for doing so.

Much of GE's past genetic engineering work has been accomplished by the technique of "cross-breeding" bacteria, which is not recombinant DNA research at all. One of the results of this research has been the development of a microbe that can digest crude oil at a relatively fast rate -- as long as water, oxygen and certain nutrients are present. This microbe may eventually be used as a means of cleaning up oil spills. It would digest the hydrocarbons, converting them into carbon dioxide, water and protein. After the thin layer of oil on the surface of the water had been consumed, the microbe would die -- or be eaten by tiny marine creatures. Here is how the microbe was created:

A number of natural microorganisms -- including members of the Pseudomonas putida family -- can digest the various hydrocarbon fractions that make up petroleum. In fact, natural bacteria function as "vacuum cleaners" that tidy up after oil spills.

This process takes a long time under normal conditions because petroleum is a mixture of many different types of hydrocarbons and any given individual strain of bacteria can digest only a few of them. Even when several different strains are present, competition between the strains slows the rate at which the oil is digested.

It occurred to our investigators that oil spills could be attacked more efficiently by a single microbial strain that possessed the genetic information for digesting several different hydrocarbons fractions at once. By means of this "cross-breeding" technique, they produced a modified Pseudomonas putida microbe that combines the hydrocarbon-metabolizing genes from four different strains of oil-digesting Pseudomonas bacteria. The new strain can digest crude oil at a rate several times faster than the parent bacteria from which it was derived.

This microorganism is still in the laboratory stage, and we do not have any immediate plans for field tests. Such tests, if they occur, will be conducted under the auspices of the Environmental Protection Agency or other appropriate agencies.

The remainder of our comments are directed to the subject of proposed Federal regulations on recombinant DNA research.

We at General Electric believe that Federal, rather than state and local, regulation of recombinant DNA research is preferable. Such regulations, while protecting the health and safety of the workers and the public, should

make it possible for this important research to continue expeditiously. The current NIH guidelines should form the basis for this regulation. As we indicated before, GE is already following these guidelines voluntarily and we intend to continue doing so.

The safety committee called for by the guidelines does raise one question, however. Such a committee (for both monitoring and reporting purposes) must be established by each institution conducting recombinant DNA research. The reporting mechanism, if incorporated into Federal regulations, involves the advance disclosure of proprietary information to the NIH or other Federal agency. This could have adverse effects on the competitive status for organizations conducting the work and on their ability to obtain patents -- unless provision is made for such disclosures to be held in strict confidence.

In summary, we believe that due care and caution must be exercised in the area of recombinant DNA research. The NIH guidelines now in existence would seem to be a reasonable and responsible basis for the protection of health and safety. The guidelines help to assure that, while the public is being protected, the acquisition of knowledge and the beneficial applications which can flow from it are not being suppressed.

# # # # #

Excerpts from—

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The Recombinant DNA Debate

For the last 3 years scientists, laymen, and responsible government institutions have engaged in open, public analysis of the hazards that might result from recombinant DNA research, hazards that remain speculative. A set of safety guidelines* for the conduct of the experiments emerged from the discussions. In the absence of all the necessary facts, the guidelines were made extremely stringent in order to compensate for possible errors in judgment, and they apply to all studies funded by the federal government. There is now a concentrated effort to obtain federal legislation imposing the guidelines on all recombinant DNA experiments in the United States, regardless of the source of funds. Similar guidelines exist in many countries.

The foregoing summary reflects neither the current level of interest, emotion, or confusion on the subject in the United States, nor the attitudes reflected in graphics accompanying relevant newspaper and magazine articles and television shows—fanciful ugly bugs crawling out of test tubes, DNA double helices ending in monster heads, chimeric animals that insult the Greek imagination.

The nature of the current debate was evident at the public forum on recombinant DNA held at the National Academy of Sciences from 7 to 9 March. The forum successfully fostered an exchange of views among scientists and the public, although the atmosphere was often charged. The encounter illuminated the arguments of both biologists and laymen who refer to themselves as "opponents" of recombinant DNA research.

It was argued that all recombinant DNA research should be stopped and the nation should instead give priority to the distribution of existing methods of health care and sound nutrition to the citizens of the world. This argument is specious. Certainly there is a need to rectify inexcusable inequities in the distribution of food and health care. But recombinant DNA research is neither an alternative to such action nor a competitor for the necessary funds. Rather it provides for future opportunities to solve problems that are unsolved or that may yet arise.

Another argument was the denial of any positive value in the research. This country, through representative government, long ago committed itself to the importance and support of basic biological research. That investment has now yielded a powerful tool for investigating basic processes relevant to understanding serious and intractable diseases. There is no other way at hand or foreseeable to investigate the structure of the genome of complex organisms. Has the purpose of the investment been forgotten? Surely when a distinguished biologist couples denial of any positive value in the research with a denial that the bacterial chromosome can be characterized as a DNA molecule or with a denial that insulin is clinically useful, we must wonder just what the debate is all about.

It was argued that because recombinant DNA techniques may make genetic engineering (deliberate modification of the genes of complex organisms, including man) feasible, all such work should stop. The argument assumes a public consensus that genetic engineering is evil, although the arguers concede, with everyone else, that the subject needs intense, universal discussion. The argument also denies historical lessons that teach us to distinguish carefully between the acquisition of knowledge and its application.

It is difficult to take demands for bans on "all" recombinant DNA research seriously when they come from those who demand to be heard but do not stay to listen. Those now labeled "proponents" of the research worked long and hard for prohibiting certain experiments and matching containment requirements to estimated risks for others. The cautious analytical approach is a discouraging tactic against uninformed fear, mysticism, and political opportunism. But it must continue; nothing less than science itself is at stake.

—MAXINE F. SINGER

*"Guidelines for research involving recombinant DNA molecules," *Fed. Regist.* **41** (No. 131) (1976).

REPORTS

Recombinant DNA: Examples of Present-Day Research

Many words have been spoken and written about DNA—almost all of them speculative, with little factual basis. This issue contains a collection of reports that sample the state of the art in the area of recombinant DNA research. The content is necessarily far from definitive, for the field is young and moving fast. The editors hoped that by assembling this material a more substantial base for discussion would be created, and that a clearer view of the containment problem might emerge. Modest progress has been made toward these objectives. This overview begins by mentioning some of the procedures by which recombinant DNA research is conducted, and then points out some of the highlights of the collection.

Some of the reports in this issue may prove difficult to read because of the technical language. Essentially, however, the concepts are simple. The term recombination as used here is a genetic term. If, for example, two strains of mutant bacteria—one unable to grow without added tryptophan and the other unable to ferment lactose—are crossed a small percentage of the progeny will be "wild-type," that is, able to grow without tryptophan and to ferment lactose. At the molecular level, this recombination between the two strains results because of breakage of the parental DNA molecules and subsequent rejoining to form the recombinants.

The recombinant DNA technology has made it possible to carry out this recombination process of breakage and rejoining of DNA molecules in the test tube. For many years geneticists have used the recombination process to study the nature of genes, but they were confined to studying recombinants of the same or closely related species. The in vitro process now makes it possible, for the first time, to join DNA molecules of unrelated organisms.

Actually recombination between distantly related species may take place in nature. Certainly mechanisms for the recombination of unrelated DNA molecules exist.

The present capability to join DNA molecules in vitro is the result of 25 years of research on the properties of DNA and the enzymes involved in cleaving and replicating it. In this process, the most important enzymes are the restriction endonucleases. These enzymes have the capability to cut DNA at sequence-specific sites. In Roberts' recent review of this subject (*1*), more than 80 such enzymes isolated from diverse strains of bacteria are described. Some of these enzymes produce staggered cuts in the double strands so that single-stranded ends are produced at each end of the fragment. All fragments produced by a given enzyme have the same self-complementary end, so that a single fragment can circularize by base-pairing or it may combine with another fragment to produce a dimer. In the latter case, if the fragments are from different sources, a recombinant molecule is produced. When this technique is used to produce recombinant DNA's, the gaps remaining in both strands are sealed by an enzyme called DNA ligase. In this issue Scheller, Dickerson, *et al.* describe new and more sophisticated methods for joining DNA molecules using synthetic adapter molecules.

It is convenient to refer to the two DNA molecules in a recombinant as the vehicle and the passenger (Fig. 1). The vehicle is a DNA molecule that is capable of self-replication in its host. When the passenger is joined to the vehicle, it behaves as a (sometimes) passive addition and is replicated together with the vehicle. Two general classes of vehicles have been employed in *Escherichia coli*. Plasmids are small, circular DNA molecules that are capable of self-replication independent of the chromosome. Most of the plasmids now in use originated from the plasmid Col EI, which is about 1/1000 the size of

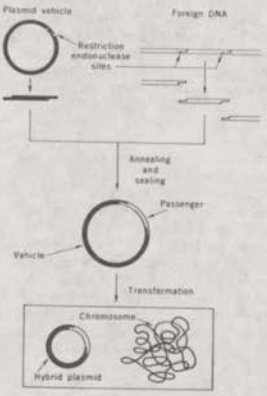


Fig. 1. Scheme of events in recombinant DNA experiments.

the *E. coli* chromosome and produces a bactericidal protein—colicin EI; this protein kills other bacteria that do not carry the plasmid. The other *E. coli* vehicle is the well-studied bacteriophage lambda (λ). Two reports, those of Blattner *et al.* and Leder *et al.* describe the construction of safe λ vectors for use as vehicles.

The recombinant DNA technology is not confined to the *E. coli* host system. It is possible to employ other vehicle-host systems, including mammalian cells in tissue culture. For this purpose, defective derivatives of the DNA tumor viruses SV40 and polyoma can be used as vehicles. These viruses contain circular DNA molecules that are about the same size as the *E. coli* plasmid vehicles. Villarreal and Berg describe their new screening methods for detecting SV40 hybrids.

When the recombination in vitro between vehicle and passenger DNA is completed, the recombinant DNA must be introduced into the host cell where it can replicate. This is done by a process known as transformation. The DNA is added to cells and is taken up at a low frequency. The plasmid vehicles are constructed so that they give the transformed cell a selective advantage. Some vehicles carry antibiotic resistance genes so that only transformed cells can grow in the presence of the antibiotic. It is the transformation process that allows one to "clone" DNA fragments. Since the frequency of transformation is low, a transformed cell is, in general, produced by the uptake of a single vehicle-passenger DNA molecule. The transformed cell multiplies to form a colony. Different colonies contain different passengers. Each colony can be propagated indefinitely, enabling one to store or produce (or both) the passenger DNA molecule at will.

Two popular misconceptions should be cleared up at this point. Cloning as referred to here and in recombinant DNA research in general is totally different from the process developed by Gurdon (*2*), where the nucleus from a somatic cell containing the complete genetic information on one individual (a frog in Gurdon's experiments) is removed and implanted into an enucleated egg. Gurdon demonstrated that this egg could in some cases develop normally to produce a frog. By this technique one could—in principle—propagate an indefinite number of identical individuals. By contrast, in DNA cloning experiments, fragments of passenger DNA represent a minute fraction of the total genetic information of the organism from which they are derived. Clones carrying recombinant plasmids are often referred to as hybrid strains.

If passenger DNA from the mouse is joined to a plasmid vehicle and transformed into *E. coli*, the resulting clones would be referred to as *E. coli*–mouse hybrids. The use of the word hybrid suggests to the layman that a significant mouse character is acquired by *E. coli*. This is, of course, not the case. The hybrid *E. coli* is still very much the organism that it was. The mouse information that it has acquired represents about one-millionth of the genetic information of the mouse—a few genes at most—and is only about one-thousandth of the genetic information of *E. coli*.

In many of the reports in this issue, cloning has been used to isolate and propagate fragments of DNA from diverse sources. When the fragments are isolated from *E. coli* or closely related bacteria, the genes are usually expressed. Thus Zieg *et al.* have isolated the genetic region of *Salmonella* that controls which of two possible flagellar forms will be expressed. The control mechanism that operates in this system is one that had not previously been described. A segment of DNA in the control region can be inverted. The flagellar type that is expressed depends on the orientation of the DNA segment. There are indications that mechanisms similar to this one may operate in higher organisms. It will now be possible to elucidate in detail how this kind of control system works.

Panasenko *et al.* have constructed a hybrid of λ and *E. coli* which carries the DNA ligase gene. This hybrid phage can be used to produce a 500-fold increase of this enzyme in the cell. This work illustrates a practical advantage that can be gained by use of recombinant DNA technology. Thus, the purification of DNA ligase is greatly simplified when there is 500 times as much enzyme per cell.

Many of the reports describe the isolation of DNA fragments from eukaryotes. Beckmann *et al.* have isolated plasmids carrying transfer RNA genes of yeast. McClements and Skalka have cloned the ribosomal RNA of chicken. Lee *et al.* and Scheller, Thomas, *et al.* have characterized fragments of sea urchin DNA, which contain middle repetitive DNA sequences. The organization of these sequences has been determined before by less direct methods. Now examination of cloned fragments confirm that repetitive sequences are interspersed between unique sequences. In one clone it was shown that the nearby repeats are not identical.

Two groups (Liu *et al.* and Wilson *et al.*) have used the enzyme reverse transcriptase to make DNA copies of hemoglobin messenger RNA (mRNA). These DNA fragments were then inserted into plasmids and cloned. The cloned DNA can be used for more convenient sequencing of the mRNA as shown by Liu *et al.* It can also be used as a pure probe to investigate the multiplicity of hemoglobin genes or to isolate the genes themselves in further cloning experiments.

It can be seen that there is plenty of activity in this field. DNA's from diverse sources are being cloned, and the cloning technique is being used to study questions of gene organization and control. With all of this work, are we in a better position to answer the question that is being asked by the public with increasing frequency? Are these organisms dangerous? It is perhaps impossible to design an experiment that can give an unambiguous answer to this important question. In one sense, the workers themselves have been guinea pigs—there have been no reports of illness associated with infection by a strain of bacteria containing cloned DNA. Cameron and Davis have looked carefully to determine whether or not a fragment of DNA from yeast or *E. coli* could confer an advantage to a λ bacteriophage vehicle which carries it. They found that foreign DNA fragments often inhibit growth of the phage, but in no case did a fragment confer an advantage. This agrees with the impression formed by many workers that some DNA fragments—especially from bacteria—may be "unclonable," suggesting that they have a lethal effect on the host. Thus, we can believe that it is probably not possible to create a strain that would overgrow the laboratory and head for town as depicted in movies of the 1950's.

One way to assess the danger of inadvertent creation of a pathogen is to design "worst case" experiments that can test the ability of *E. coli* to act as a pathogen. An example of such an experiment would be to test whether strains of *E. coli* carrying polyoma DNA as a passenger can cause tumors in mice (polyoma is a virus which can do this). This experiment is being planned at the National Institutes of Health (NIH), but it must take place under the highest containment conditions (P4), and therefore it will be some time before the results will be available (*3*). The pathogenic character of a species is the result of complicated interactions between the organism and its environment. The "worst case" experiments represent a way to test the possibility that *E. coli* K12 can be converted into a pathogen—at least in special cases. One hopes that more of these experiments will be conducted.

The NIH guidelines are the result of the compromise between the need to protect the public from conjectural hazards and to provide them with the benefits that can be gained from fundamental knowledge in biology. The guidelines provide a sliding scale of biological and physical protection that increases for experiments with higher organisms.

Two reports in this issue deal with the construction and testing of bacteriophage λ vehicles that have been awarded the EK2 designation (medium biological containment) by the NIH advisory committee (*4*). The standards used in evaluating these vehicles have been rigorous and imaginative. They require that the EK2 vehicle pass a series of "worst case" tests that ensure that the chance of propagation of an escaped particle is less than 10^{-8}. These vehicles make it possible to carry out experiments with mammalian DNA under P3 conditions.

The ability to clone and propagate DNA fragments from higher organisms is a genetic technique for which there was no previous counterpart. It was simply impossible to design an experiment for obtaining the gene for the α chain of hemoglobin, for example. Two new technologies—recombinant DNA research and DNA sequencing—now make it possible to examine the structure and organization of eukaryotic genes on a scale not imaginable 5 years ago.

The following reports describe the beginning of this research. Five years from now we will have taken a quantum jump in our understanding of chromosome structure and gene organization.

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References and Notes

1. R. J. Roberts, *Crit. Rev. Biochem.* **3**, 123 (1976).
2. J. B. Gurdon, *J. Embryol. Exp. Morphol.* **10**, 622 (1962).
3. **P1** containment specifies sterile techniques such as hand washing, decontamination of the work area, and exclusion of food and smoking materials. Waste must be decontaminated before disposal. **P2** containment is similar to P1, except that more stringent procedures must be followed and access to the laboratory must be limited to authorized personnel who have been advised of the hazard. In **P3** containment, in addition to the above, experiments requiring P3 must be done in a negative pressure laboratory in an isolated room separated from other work areas. All procedures involving opening of vessels must be performed in a biological safety cabinet. Gloves must be worn. No recombinant organisms (or molecules) are permitted to leave the P3 in viable form, except in a sealed, unbreakable, externally decontaminated container. **P4** is an elaborate containment for handling the most dangerous pathogens known.
4. Biological containment is as follows. **EK1** requires, for example, the use of *E. coli* K12, a strain generally conceded to be unable to colonize the human bowel. **EK2** systems require, for example, the use of genetically altered K12 strains, so that they will not grow under usual conditions for *E. coli*. **EK3** is the use of an EK2 system and, in addition, requires the system be tested in animals for safety.

24 March 1977

Charon Phages: Safer Derivatives of Bacteriophage Lambda for DNA Cloning

Abstract. *The Charon λ bacteriophages have been developed as vectors for cloning. Their construction incorporates mutations that make them simple to use and also greatly increases their safety for the biological containment of cloned recombinant DNA. Three of the Charon vector phages, 3A, 4A, and 16A, have been certified for use as EK2 vector-host systems, when propagated in bulk in a special bacterial host, DP50SupF. We present here some of the data on which the safety of these systems was evaluated. DNA fragments ranging in size from 0 to 2.2 × 10⁶ base pairs can be cloned in these EK2 Charon phages.*

The desirability of using vectors for DNA cloning which have poor survival in natural environments and which require special conditions for their replication has been amply emphasized in the recent past (*1*, *2*). From the point of view of containment, DNA cloning with lytic phages offers a natural advantage, since two components—phage and sensitive bacteria—must repeatedly come together for significant replication to take place. Phage vectors are also convenient because the chimeric DNA is delivered to the experimenter in a package. Since the phage and bacteria coexist only briefly, a cloned segment need not be compatible with *Escherichia coli* metabolism for extended periods of time.

Bacteriophage λ is particularly well suited for adaptation to make it useful as a lytic cloning vehicle. This is because one-third of its DNA, which forms a continuous block in the middle of the genome, can be replaced without the phage losing its ability to grow lytically (Fig. 1). Several groups of investigators, including our own, have constructed derivatives of λ specifically adapted for DNA cloning (*3*–*5*). The aim in all these adaptations is to construct phages so that a restriction endonuclease can make a cut (or cuts) only in the dispensable one-third of the λ DNA to permit subsequent addition of (or replacement by) a foreign DNA segment. Point mutations, substitutions, and deletions are, therefore, introduced into wild-type λ to alter the distribution of restriction sites and to eliminate sites in the essential regions of the phage genome. Other objectives of vector engineering include (i) cloning a variety of sizes of DNA fragments; (ii) cloning with more than one restriction enzyme; (iii) indicating by plaque type whether or not a phage has incorporated a DNA fragment; (iv) cloning with minimal manipulations; (v) controlling transcription of the cloned fragment from promoters on the vector; (vi) growing vectors and clones to a high

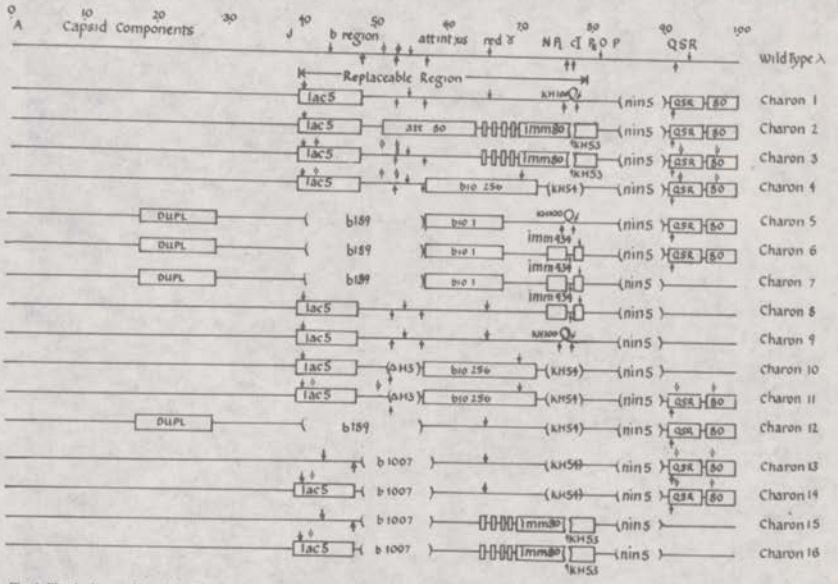


Fig. 1. Physical map of λ and the Charon phages. The phage λ map which we now estimate to be 49,400 ± 600 (S.E.M.) base pairs (*34*) is drawn to scale with the replaceable region indicated relative to essential genes for lytic growth. Beneath it are the Charon phage vectors with portions from λ (indicated by lines) aligned with the λ map. Boxes indicate substitutions, but the lengths of DNA substituted are not shown to scale. The length of DNA in each vector is shown in Table 1. Downward and upward arrows are Eco RI and Hind III sites, respectively. The φ symbol shows known Sst I sites; Charon 15 has none. (Sst I sites were not determined for phages 1, 5, 6, 7, 8, 9, 10, and 12.) DUPL is a duplicated piece of phage λ. *lac*5, shown by four small boxes at around 70 in some phages is from φ80; it is partially homologous to λ. The parenthesized b189, b1007, ΔH3, KH53, KH54, and *nin*5 are deletions; KH100 is an insertion; *imm*434 is a replacement for imm λ [see (*6*)].

yield; (vii) readily recovering cloned DNA; and (viii) features contributing to biological containment.

The λ vectors that we have developed during the past 3 years have been designated Charon phages, after the mythological boatsman of the River Styx. They have been numbered sequentially as constructed. The structures of Charon phages 1 to 16 are shown in Fig. 1. The properties that make them useful as vectors are summarized in Table 1.

Construction of Charon phages. Most of the mutations that were incorporated into Charon phages have been described by us (*6*) and others (*7*). These were assembled into the desired arrangements by standard genetic crosses (*8*). We describe here only those mutations that were isolated for this project and have not previously been described.

The deletion KH53 in the immunity region of φ80 was isolated by selecting for elimination of the restriction endonuclease Eco RI site in the φ80 immunity region according to the method of Arber and Kühnlein (*9*). Concomitant with loss of the restriction site, the plaque morphology changed from turbid to clear. Examination of heteroduplexes (Fig. 2k) between the DNA of KH53 and of a phage with the wild-type φ80 immunity region revealed a single-stranded loop of approximately 750 base pairs near the position of the restriction site. KH53 was shown by measuring its buoyant density to be a deletion rather than an insertion; it probably deletes part of the φ80 analog of the λ cI gene. However, since little is known about the genetic organization of the immunity region of φ80, other explanations are possible. As a practical matter, KH53 is nonrevertible and drastically reduces the formation of lysogens (see safety tests).

We selected two mutations, BW1 and BW2, which eliminated the Eco RI sites just to the right of the immunity regions of λ and φ80, respectively. Another mutation isolated for this study is the duplication (DUPL) located on the left arm of Charons 5, 6, 7, and 12. This appears to be similar to duplications described by Bellett *et al.* (*10*) and has been useful for increasing the size of that arm.

The no-cut right end (NRE) of Charons 7, 8, 9, 10, 15, and 16 was specially constructed by combining the *nin*5 deletion with a hybrid isolated by Murray and Murray (*5*) which removes the Hind III site near gene *Q*, and then selecting a new point mutation (DK1) that eliminates the Eco RI site near gene *S*. The NRE, unlike

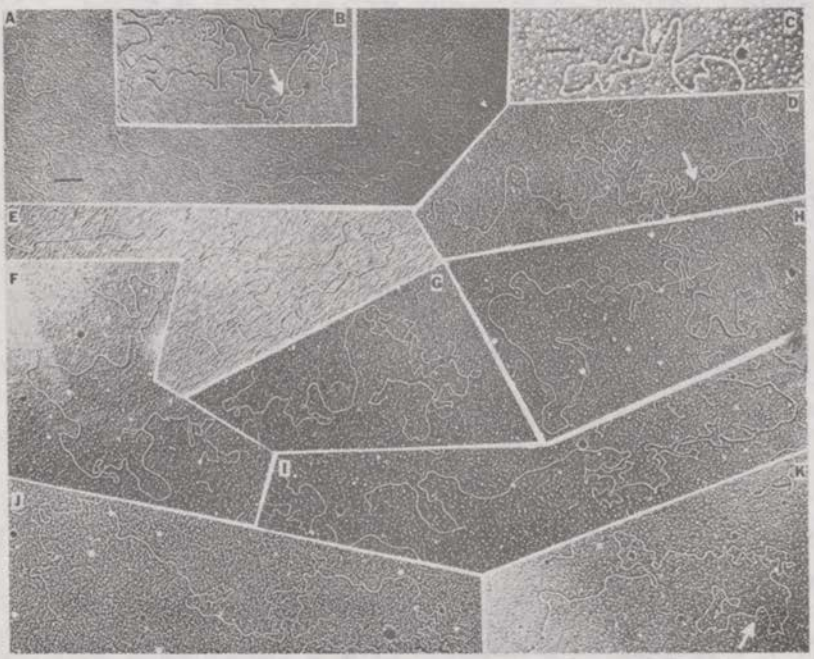


Fig. 2. Heteroduplex analysis of Charon phages. Heteroduplexes made by combining DNA strands from genetically different phages (*11*) were examined in the electron microscope. The line diagram identifies each heteroduplex and labels the loops and bifurcations where DNA strands do not pair. The calibration bar in panel (A) refers to double-stranded DNA and applies reasonably to all panels except (C). The calibration bar in panel (C) refers to single strands and applies only to that panel. The identification of phages not shown in Fig. 1 is as follows: Ch3ΔLac was made from Charon 3 by deleting its Eco RI Lac operator-containing fragment. Bio16a, panel (C), is equivalent to λ except for a small substitution of *bio* DNA to the right of *att*. 4M121 was obtained by inserting unrelated DNA from phage mu into Charon 4 (*12*). bio256nin has the same *bio* substitution and *nin*

the QSR80 substitution, has no sites to the right of *nin*5 for any of nine restriction enzymes we have tested that are potentially suitable for cloning (*11*). NRE has no visible nonhomology with λ in heteroduplexes (see Fig. 2, i and j), suggesting that the contribution of φ80 is slight.

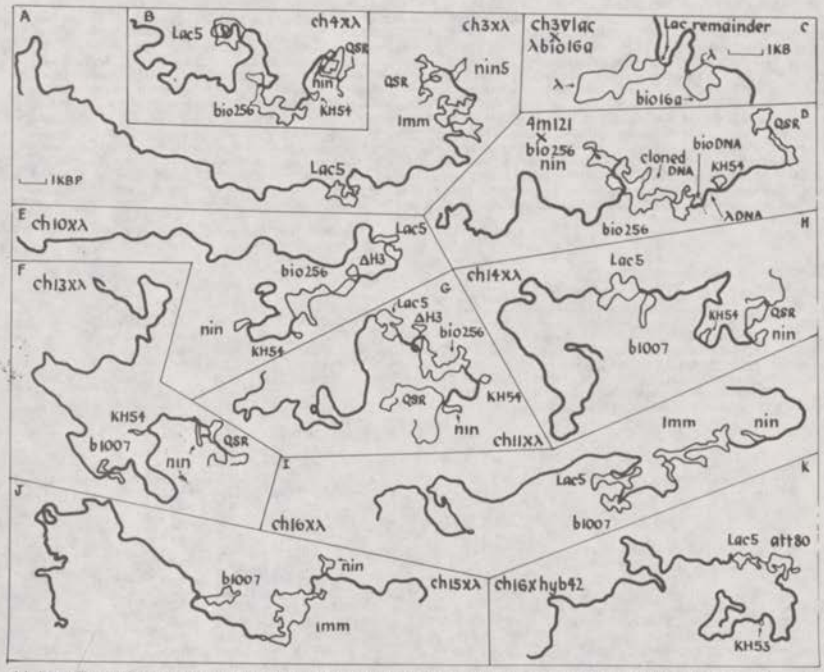
Some of the Charon phages have had amber mutations, *A*am32 and *B*am1 (*12*), introduced in their capsid genes in order to enhance biological containment. These phages are designated with an A (for example, Charon 3A is derived from Charon 3 by incorporation of these mutations).

Verification of vector structure. It is most important to verify the structure of each vector. The best way to determine whether deletions and substitutions are present and to detect unexpected structural changes is by heteroduplex mapping (*31*). All 16 vectors were analyzed by this technique. The forms observed in the electron micrographs were completely consistent with the structures of the phages shown in Fig. 1. Electron micrographs of the heteroduplexes between DNA from wild-type λ and from eight of the vectors are shown in Fig. 2, together with three other informative heteroduplexes.

Heteroduplex analysis has also proved valuable for determining the amount of bacterial DNA remaining in some of the vectors after they have been used for cloning with Eco RI. An example of this is shown in Fig. 2c, where the bacterial DNA remaining from *lac*5 after the dispensable portion of Charon 3 had been excised with Eco RI is displayed directly as a single strand; it is less than 200 base pairs in length. The bacterial DNA remaining after Eco RI cloning with vectors that contain *bio*256 was similarly determined by subtracting the duplex lengths indicated by arrows in Fig. 2, d and b; this measured 980 ± 40 base pairs.

Genetic characteristics of all the vectors have been verified by plating on suitable indicator strains. Characteristics examined included the immunity type, the presence of the *lac*5 and *bio*256 substitutions, and the presence of amber mutations (*13*).

Each vector DNA was also digested with restriction enzymes, and the sizes of DNA fragments were determined on agarose gels. By analyzing these, and other related data, the positions of the restriction sites indicated in Fig. 1 were determined. The Sst I sites of Charon phages 1, 2, 5, 6, 7, 8, 9, 10, and 12 have not yet been determined.



deletion as Charon 4 but lacks the KH54 deletion; this allows the deletion to be displayed in panel (D). hyb42 is λ*att*80*imm*80*nin*; its wild-type φ80 immunity region permitted display of the KH53 deletion. The arrows in the photograph panels (D) and (B) indicate duplex segments next to the KH54 marker; subtraction of their lengths provided a measurement of *E. coli* DNA remaining in Charon 4 clones; this residual *bio* DNA is indicated in the tracing of panel (D). The arrow in the photograph panel (C) indicates *E. coli* DNA remaining in Charon 3 clones. The arrow in photograph panel (D) shows the KH53 deletion.

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Vectors for cloning a variety of sizes of DNA fragments. Since λ capsids cannot accommodate DNA molecules outside the size range 38 to 53 kilobase pairs (kbp) (*4*, *10*, *14*, *34*), each λ phage vector has a maximum and some have a minimum size of DNA fragment that can be cloned with a given restriction enzyme. The size limits for each of the Charon phages are listed in Table 1. These ranges depend not only on the positions of restriction cuts but also on the sizes of deletions and substitutions in the essential region of the vector. Any decrease in the size of the vector genome results in a corresponding increase in the maximum fragment that can be cloned, and may result in a greater minimum size. Charons 1, 4, 8, 9, 10, 11, 13, and 14 are all suitable for cloning large fragments. Charons 8 and 9 have the largest capacities, and approach the theoretical maximum for a nondefective λ vector. Among the EK2-certified Charon phages, Charon 4A can be used for cloning large Eco RI fragments.

Vectors for cloning with more than one restriction enzyme. When small DNA fragments are to be cloned, a single restriction site in a nonessential portion of the phage DNA is sufficient. Several of the Charon vectors can be used with more than one restriction enzyme in this way (Table 1). If only one site occurs for each enzyme, the heterogeneously terminated fragments obtained by digestion with all possible pairs of the enzymes can be cloned. Heterogeneously terminated vectors and fragments prevent self-cyclization reactions. The EK2-certified phage Charon 16A can be used in this way with Eco RI and Sst I.

For cloning large fragments with multiple enzymes, a "magic" arrangement would be to have a series of different restriction sites arranged in the same order at each end of the dispensable region. With such an arrangement, restriction enzymes could be used either singly or in any possible paired combinations to clone large fragments. Charon 9 is the closest approximation to such a vector in our series.

Vector functions indicating successful cloning. A powerful indicator of successful cloning is available in Charon phages 1, 4, 8, 9, 10, 11, 13, and 14, which all have a lower limit to the size of cloned DNA they can accept (see Table 1). In these phages, when the dispensable portion of

Table 1. Characteristics of Charon cloning phages. The DNA cloning vectors and their genotypes are presented. The individual mutations are discussed in the text and in (*6*). Typical growth titers obtained by the PDS method are given, and they do not differ significantly with either the A series or the clone-bearing derivatives of the vectors. The total DNA length of each vector is given as a percentage of wild-type λ, determined from buoyant densities. These vectors can be used with various restriction enzymes. For each of the useful vector-enzyme combinations, the left- and right-end fragment lengths are given as percentage of λ, as determined by agarose gel electrophoresis or electron microscopy (or both). The corresponding cloning capacity is given in kilobase pairs, assuming that the packaging limits on the genome are from 38 to 53 kbp. The symbols p_L and p_{Lacr}, under the heading "promoter control," indicate whether or not these major vector promoters would be expected to transcribe the cloned fragment. Other promoters (p_R', p_{tet}, or p_{ant}) might also be useful in some cases. Suggested genetic tests to indicate successful cloning are: (a) colorless plaques on Lac$^-$ cells provide definite indication of foreign DNA insertion; (b) colorless plaques on Lac$^+$ cells provide definite indication of foreign DNA insertion without reinsertion of dispensable vector fragment; (c) clear plaques provide definite indication of foreign DNA insertion; (d) combination of colorless plaques in Lac$^+$ cells with Bio$^-$ phenotype provides definite indication of foreign DNA substitution without reinsertion of either dispensable fragment. Bio$^+$ phenotype is detected by a ring of bacterial growth around plaques on Bio$^-$ cells on biotin-deficient plates; (e) lack of ability to plate on *polA*$^-$ cells provides definite indication of foreign DNA insertion; (f) colorless plaques on Lac$^+$ cells indicate removal of dispensable fragment but does not ensure that insertion of foreign DNA has taken place; (g) plaque formation indicates DNA insertion but does not ensure that reincorporation of dispensable fragments has not taken place; and (h) no particularly useful tests currently available. Not all genetic tests have been verified in all cases.

Vector	λ genotype	Typical growth	Total DNA length (%λ)	Fragment lengths in percent λ			Cloning capacity (kbp)	Promoter control	Genetic tests
					Left end	Right end			
Charon 1	*lac*5 KH100 BW1 *nin*5 *QSR*80	3×10^{10}	100.5	Eco RI:	39.5	20.6	9.1–23.2		(g)
Charon 2	*lac*5 *att*80 *imm*80 KH53 BW2 *nin*5 *QSR*80	2×10^{10}	95.1	Eco RI:	39.5	55.6	0–5.8	p_{Lacr}	(a)
Charon 3	*lac*5 *imm*80 KH53 BW2 *nin*5 *QSR*80	6×10^{10}	98.7	Eco RI:	39.5	42.6	0–12.3	p_L	(f)
Charon 4	*lac*5 *bio*256 KH54 BW1 *nin*5 *QSR*80	1×10^{10}	93.6	Eco RI:	39.5	22.5	8.2–22.2	p_L	(d)
Charon 5	DUPL b189 *bio*1 KH100 BW1 *nin*5 *QSR*80	2×10^{10}	93.5	Eco RI:	72.7	20.8	0–7.1		(h)
Charon 6	DUPL b189 *bio*1 *imm*434 BW1 *nin*5 *QSR*80	1×10^{10}	88.6	Eco RI:	68.3	19.9	0–9.2		(c)
Charon 7	DUPL b189 *bio*1 *imm*434 BW1 *nin*5 NRE	2×10^{10}	86.2	Eco RI:	68.3	17.6	0–9.9		(c)
				Hind III:	67.3	18.6	0–9.9		(c)
				Eco RI/Hind III:	67.3	17.6	0–10.4		(c)
Charon 8	*lac*5 *imm*434 BW1 *nin*5 NRE	5×10^{10}	92.8	Eco RI:	39.5	17.6	10.6–24.6		(g)
				Hind III:	48.5	18.5	5.7–19.8		(g)
Charon 9	*lac*5 KH100 BW1 *nin*5 NRE	2×10^{11}	97.6	Eco RI:	39.5	18.3	10.2–24.3		(g)
				Hind III:	48.5	17.6	6.2–20.2		(g)
				Eco RI/Hind III:	39.5	17.6	10.6–24.6		(g)
				Sst:	42.0		≥ 13.2	p_L	(h)
				Sst/Hind III:	42.0	17.6	9.3–23.4		(g)
Charon 10	*lac*5 ΔH3 *bio*256 KH54 BW1 *nin*5 NRE	3×10^{10}	85.3	Eco RI:	39.5	20.2	9.2–23.4	p_L	(b)
				Hind III:	52.2	33.1	0–10.7	p_L	(h)
				Sst:	42.0	34.0	1.3–15.3	p_L	(b)
				Hind III/Sst:	42.0	33.1	1.7–15.7	p_L	(b)
Charon 11	*lac* 5 ΔH3 *bio* 256 KH54 BW1 *nin* 5 *QSR*80	5×10^{10}	89.0	Eco RI:	39.5	22.5	8.2–22.2	p_L	(b)
Charon 12	DUPL b189 KH54 BW1 *nin*5 *QSR*80	1×10^{11}	91.0	Eco RI:	64.2	26.8	0–7.9	p_L	(e)
Charon 13	b1007 KH54 BW1 *nin*5 *QSR*80	1×10^{11}	83.5	Eco RI:	44.5	26.8	2.4–16.7	p_L	(g)
Charon 14	*lac*5 b1007 KH54 BW1 *nin*5 *QSR*80	2×10^{10}	83.7	Eco RI:	39.5	26.8	5.0–19.1	p_L	(b)
Charon 15	b1007 *imm*80 KH53 BW2 *nin*5 NRE	1×10^{11}	86.0	Eco RI:	44.5	41.5	0–10.4	p_L	(h)
				Hind III:	48.5	37.5	0–10.4	p_L	(h)
				Eco RI/Hind III:	44.5	37.5	0–12.3	p_L	(h)
Charon 16	*lac*5 b1007 *imm*80 KH53 BW2 *nin*5 NRE	3×10^{10}	86.0	Eco RI:	39.5	46.5	0–10.4	p_{Lacr}	(a)
				Sst:	42.0	44.0	0–10.4	p_{Lacr}	(a)
				Eco RI/Sst:	39.5	44.0	0–11.6	p_{Lacr}	(f)

DNA has been removed, the resulting vector DNA falls below 38 kbp and therefore is too small to be packaged. Only when an additional piece of DNA is inserted into the vector will a viable phage be produced (*4*). The ability to form a plaque is, therefore, a strong indicator of successful cloning. Reinsertion of a fragment or fragments from the dispensable portion will, of course, also yield viable phage. Since it is difficult to remove these dispensable fragments physically, it is advantageous to be able to detect their reinsertion genetically. It is also desirable to reduce the number and kinds of fragments from the dispensable region, so as to reduce the number of genetic tests and to reduce competition during cloning between the undesirable reinsertion fragments and the desired foreign DNA fragments. Charons 10 and 11 have a single dispensable Eco RI fragment that can be detected, when inserted in either orientation, either by plaque color on XG (defined below) plates or by tests for *bio* function.

For cloning small fragments, vectors must be used that do not have a minimum size limit. Indicators of successful cloning in these vectors can be obtained by arranging for the cloning site to be within a nonessential gene whose function can be monitored. Insertional inactivation of the nonessential gene by the cloned fragment will then indicate success. Three such indicator systems are potentially available for Charon phages. Insertion into Charon phages 6 and 7 should make the plaques clear. Insertion into the *red* gene of Charon 12 should make the phage unable to plate on *polA*$^-$ cells. Insertion into the *lac z* gene of Charons 2 or 16 should render the plaques colorless on Lac$^-$ cells on XG plates.

Use of lac5 *as an indicator function*. As was discussed above, several of the Charon vectors, including all three EK2-certified phages, contain the *lac5* substitution, which carries the *E. coli* gene for β-galactosidase (*lac z*). When the chromogenic substrate 5-chloro-4-bromo-3-indolyl-β-D-galactoside (XG) is included in the plating medium, phage carrying *lac5* give vivid blue (indigo) plaques. In order to use this reaction for indication of potential success in cloning, it was necessary to gain some understanding of the mechanism of formation of blue plaques. In *lac5* the promoter, operator, and *z* gene are oriented leftward, and an Eco RI site is located within the *z* gene (*15*). This cut is within 200 base pairs of the left end of the substitution. To determine the effect of an interrupted *lac z* gene on plaque color, phages were constructed from Charon 3 and Charon 11 in which the Lac region had been cut out by Eco RI, turned around, and reinserted so that its orientation (see below) was changed from "n" to "u," and the *z* gene was interrupted. These phages, the parents, and other containing insertions of unrelated DNA were tested on Lac$^+$ and Lac$^-$ indicator bacteria on plates containing XG (80 μg/ml). We found that the development of blue plaques depended on the *lac* genotype of the plating bacteria as well as that of the phage. Dark blue plaques were always produced when the phage contained *lac5* in the normal arrangement, and the plaques were always colorless when the fragment containing *lac5* was removed. However, the phages with an interrupted *z* gene gave pale blue plaques on Lac$^+$ cells but colorless plaques when the indicator bacteria were Lac$^-$ (see cover). We conclude that a blue plaque can be produced either directly by expression of a β-galactosidase gene supplied by the infecting phage or indirectly when the many copies of the Lac operator DNA produced during phage growth bind the Lac repressor available in the cell, causing derepression of the bacterial *lac* operon.

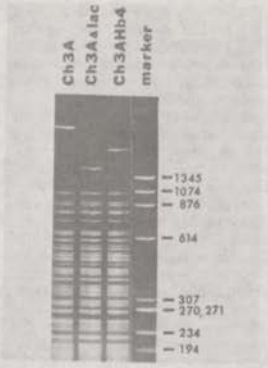


Fig. 3. Detection of inserted DNA in Charon 3A by Hha I digestion. Charon 3AHb4 was made by replacing the Eco RI Lac operator–containing fragment of Charon 3A with mouse globin cDNA. Charon 3AΔLac is Charon 3A without the Eco RI fragment. DNA from each of these phages was digested with Hha I and compared by 6 percent polyacrylamide gel electrophoresis. A Hae III digest of φX174 DNA was used as a calibration mixture. The marker sizes are indicated in base pairs. The largest fragment (> 1345 base pairs) is the piece of interest. If it is larger than that in Charon 3AΔLac, then an insert is present (as in Charon 3AHb4). If it is replaced or accompanied by new smaller pieces, then an insert with an Hha I site (or sites) is present (no example of this is shown).

To summarize, if we use as examples Eco RI cloning with the EK2-certified vectors, potential success with Charon 16A is indicated by loss of the Lac function detected by colorless plaques on XG plates with Lac$^-$ bacteria. Potential success with Charon 4A is indicated by obtaining viable phages giving colorless plaques on XG plates with Lac$^+$ bacteria. [A separate test is required for the absence of the Bio fragment (*13*).] Potential success with Charon 3A is indicated by colorless plaques on XG plates with Lac$^+$ bacteria. Since Charon 3A phages that have lost the Lac fragment without insertion of foreign DNA cannot be distinguished genetically from a clone, additional tests are required, such as hybridization to a suitable probe or gel electrophoresis of a restriction enzyme digest of the phage DNA (Fig. 3).

Controlled transcription of cloned fragment. The cloning sites in many of the Charon phages can have their transcription controlled by the phage leftward promoter (p_L) or the Lac promoter. This property has already proved valuable in mapping promoters in *E. coli* fragments specifying ribosomal proteins that were cloned in Charon phages 3 and 4 (*16*).

Cloning with Charon phages. The procedure that we have developed for constructing clones differs significantly from those previously described; the conditions for ligation of fragments have been chosen to favor assimilation of all target fragments into giant concatenates rather than to attempt to limit the reaction at the dimer stage. The vector phage DNA is annealed to join its ends. (Lambdoid phages have mutually complementary single-stranded sequences on the ends of their DNA molecules.) The vector and target DNA's are digested with the desired restriction enzyme, which is then inactivated with diethyl pyrocarbonate (*17*). In order to generate recombinant DNA molecules, the vector and target DNA's are mixed. Their relative proportions are adjusted so that the molar concentration of target DNA fragments does not exceed that of the vector DNA, and the total DNA concentration is kept high (*18*) to ensure that the formation of recombinant molecules competes effectively with monomolecular cyclization reactions, particularly of short target fragments. The mixture is incubated with T4 ligase to join the molecules covalently. Viable phages can then be obtained by transfection of *E. coli* spheroplasts (*19*) or calcium-treated *E. coli* (*20*), or by in vitro encapsidation into λ capsids (*21*). We presume that λ-sized chimeric molecules are either extracted directly from concatenates by the λ packaging function, or

that circles are first generated by recombination within the transfected *E. coli* cell.

Vectors with a high yield. An important safety feature for any cloning vector is its ability to be grown in high yield, so that only small volumes of culture are required for production purposes. The Charon phages and clones derived from them can usually be propagated to at least 10^{10} phage per milliliter by our PDS (pre-adsorb-dilute-shake) method (*22*). After lysis, 3 ml of chloroform is added; this immediately kills all surviving bacteria without affecting the phage. Typical yields for some of the vectors are shown in Table 1. Yields of phages containing cloned fragments have usually been in the same range.

Recovery of cloned DNA. Two convenient methods have been devised for recovering cloned DNA fragments from Charon phages. The first method is general and applies to any clones containing a single restriction enzyme fragment smaller than about 2 kbp. In this method the clone-bearing phage DNA is prepared, heated at 55°C in 2*M* NaCl to anneal the cohesive ends, dialyzed, cut with the restriction enzyme, and centrifuged for 4 hours at 40,000 rev/min (SW50.1 rotor) at 15°C. The small cloned fragment remains in the supernatant and can be recovered free of the large vector fragment by ethanol precipitation.

The second method is useful in selected cases, and we have used it for studying mouse globin complementary DNA (cDNA) clones that were obtained by procedures similar to those that have been used with plasmids (*23*). The purification method is based on the fact that the largest DNA fragment in Hha I restriction enzyme digests of Charon 3A ΔLac (see Fig. 2 legend) contains the single Eco RI site of this phage. Provided that the cloned piece of DNA inserted into this site does not contain an Hha I site, the size of the cloned DNA fragment can easily be determined from the size of the largest fragment in an Hha I digest. The position of this fragment after polyacrylamide gel electrophoresis is convenient for preparative purposes. Figure 3 illustrates this point with one of our mouse globin cDNA clones.

Hybrid phage nomenclature. Charon clones are named by appending to the name of the vector one or more letters denoting the source of inserted DNA, followed by an isolation number. For example, Charon 3AHb4 was our fourth isolation candidate for a mouse globin clone. The orientation of an inserted fragment can be denoted "n" (natural) or "u" (unnatural), depending on whether the coordinate system assigned to the target fragment increases from left to right in agreement with λ's. In Charon 11, the Lac and Bio DNA's are in the "n" orientation since the clockwise "time-increasing" direction of the *E. coli* map corresponds to the left-right direction in λ.

Biological containment. Guidelines have been set up by the National Institutes of Health for assessing the suitability of host-vector systems for the cloning of DNA from different sources (*2*). When DNA from higher organisms, such as mammals, is being cloned, a host-vector system with a biological rating of EK2 is required.

The NIH guidelines specify (*2*) that: "For EK2 host-vector systems in which the vector is a phage, no more than one in 10^8 phage particles should be able to perpetuate itself and/or a cloned DNA fragment under nonpermissive conditions designed to represent the natural environment either (a) as a prophage or plasmid in the laboratory host used for phage propagation or (b) by surviving in natural environments and transferring itself and/or a cloned DNA fragment to a host (or its resident lambdoid prophage) with properties common to those in the natural environment." Charon phages 3A, 4A, and 16A in conjunction with host bacteria DP50 (χ1953), or DP50SupF (χ2098) have now been certified as meeting these criteria by the director of NIH following the recommendation of his Recombinant DNA Advisory Committee.

The accumulation of data on safety and the certification process itself took more than a year and involved our submitting eight documents detailing experimental results concerning safety (*24*). A phage working group, appointed by the NIH Recombinant DNA Advisory Committee and headed by W. Szybalski, met three times to analyze the results. They made several useful suggestions to us for additional experiments prior to recommending that the systems be certified.

In the following, we present a brief synopsis of the safety features of the λ vectors, including (Table 2) the results of three safety experiments that were considered particularly germane by the phage working group.

General safety features of λ phages. Foreign DNA cloned in a λ phage might be propagated outside the laboratory in

Table 2. Gene transfer between Charon A phages and bacteria. Exchange of genes in both directions between Charon A phages and bacteria was estimated by considering three cases. (Experiment 1) Gene transfer from phage to productive host: Lysates of Charon A phages were prepared and the titers for phage (at lysis) and for total bacteria (after overgrowth) were determined; the λ*lacz*$^-$*y*$^+$*sup*E bacterium CSH18 was used as host. Lac$^+$ bacteria (those that had stably incorporated the *lacz* gene function from the phage) were measured in the overgrown cultures. The ratio of the stable associates after overgrowth to the phage titers at lysis were calculated. (Experiment 2) Gene transfer from phage to nonproductive host: Charon A phages were adsorbed at a 1 : 1 ratio to λ^{q}Lacz$^-$y$^+$sup^o bacteria (M96CF^r) and to two heteroimmune lysogens of this strain (φ80*imm*21 and λ*imm*21). Lac$^+$ bacteria produced by the transfer of *lacz* from phage to host were measured and expressed as a fraction of the phage input. (Experiment 3) Gene transfer from nonproductive host to phage: The experiment was similar to experiment 2 except that the phage produced by the infected cells were studied. Phages that had either of the safety features, immunity deletion or A^-B^-, replaced by genes from the host lysogens (marker rescue) were assayed. Transfer of *imm*21 to Charon 4A phage was measured by titration on a λ lysogen of the *sup*F strain Ymel; for Charons 3A and 16A, a φ80 lysogen was used. Transfer of A^+B^+ genes from lysogen to Charon phage was assayed by counting the number of blue plaques on the *sup*^{o} strain W3350 on XG plates. N.T., not tested.

Vector	Ch3A	Ch4A	Ch16A
	Experiment 1. Phage to productive host		
Phage/ml*	1.0×10^{11}	1.0×10^{10}	3.0×10^{10}
Bacteria/ml†	5.8×10^{8}	4.7×10^{8}	3.1×10^{10}
Lac$^+$bacteria/ml	4.9×10^{4}	5.2×10^{4}	1.5×10^{4}
Lac$^+$bacteria/phage	4.9×10^{-7}	5.0×10^{-6}	5.0×10^{-7}
	Experiment 2. Phage to nonproductive host (Lac$^+$ cells obtained per input phage)		
Nonlysogen	8.4×10^{-5}	1.8×10^{-4}	N.T.
φ80*imm*21 lysogen	7.0×10^{-7}	2.5×10^{-7}	$<2.9 \times 10^{-8}$
λ*imm*21 lysogen	4.5×10^{-6}	1.2×10^{-6}	2.7×10^{-6}
	Experiment 3. Nonproductive host to phage (output phage with rescued imm21 *or* A^+B^+ *per input phage)*		
Nonlysogen	$<10^{-6}$	$<10^{-6}$	$<10^{-6}$
φ80*imm*21 lysogen:			
*imm*21			1.0×10^{-5}
A^+B^+	3.6×10^{-4}	2.0×10^{-5}	1.3×10^{-5}
λ*imm*21 lysogen:			
*imm*21	$<5.7 \times 10^{-5}$	$<3.9 \times 10^{-5}$	1.2×10^{-4}
A^+B^+	1.1×10^{-3}	2.4×10^{-3}	5.9×10^{-3}

*At harvest. †After overgrowth.

two ways: (i) lytically, if the clone-bearing phage found a suitable host in the environment and started a lytic replication cycle before it had been inactivated; or (ii) as a lysogen or plasmid, if the phage DNA containing the cloned segment became incorporated into the genome of a bacterium, or if the phage DNA became established as a defective (nonlytic) plasmid in a bacterium, and if the bacterium could then grow outside the laboratory.

In order for λ to be propagated lytically outside the laboratory, phage particles must be capable of surviving in the natural environment. We find that fewer than 1 in 10^{10} phages survive exposure to *p*H 3 for 2.75 hours (mimicking stomach conditions); fewer than 2 in 10^7 survive 30 minutes in detergent (1 percent sodium dodecyl sulfate); fewer than 2 in 10^4 survive 6 hours of drying on a laboratory bench; fewer than 3 in 10^6 survive 120 hours in raw sewage. Thus, there are initial inactivation barriers to dissemination.

In order for λ to multiply in the natural environment, the phage must repeatedly encounter sensitive bacterial hosts. Typical raw sewage contains 10^7 bacteria per milliliter. Calculations show that self-sustaining lytic replication requires at least 10^8 sensitive bacteria per milliliter, so that phages can encounter bacteria sufficiently often for replication to overcome inactivation (*25*). Yet, of more than 2000 strains of *E. coli* independently isolated from the wild or from hospital patients, none was λ sensitive (*26*). Consequently sewage is unlikely to be able to sustain a lytic replication of λ. The lack of ability of *E. coli* in the wild to replicate λ phages is borne out by our measurements of decreasing titers of λ phages on shaking with raw sewage (Fig. 4). Furthermore, λ cannot inject its DNA into bacteria below 15°C (*27*).

With the Charon phages, it has also been possible to decrease the number of potential natural hosts by including amber mutations in the phages. Such phages require a suppressor transfer RNA (tRNA) in the host bacterium of a type not usually found in wild-type *E. coli*. A further decrease in the number of potential natural hosts is obtained by propagating the phage in the laboratory on bacteria that do not modify the phage DNA; the DNA is then vulnerable to attack by the restriction endonucleases found in many wild-type *E. coli* strains.

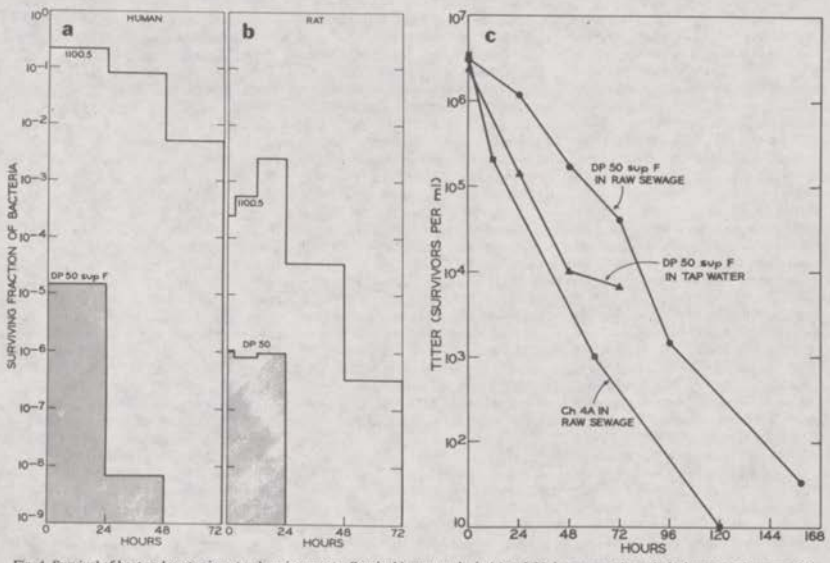


Fig. 4. Survival of host and vector in natural environments. Survival in mammals: in (a) and (b) the passage through the human and rat gut of the debilitated laboratory hosts DP50 and DP50SupF and, for comparison, a nondebilitated strain of *E. coli* K12 [1100.5 (*28*)] was examined. These strains are resistant to nalidixic acid, and titers can be determined on eosin–methylene blue–minimal galactose plates supplemented with vitamin-free Casamino acids and containing nalidixic acid (100 μg/ml). Such plates eliminate growth of native gut bacteria much better than plates made with yeast extract, and permit discrimination between Gal^+ (1100.5) and Gal^- (DP50 and DP50SupF) bacteria. (a) A mixture of 3×10^{11} DP50SupF and 9.4×10^8 1100.5 was fed in 250 ml of milk to three humans. Bar heights represent the averaged fraction of bacteria surviving gut passage during the time intervals defined by the end points of each bar. The total fraction of DP50SupF surviving is 1.4×10^{-5}, which is 4.7×10^{-4} that of 1100.5. (b) Broth (0.25 ml) containing 8×10^9 DP50 was placed on the tongues of three rats, and 1.6×10^8 1100.5 bacteria were fed to three other rats in the same manner. The total fraction of DP50 surviving is 2.9×10^{-4}, which is 8.3×10^{-4} that of 1100.5. In neither species was there any indication of colonization by any K12 strain. Survival in sewage. In (c) City of Madison raw sewage, including University of Wisconsin effluent, was collected from a pond immediately upstream of the treatment plant; it had a titer of 10^7 bacteria per milliliter measured on L plates. The inactivation of the EK2 vector, Charon 4A, shaken in raw sewage at 37°C, and of the debilitated host, DP50SupF, shaken in raw sewage and tap water at room temperature, was examined. The titer of Charon 4A was determined by counting blue plaques on XG plates; DP50SupF was determined as above. The initial first-order rate constants for inactivation in sewage were 1.0 day^{-1} (bacteria) and 5.6 day^{-1} (phage). The averages over the entire experiment were 1.7 day^{-1} (bacteria) and 2.6 day^{-1} (phage).

We conclude that, as far as dissemination by lytic processes is concerned, λ offers an inherently high degree of biological containment, which has been further increased in the Charon phages.

Escape of the phage-cloned DNA in a bacterial lysogen or plasmid must be considered from two aspects—the bacterium might be the host used for propagation in the laboratory, or it might be a naturally occurring one encountered by a clone-bearing phage after accidental release into the environment. In either case the self-replicating ability of bacteria, in contrast with the inability of phage to replicate without a host, makes dissemination of a cloned fragment by the bacterial route potentially serious. We have, therefore, focused much of our safety strategy on designing the vector so that its replication will be confined to the lytic mode.

The ability to form lysogens or plasmids has been reduced in Charon phages 3A, 4A, and 16A by deletions KH53 or KH54 in the immunity regions of the phages, by the *nin*5 deletion (coupled with wild-type N function) which enhances lytic functions, and, in the case of Charon phages 4A and 16A, by the elimination of the gene *int* and the *att* site, which are needed for efficient insertion of the phage genome into the bacterial chromosome.

The effectiveness of these strategies can be measured by determining what proportion of bacteria surviving an encounter with a Charon phage acquire the cloned fragment of DNA in some stable association. The *lac*5 *E. coli* substitution carried on Charons 3A, 4A, and 16A is a convenient model cloned segment, provided that test bacteria are used which provide the *lacy* (permease) function and do not contain the *lacz* (β-galactosidase) function. Appropriate test bacteria were constructed for this purpose (*28*).

The first experiment shown in Table 2 was designed to measure the formation of stable associates with a laboratory host during productive growth. Charon phages were grown by the PDS method on Lacz⁻y⁺cells. To mimic a worse case, the lysates were not harvested at the normal time but, instead, were allowed to overgrow until surviving bacteria had reached a high titer. Chloroform was not added. Surviving cells were separated from the free phage, diluted into broth, and shaken for 90 minutes to permit stable associates to become established and to permit unstable associates to segregate. The bacteria were then plated on XG indicator plates with lactose as the sole carbon source. Reconstruction experiments were done to verify our ability to demonstrate very low levels of Lac⁺ bacteria in lysates. The titers of phage, total bacteria, and Lac⁺ bacteria are listed in Table 2 (experiment 1). The results show that the ratio of undesirable products (Lac⁺ bacteria) to desirable products (phages) is of the order of 10^{-6}. (The majority of the bacteria surviving these lytic infections were shown to be λ-resistant.) When lysates were harvested at the proper time, these ratios were more favorable by a factor of 10^3, and chloroform is routinely used to kill all the bacteria in lysates. Nevertheless, the phage working group recommended using a host bacterium for the productive growth of the phage that is able to provide an additional safety factor over that provided by *E. coli* K12. This recommendation was implemented by our using DP50, as described below.

The second experiment in Table 2 was undertaken to test the likelihood that a recombinant phage that escaped into nature might subsequently be stably integrated into a naturally occurring bacterium. Although the probability of an encounter under dilute conditions with a nonpermissive bacterium that could adsorb λ would be low, as discussed above, it might be cause for concern if the integration step turned out to be highly efficient. Again worse cases were tested in which the bacteria were λ-sensitive, lacked an amber suppressor, and carried prophages having a high homology (λ *imm*21) or moderate homology (ϕ80 *imm*21) with the Charon phages. We also tested nonlysogenic bacteria. In these experiments, Charon phages were adsorbed efficiently to the bacteria at a ratio of about 1 : 1 and then diluted into broth as before. The efficiency of transfer of a model cloned segment to the nonpermissive lysogen relative to the number of input phage is listed in Table 2 (experiment 2). These data show that the formation of stable associations is far from being efficient.

The third experiment in Table 2, suggested by the phage working group, provides data on marker rescue. The marker rescue experiments were designed to test the possible outcome of a phage escaping as a particle, and surviving in the natural environment long enough to find a rare bacterium (to which it can adsorb) which harbors a prophage that can remove by marker rescue the phage mutations introduced for safety. Recombination between such a prophage and the clone-bearing phage could create a new phage with some of its safety features compromised. The protocol for this experiment again involved adsorbing Charon A phages to λ-sensitive lysogens lacking an amber suppressor. The release of Lac⁺-containing phages that had lost either the amber mutations or the immunity deletions was then measured (Table 2, experiment 3).

The results show that the frequencies of loss by marker rescue of the amber mutations on the left side of the cloned segment are around 3×10^{-3} and of the immunity mutations on the right side are around 5×10^{-5}. Both of these events would not fully compromise the safety of the Charon system, and they would be but a small part of an extensive chain of improbable events. The results indicate that these probabilities are indeed worst case estimates because when the homology between the vector and the prophage was reduced, as would be the case in most strains from nature, the rescue frequencies were reduced.

Bacterial hosts. The bacterial hosts, DP50 and DP50SupF, were selected for the Charon phage system because of their additional contributions to the problem of biological containment. DP50 (also called χ1953) was constructed by Pereira and Curtiss [see (*29*)], University of Alabama. DP50SupF (also called χ2098) was constructed from DP50 by Leder *et al*. (*30*), and has the same genotype as DP50 except for the addition of *supF*58 (*su*III). The genotypes of the strains are: F⁻, *dap*D8, *lacy*, Δ*gal-uvrB*, Δ*thyA*, *nalA*^{r}, *hsdS*, *supE*44 (plus *supF*58 in DP50SupF). The *dap*D8, Δ*thyA*, and Δ*gal-uvr*B mutations were introduced so that the bacteria themselves have a low survival in nature. This provides an extra margin of safety if the normal harvesting procedure, including chloroform treatment of the lysates, is not executed on time, and the entire culture including phage, bacteria, and a low frequency of plasmids or lysogens is inadvertantly released into the natural environment. The survival of these bacteria during shaking in raw sewage and during intestinal passage through humans and rats is shown in Fig. 4. Relative to *E. coli* K12, the survival of these strains is reduced about a thousandfold. None of the tested bacteria colonized either the rat or human gut.

The reversion rates of the bacterial mutations important for safety, *dap*D8, Δ*thy*A, and Δ*gal-uvr*B, must be considered. The *thy* and *gal* mutations are deletions and do not revert. Dap⁺ revertants were found at about 10^{-8} in stock cultures grown from a single colony. They only accumulated to 5×10^{-8} even after 10^{12}-fold growth, so that Dap⁺ revertants do not have any appreciable selective advantage in dap-containing medium. Moreover, none of 22 tested bacteria surviving human or rat gut passage were Dap⁺. Thus, reversion of *dap*D8 is not likely to

compromise the safety of the bacteria to any appreciable extent. Nevertheless, as with phage, it is advisable to frequently check the genotype of the bacteria in order to avoid contamination and mistakes as well as revertants.

Since both bacteria lack the *E. coli* K modification system, only a very small proportion, about 10^{-4}, of phage propagated on them would grow on bacteria from nature having a restriction system similar to that of *E. coli* K12. Both DP50 and DP50SupF will grow the Charon A phages efficiently.

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References and Notes

1. P. Berg, D. Baltimore, S. Brenner, R. O. Rilin III, M. O. Singer, *Science* **188**, 991 (1975); W. Szybalski, in *Genetic Alteration: Impact of Recombinant Molecules on Genetic Research*, R. Beers, Ed. (Raven, New York, in press).
2. *Fed. Regist.* **41**, 27902 (1975).
3. N. E. Murray and K. Murray, *Nature (London)* **251**, 476 (1974); A. Rambach and P. Tiollais, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 3927 (1974); L. Enquist, D. Tiemeier, P. Leder, B. Weisberg, N. Sternberg, *Nature (London)* **259**, 596 (1976); B. G. Williams, D. D. Moore, J. W. Schumm, D. J. Grunwald, A. E. Blechl, F. R. Blattner, in *Genetic Alteration: Impact of Recombinant Molecules in Genetic Research*, R. Beers, Ed. (Raven, New York, in press); D. J. Donoghue and P. A. Sharp, in preparation; D. Tiemeier, L. Enquist, P. Leder, *Nature (London)* **263**, 526 (1976).
4. M. Thomas, J. R. Cameron, R. W. Davis, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 4579 (1974).
5. K. Murray and N. E. Murray, *J. Mol. Biol.* **98**, 551 (1975).
6. KH100 and KH54 were described by F. R. Blattner, M. Fiandt, K. K. Haas, P. A. Twouse, W. Szybalski, *Virology* **62**, 458 (1974).
7. *bio1* was described by K. F. Manly, E. R. Signer, C. M. Radding, *ibid.* **39**, 137 (1969); *imm*434, by A. D. Kaiser and F. Jacob, *ibid.* **34**, 509 (1969); *nin*5, by D. Court and K. Sato, *ibid.* **39**, 348 (1969); Q*SR*80, by N. Franklin, W. Dove, C. Yanofsky, *Biochem. Biophys. Res. Commun.* **18**, 910 (1965); *lac*3, by J. Shapiro, L. MacHattie, L. Eron, G. Ihler, K. Ippen, J. Beckwith, R. Arditti, W. Reznikoff, R. MacGillivray, *Nature (London)* **224**, 768 (1969); *b*189, by J. S. Parkinson and R. J. Huskey, *J. Mol. Biol.* **56**, 369 (1971); *b*1007, by D. Henderson and J. Weil, *Virology* **67**, 124 (1975); *imm*80, *att*80, and hybrid 42, by J. Szpirer, R. Thomas, C. M. Radding, *ibid.* **37**, 585 (1969); Δ*H*3 is a deletion of the *Hind* III fragment immediately to the left of *att*, and was the gift of D. Kampf and R. Kahn.
8. B. G. Williams, thesis, University of Wisconsin, Madison (1977).
9. W. Arber and U. Kühnlein, *Pathol. Microbiol.* **30**, 946 (1967).
10. A. J. D. Bellett, H. G. Busse, R. L. Baldwin, in *The Bacteriophage Lambda*, A. D. Hershey, Ed. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1971), p. 501.
11. These enzymes are as follows. Eco RI [M. Thomas and R. W. Davis, *J. Mol. Biol.* **91**, 315 (1975)] was purified by a modification of the method of D. Smith and J. Davies (personal communication). Hind III [P. H. Roy and H. O. Smith, *J. Mol. Biol.* **81**, 427 (1973)] was obtained from New England Bio-Labs (Beverly, Mass.). Bam H1 [G. A. Wilson and F. E. Young, *J. Mol. Biol.* **97**, 123 (1975)] was obtained from New England Bio-Labs. Kpn I and Pst I [D. Smith, F. R. Blattner, J. Davies, *Nucleic Acids Res.* **3**, 343 (1976)] were both obtained from New England Bio-Labs. Sal I (J. Arand, P. Meyers, R. Roberts, personal communication) was obtained from New England Bio-Labs. Bgl II [G. A. Wilson and F. E. Young, in *Microbiology, 1976*, D. Schlessinger, Ed. (American Society of Microbiology, Washington, D.C., 1976), p. 350] was obtained from Bethesda Research Laboratories (Rockville, Md.). Xma I (R. Roberts, personal communication) was a gift from F. W. Farrelly.
12. A. Campbell, *Virology* **14**, 22 (1961).
13. The immunity specificity of the vectors was determined by plating on appropriate homoimmune and heteroimmune lysogens, and by examination of plaque type (turbid or clear) [A. D. Hershey and W. Dove, in *The Bacteriophage Lambda*, A. D. Hershey, Ed. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1971), p. 3]. The presence or absence of amber mutations was determined by plating on hosts containing or lacking an appropriate amber suppressor (*12*). For phages with λ or 434 immunity, the presence of the *nin*5 deletion was demonstrated by the ability to plate on GroN host strains [C. P. Georgopolous, in *The Bacteriophage Lambda*, A. D. Hershey, Ed. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1971), p. 639]. The presence or absence of the λ genes *exo*, *β*, and γ was determined by ability of phages to plate on RecA$^-$ host strains (Frec phenotype (J. Zissler, E. Signer, F. Schaefer, in *ibid.*, p. 455) or hosts lysogenic for phage P2 [Spi phenotype (J. Zissler, E. Signer, F. Schaefer, in *ibid.*, p. 469). The presence of the *lac*5 substitution was determined by plating on XG dye indicator plates, as described in the text (*37*, p. 48)]. The presence of *bio* substitutions in phages was determined by ability to promote growth of Bio$^-$ hosts on biotin-deficient plates [G. Kayajanian, *Virology* **36**, 732 (1968)].
14. N. Sternberg and R. Weisberg (personal communication); M. Shulman and M. Gottesman, in *The Bacteriophage Lambda*, A. D. Hershey, Ed. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1971), p. 477; M. Gottesman and M. Weisberg, in *ibid.*, p. 113.
15. R. B. Helling, H. M. Goodman, H. W. Boyer, *J. Virol.* **14**, 1235 (1974); D. H. Gelfand and P. O'Farrell, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3900 (1976).
16. S. R. Jaskunas, A. M. Fallon, M. Nomura, B. G. Williams, F. R. Blattner, in preparation.
17. The reaction mixture is made 0.1 percent diethyl pyrocarbonate (Calbiochem) by adding 0.1 volume of a freshly diluted 10 percent solution in ethanol. After incubation for 10 minutes at 37°C, other enzymes may then be added as desired.
18. The concentration *C* (micrograms per milliliter) of vector DNA, which equals the intramolecular concentration of target fragment termini, is given by $C = 46 \, V/L^2$ [derived from C. Wang and N. Davidson, *J. Mol. Biol.* **19**, 469 (1969)], where *L* is the size of target molecule in kilobase pairs, and *V* is the length of the vector DNA as percentage of λ (including any dispensable fragments) other than the readily removed D. For example, to clone a 5-kbp fragment in Charon 16, vector DNA should be at or above 354 μg/ml.
19. W. D. Henner, I. Kleber, R. Benzinger, *J. Virol.* **12**, 741 (1973).
20. M. Mandel and A. Higa, *J. Mol. Biol.* **53**, 159 (1970).
21. D. Kaiser, M. Syvanen, T. Masuda, *ibid.* **91**, 175 (1975); B. Hohn and T. Hohn, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 2372 (1974); N. Sternberg, D. Tiemeier, L. Enquist, *Gene*, in press.
22. The PDS method was modified from that described by Blattner *et al.* (*6*). The phage (10^9 to 10^{11} plaque-forming units) was mixed with 0.3 to 1.0 ml of stationary culture of cells plus an equal volume of 0.01*M* $MgCl_2$ and then incubated at 37°C for 10 minutes; the mixture was diluted into a liter of warmed NZY or NZYDT (*see* below) broth, and shaken overnight at 37°C; NZY broth contains (per liter) 10 g of NZamine (a casein hydrolyzate available from Humko-Sheffield, Linnhurst, N.J.), 5 g of yeast extract (Difco), 5 g of NaCl, and 2 g of $MgCl_2 \cdot 6\,H_2O$. NZYDT broth contains, in addition to NZY constituents, 0.04 g of thymidine and 0.1 g of diaminopimelic acid. We have found many batches of Bacto-tryptone (Difco) that do not work when substituted for NZamine. The exact ratio of phages to bacteria is generally not critical.
23. F. Rougeón, P. Kourilsky, B. Mach, *Nucleic Acids Res.* **2**, 2365 (1975); R. Higuchi, G. V. Paddock, R. Wall, W. Salser, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3146 (1976); T. Maniatis, S. G. Kee, A. Efstradiatis, F. Kafatos, *Cell* **8**, 163 (1976); T. H. Rabbitts, *Nature (London)* **260**, 221 (1976).
24. The original application, submitted 1 June 1976 is: "Application for EK2 Certification of a Host Vector System for DNA Cloning," B. G. Williams, D. D. Moore, J. W. Schumm, D. J. Grunwald, A. E. Blechl, and F. R. Blattner; Supplement I: "Part I *New dap*$^-$ Bacterial Host, DP50, for Productive Growth of Charon 3A and 4A Phages. Part II Two Phages Derived from Charon 3A and 4A (16A and 14A) with Deleted Attachment Sites; and Two (15A and 13A) Which Are in Addition Devoid of Bacterial DNA," F. R. Blattner, A. E. Blechl, H. E. Faber, L. A. Furlong, D. J. Grunwald, D. O. Kiefer, E. L. Sheldon, and O. Smithies; Supplement II: "Marker Rescue Tests," F. R. Blattner, D. O. Kiefer, and D. D. Moore. Supplement III: "Additional Marker Rescue Data on Charon 3A and Charon 4A," F. R. Blattner, D. O. Kiefer, and D. D. Moore; Supplement IV: "Further Tests on DP50 (γ 1953) and DP50 : SupF (χ 2098)," O. Smithies, F. R. Blattner, A. Blechl, H. E. Faber, E. L. Sheldon, B. G. Williams, and L. A. Furlong; Supplement V: "Additional Data Requested by the Phage Working Group," F. R. Blattner, B. G. Williams, D. O. Kiefer; Supplement VI: "Rearmament of Charon Phages in Sewage," F. R. Blattner, D. O. Kiefer, H. E. Faber, and O. Smithies; Supplement VII: "Marker Rescue Tests on Charon Phages Having Amber Mutations in Genes W and E Instead of Genes A and B," F. R. Blattner and D. O. Kiefer.
25. For net replication to occur, $(K_s)(\theta)(\Gamma)(\kappa,) > 1$, where $K_s = 5.6\,day^{-1}$ is the initial first-order rate constant for phage inactivation by City of Madison sewage, β is the concentration of *h*-sensitive bacteria in sewage, $\Gamma = 100$ is the yield of phage per infected bacterium, and $K_a = 7.2 \times 10^{-7}$ ml per bacterium per day (J. Salstrom, personal communication) is the second-order rate constant for phage-bacteria collision and infection.
26. R. W. Davis, personal communication; P. Leder, personal communication; J. S. Parkinson, personal communication: 2000 is the sum of determinations made by the above three persons.
27. D. McKay and V. Bode, *Virology* **36**, 732 (1968).
28. CSH18 is *Δlac pro*) X111 *supE* (F′ *lac z* Y* *proA*$^+$B$^+$). The F′ carries *lacz* deletion H125. CSH46 = M96 and is *Δlac pro*) X111 *sup*$^+$ (*sc*I857 Sus68 Δ80 *lacIz*). CSH18 and CSH46 are described in (*37*, p. 16). M96CF$^-$ was constructed by curing strain CSH46 of the prophage and introducing the F′ from CSH18. In Table 2, "λ *imm*21 lysogen" refers to λ *h*λ *att*80 *imm*21 Q*SR*80 (for tests with Charon 3A and Charon 4A) and λ *h*λ *imm*21 Q*SR*80 (for tests with Charon 16A) in M96CF$^-$. "Φ80 *imm*21 lysogen" refers to Φ80 Δ80 *att*80 *imm*21 Q*SR*80 in M96CF$^-$. Strain 1100.5 (*nalA*^{r} *endA*$^-$), used in the mammalian ingestion experiments, was obtained from J. Davies.
29. As described by R. Curtiss, III, M. Inoue, D. Pereira, J. Hsu, L. Alexander, L. Rock, in *Molecular Cloning of Recombinant DNA*, W. Scott and R. Lerner, Eds. (Academic Press, New York, in press).
30. P. Leder, D. Tiemeier, L. Enquist, *Science* **196**, 175 (1977).
31. B. C. Westmoreland, W. Szybalski, H. Ris, *Science* **163**, 1343 (1969); R. W. Davis, M. Simon, N. Davidson, *Methods Enzymol.* **21**, 413 (1971).
32. D. D. Moore, J. W. Schumm, M. M. Howe, F. R. Blattner, in *DNA Insertions*, S. Adhya and A. Bukhari, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1977).
33. J. H. Miller, *Experiments in Molecular Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1972).
34. Measured in our laboratory by D. Daniels. Circles of P1*vir* DNA (20.44 percent of λ*c*72 length, M. Fiandt, personal communication) were compared to ϕX174 replicative form, assumed to be 5375 bp from the complete DNA sequence, as determined by F. Sanger *et al.*, *Nature (London)* **265**, 687 (1977).
35. The experiments related to cloning mouse globin cDNA were done under Asilomar guidelines; we used P3 physical containment with the (now) certified EK2 vector Charon 3A. We thank many friends who have given us samples of the phages and bacterial strains, J. Nulter for the cooperation of NIAID, and W. Szybalski and W. F. Dove for critical reading of the manuscript. The name Charon phages was suggested by Eric C. Rosenvold. We thank C. Morita, D. Stephensen and V. Farkas for technical assistance; J. Richards, S. Nitz, and A. Johnson provided help with illustrations. Supported by NIH contract AI 62506, and grants GM 21812 and GM 20069. This is Paper No. 2125 from the Laboratory of Genetics, University of Wisconsin, Madison.

7 February 1977

Recombinational Switch for Gene Expression

Abstract. *Flagellar antigens are specified by two genes, H1 and H2. The expression of these genes is regulated such that only one gene activity, or phase, is expressed at a given time. Molecular cloning techniques were used to isolate the segments of* Salmonella *DNA which contain these genetic loci. Heteroduplex analyses revealed an anomaly in the cloned fragment, that is, an apparent inversion, which was shown to be adjacent to the H2 gene. A correlation was demonstrated between the phase state of the H2 gene and the sequence of the adjacent segment. We propose that an inversion of this region is the phase-determining event in flagellar gene expression in* Salmonella.

Phase variation in *Salmonella* is an example of a regulatory mechanism that involves a switch in gene expression mediated by a metastable change at the level of the DNA. This was initially referred to by Lederberg and Iino (*1*) as a change in the "local state" of the gene, and it was suggested that such changes could be involved in cell differentiation and development. The molecular mechanism of this change is not known. However, it has become possible to obtain relatively large amounts of the specific DNA that carries the phase switch so that the DNA can be examined directly. We describe experiments that suggest that phase variation results from a directed inversion adjacent to one of the genes that controls the structure of the *Salmonella* flagellar antigen.

Phase variation was discovered by Andrewes 55 years ago (*2*). He found that mass cultures derived from a single clone of *Salmonella typhimurium* would agglutinate with two different antiserums directed against their flagellar filaments. Twenty-five years later, Stocker (*3*) showed that the variation was the result of the ability of *Salmonella* strains to switch from one flagellar antigen (phase) to another with a small probability which was two to three orders of magnitude higher than the frequency of mutation. Lederberg and Iino (*1*) investigated the nature of the switch by genetic techniques. They concluded that (i) the structure of the flagellar filament was specified by two genes, H1 and H2; (ii) the ability to switch from the expression of one gene to another was controlled by a genetic element linked to the H2 gene; (iii) the state of expression of the H2 gene could be transduced, and, therefore, the switch involved a change at the DNA level. The kinds of mechanisms that have been proposed to account for phase variation fall

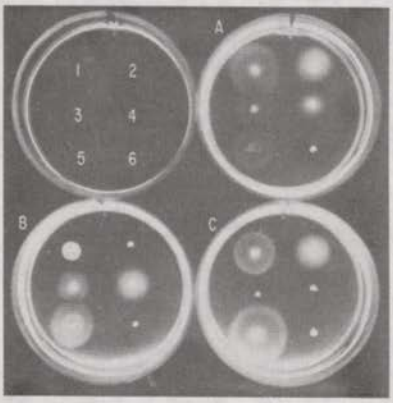
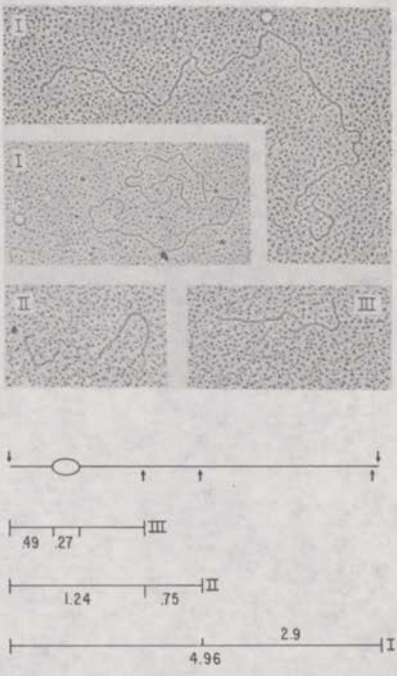


Fig. 1 (above). Serological tests illustrating flagellar antigen expression. No antiserum was added to the motility agar in plate A; antiserums to $H1^b$ and to $H2^{enx}$ were added to plates B and C, respectively. Anti-$H1^b$ was obtained from Burroughs-Wellcome Co., Greenville, North Carolina, and anti-$H2^{enx}$ was obtained from Lee Laboratories, Grayson, Georgia. Identification of the clones is as follows: (position 1) *Salmonella* phase 1–on (SL4213), (position 2) $H1^b$ clone ($C600\ Hag^-$), (position 3) *Salmonella* phase 2–on (SL4213), (position 4) $H2^{enx}$ clone ($C600\ Hag^-$), (position 5) $C600\ Hag^+$, (position 6) $C600\ Hag^-$. $H2^{enx}$ clones were immobilized only in anti-$H2^{enx}$ while $H1^b$ clones were immobilized only in anti-$H1^b$. Motility of $C600\ Hag^-$ was not affected by either antiserum. Fig. 2 (right). Heteroduplex analysis of cloned DNA. Electron micrographs: I and insert show reannealed fragment I derived from Eco RI endonuclease treatment of hybrid plasmid containing H2 gene; II and III show fragments derived by Eco RI and partial Sal I digestion. Restriction enzyme Eco RI was obtained from D. Helinski; Sal I was purchased from New England Bio-Labs, Beverly, Massachusetts. Below is a map of the $H2^{enx}$ DNA indicating positions of Eco RI sites (arrows above line) and Sal I sites (arrows below line). Covalently closed Col E1 was used as an internal standard in the measurements. Dimensions are shown in microns.



into two general classes: (i) an alteration in the genetic arrangement at the H2 locus, for example, the insertion of a heterologous sequence or the inversion of an adjacent region (*4*, *5*); and (ii) modification of a regulatory sequence (*6*), for example, specific methylation of the promotor region in the H2 gene. In order to test these hypotheses, the region carrying phase variation in *Salmonella* was cloned. A strain of *Escherichia coli* which did not synthesize the flagellar antigen (C600 mK^-rK^-, Hag^-) was used as recipient. This strain was shown to form abortive transductants with transducing phage grown on *Salmonella*. The transductants expressed either phase 1 or phase 2 of *Salmonella* in *E. coli*. The donor material was prepared by treating *Salmonella* DNA from strain SL 4213 (*7*) with Eco RI endonuclease and forming hybrid molecules with restricted colicinogenic factor E1 (Col E1). The mixture of transformed (*8*) cells was streaked onto motility agar containing colicin, and the swarms that emerged were picked and tested with antiserums specific for *Salmonella* phase 1 ($H1^b$) flagella (anti-$H1^b$) and *Salmonella* phase 2 ($H2^{enx}$) flagella (anti-$H2^{enx}$). Figure 1 shows that there were two kinds of clones: (i) those that were able to swim in the presence of anti-$H2^{enx}$ but not anti-$H1^b$ and, therefore, carried the structural gene for $H1^b$ and (ii) those that were able to swim in the presence of anti-$H1^b$ but not anti-$H2^{enx}$ and carried the gene for $H2^{enx}$. The ability to show phase variation is tightly linked to the $H2^{enx}$ gene. DNA was isolated from this strain and used to retransform the C600 Hag^- cells. One hundred single colonies were picked and stabbed onto motility agar. After 1 day, 98 of these showed some level of motility, and two were apparently nonmotile. However, when the nonmotile clones were again streaked on agar they showed motile swarms. The heterogeneity of response suggested that the strain was undergoing phase transition. Thus, the DNA prepared from this strain should be a mixture of molecules carrying phase 2 in the on-state and phase 2 in the off-state. If the switch were the result of an anomaly at the DNA level, we should be able to see it by denaturing and reannealing the $H2^{enx}$ DNA. If phase transition involved the insertion, deletion, or inversion of DNA, a fraction of the denatured DNA would consist of heteroduplexes containing a "deletion loop" or an "inversion bubble." On the other hand, if modification, that is, methylation, were involved, we would not expect to see anything distinctive about the reannealed DNA. Before denaturation and renaturation, the DNA had no anomalous features. However, after renaturation between 5 to 10 percent of the molecules had a structure similar to an "inversion bubble" (*9*) (Fig. 2).

The DNA was further characterized by using restriction endonucleases. Treatment with .Eco RI endonuclease separated the *Salmonella* DNA (fragment I) from the Col factor DNA. The *Salmonella* DNA was 4.96 ± .04 μm in length, corresponding to a molecular weight of 9.9 × 10^6. The "inversion bubble" was 0.27 ± .03 μm in length corresponding to 750 bases. The short arm adjacent to the inversion was 0.49 ± .04 μm corresponding to 1450 base pairs. The DNA had three sites for Sal I endonuclease. Figure 2 shows the positions of the Sal I sites as

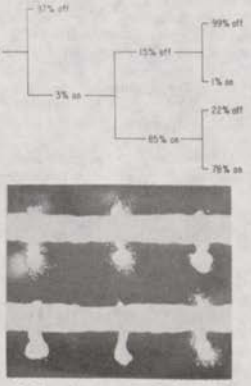


Fig. 3. Genetic properties of λ H2. Transductional crosses between λ H2 and an *E. coli* strain defective in flagellin synthesis is shown at the bottom. A tester strain, MS5014, was spread on motility agar and cross streaked with phage from individual plaques. Transduction of the H2 gene had two consequences: (i) swarms of motile cells occurred by recombination or by integration of the transducing phage; (ii) trails of immotile clones emanated from the region of infection. These resulted from abortive inheritance of the H2 gene and indicated complementation of the flagellin defect. λ H2 clones were of two types: (i) those that gave high levels of complementation, λ H2-on, that is, top left, top middle, and bottom right; (ii) those that gave very low levels of complementation, λ H2-off, that is, top right, bottom left, and bottom middle. The variation in the genetic properties of λ H2 clones is shown above. λ H2 was grown on overlay plates and harvested, and single plaques were selected. These were grown sufficiently to obtain enough phage for complementation tests. The forks in the diagram summarize the distribution of phenotypes obtained from single plaques.

determined from electron microscopy measurements and agarose gel electrophoresis.

If the "inversion bubble" were related to H2 gene expression, we might expect it to be adjacent to the H2 gene. The Col E1 factor does not have a site for Sal I endonuclease. Therefore, if we treat the plasmid DNA with Sal I, isolate the bands that are formed on agarose gel electrophoresis, and retransform with this DNA, we can delete parts of the *Salmonella* DNA and determine the effect of the deletion on the expression of the $H2^{enx}$ gene. When Col E1 factor carrying *Salmonella* DNA fragment II or fragment III was used for transformation they both gave motile enx-specific transformants. Single clones were picked from these transformants and the plasmid DNA was again isolated. It was found to carry only fragment II or fragment III. Therefore, both fragments must carry the $H2^{enx}$ structural gene. It is difficult to distinguish between the arms on either side of the "inversion bubble" in fragment III because they are of about the same length. However, flagellin, the product of the H2 gene, has an apparent molecular weight of about 50,000 (*10*), and either arm has DNA which is just sufficient to code for a polypeptide of that size. Therefore, the region carrying the inversion must either be part of the H2 structural gene or it must lie just outside the region that codes for H2. The inversion segment is therefore closely associated with the H2 gene.

The next question is whether the event that generates the "inversion bubble" correlates with phase transition. It is difficult to follow phase transition in cells carrying the hybrid-Col factor since each cell has a large number of copies (from 10 to 20) of the factor. We therefore transferred the DNA onto a λ phage vehicle. The λ phage Charon 1 designed by Blattner (*11*) could accept heterologous DNA as large as fragment I. The DNA was ligated with Eco RI–treated Charon 1 phage DNA and the mixture was used to transfect a recipient strain (C600 rK^-mK^-). Plaques were picked, and the phage were tested for their ability to transduce $H2^{enx}$ to *E. coli* that lacked the flagellin gene (strain MS5014) (*12*). Two kinds of plaques that transduced the H2 gene were found, those that gave high levels of transduction and those that gave low levels of transduction (Fig. 3). When single plaques from the λ population that gave low levels of transduction were retested, 1 percent were found to give high levels. When plaques from phage that gave high levels of complementation were retested, 80 percent of them gave high levels and 20

percent gave low levels. Figure 3 summarizes this analysis and shows the behavior of both classes of λ H2 phage in transductional tests. We assume that these two types represented the behavior of phage carrying phase 2–on, or phase 2–off. The process of phase transition was asymmetric, that is, the rate of transition from on to off appeared to be at least tenfold higher relative to the rate in the reverse direction (off to on). The picture is similar to that initially observed by Stocker (*3*). He found that the transition from phase 2–on to phase 2–off occurred at about ten times the frequency of the reverse transition. On the basis of these experiments, we can define two populations of λ phage, one that carries H2–off in almost all of the population and another which has mostly H2–on.

To determine whether the "inversion bubble" is correlated with expression of the different states of H2, DNA's from the different λ populations were prepared, denatured, and reannealed; and the frequency of inversions was measured. Three hundred molecules of λ H2–off DNA were scored, and 2.5 percent had the "inversion bubble." One hundred and fifty molecules of DNA carrying λ H2–on were scored, and 12 percent were found to carry the inversion. When equal amounts of these preparations were mixed, denatured, and reannealed, 34 percent of 150 molecules were found to have the inversion. The large increase in frequency obtained when the two populations were mixed indicated that it was the heteroduplex between phase 2–on and phase 2–off regions that formed the "inversion bubble." On the basis of these experiments we suggest that phase variation is determined by a specific region adjacent to the H2 gene which can undergo an inversion. In one configuration it allows the H2 gene and perhaps adjacent genes (*13*) to be transcribed. In the opposite configuration it does not allow H2 transcription. This hypothesis is shown schematically in Fig. 4. We cannot formally rule out the possibility that a specific transposition of heterologous DNA into the phase-controlling site is responsible for the "inversion bubble." However, in recent experiments DNA molecules associated by a duplex region which corresponded to the "inversion bubble" with nonhomologous single-stranded ends, have been observed. These results suggest that with respect to the "bubble" the notion that it is generated by an inversion is the simplest model that explains all of our observations. It raises questions about the specific mechanisms involved in regulating the transition. These can be approached experimentally with the use of the cloned DNA.

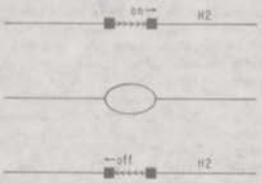


Fig. 4. A model for phase variation. An inversion of the sequence between the dark blocks could result in a change in the orientation of a promoter contained within the region or it could allow read through from a promoter outside the region.

Evidence in a variety of different systems has accumulated suggesting that a recombinational event, an inversion (*14*), or a transposition (*15*) can be involved in regulating gene expression. These findings taken together with our present view of phase variation suggest that it may be worthwhile to reexamine the notion that directed recombinational events play a role in cell differentiation and development.

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References and Notes

1. J. Lederberg and T. Iino, *Genetics* **41**, 744 (1956).
2. F. W. Andrewes, *J. Pathol. Bacteriol.* **25**, 515 (1922).
3. B. A. D. Stocker, *J. Hyg.* **47**, 398 (1949).
4. U. B. Pearce and B. A. D. Stocker, *J. Gen. Microbiol.* **49**, 335 (1967).
5. T. Iino, *Bacteriol. Rev.* **33**, 454 (1969).
6. R. Holliday and J. E. Pugh, *Science* **187**, 226 (1975).
7. This strain showed H2^{on}–H1^{a} variation and was obtained from B. A. D. Stocker; M. Enomoto and B. A. D. Stocker, *Genetics* **81**, 595 (1975).
8. V. Hershfield, M. W. Moyer, C. Yanofsky, M. Lovett, D. Helinski, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 3455 (1975).
9. We refer to this structure as an "inversion bubble." However, it need not be the result of an inversion, it could result from another process, for example, the specific transposition of a heterologous sequence.
10. H. Kondoh and H. Hotani, *Biochim. Biophys. Acta* **336**, 117 (1974).
11. F. R. Blattner, B. G. Williams, A. E. Blechl, K. Denniston-Thompson, H. E. Faber, L.-A. Furlong, D. J. Grunwald, D. O. Kiefer, D. D. Moore, J. W. Schumm, E. L. Sheldon, O. Smithies, *Science* **196**, 161 (1977).
12. M. Silverman, P. Matsumura, M. Simon, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3126 (1976).
13. H. Fujita, S. Yamaguchi, T. Iino, *J. Gen. Microbiol.* **76**, 127 (1973).
14. H. Saedler, H. J. Reif, S. Hu, N. Davidson, *Mol. Gen. Genet.* **132**, 265 (1974); A. Toussaint, *Virology* **70**, 17 (1976); S. Tonegawa, N. Hozumi, G. Matthyssens, R. Schuller, *Cold Spring Harbor Symp. Quant. Biol.*, in press.
15. B. McClintock, *Cold Spring Harbor Symp. Quant. Biol.* **21**, 197 (1956); *Symp. Soc. Dev. Biol.* **184** (1967); J. B. Hicks and I. Herskowitz, *Genetics*, in press; J. B. Hicks, J. N. Strathern, I. Herskowitz, in *DNA Insertion Elements, Plasmids and Episomes*, A. J. Shapiro and S. Adhya, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., in press).
16. We thank John Abelson for his enthusiasm and for numerous helpful conversations. The work was supported by NSF grant PCM 76-17197 and NIH grant AI 13008-01).

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Gene Cloning and Containment Properties of Plasmid Col E1 and Its Derivatives

Abstract. *Colicinogenic plasmid E1 (Col E1) and Col E1 derivatives offer advantages as plasmid cloning vehicles with regard to both utility and biological containment. The Col E1 derivative pCR1 does not alter those essential characteristics of the enfeebled* Escherichia coli *strain χ1776 that make this strain particularly useful as a host-vehicle system for recombinant DNA research.*

In this report we discuss the properties of colicinogenic plasmid E1 (Col E1) and certain Col E1 derivatives obtained in our laboratory that make these plasmids particularly suited as cloning vehicles for recombinant DNA research.

Col E1 is a relatively small, covalently closed, circular DNA molecule (4.2 megadaltons in size) (*1*) that is present in multiple copies in *Escherichia coli*—about 20 to 30 molecules per cell (*2*). When grown in the presence of chloramphenicol, Col E1 is amplified; that is, bacterial chromosomal replication ceases, but Col E1 continues to replicate until approximately 45 percent of the cellular DNA is Col E1 DNA (*2*, *3*). With the use of this amplification step, high yields of both Col E1 DNA and also any DNA sequence that is inserted into the plasmid are readily obtained (*4*).

Since Col E1 contains a nonessential region, a single site sensitive to the restriction endonuclease Eco RI, Eco RI–digested DNA can be inserted into the plasmid after cleavage with this restriction enzyme. Recombinant plasmids constructed by insertion at the Eco RI site are readily detected since they no longer produce the antibiotic protein colicin E1 (*4*). Col E1 is a nonconjugative plasmid; that is, it is unable to promote its own transfer from one bacterial cell to another (*5*). However, the conjugal transfer of Col E1 is promoted by self-transmissible (conjugative) plasmids (*6*). Conjugative plasmids vary greatly in their ability to promote the transfer of Col E1.

There are several unique derivatives of Col E1 that have been developed for use

as cloning vehicles. Mini-Col E1 (pVH51) is a spontaneously-isolated plasmid (2.1 megadaltons) in which approximately one-half of Col E1 DNA is deleted (7). pVH51 has an even higher copy number than does Col E1 (approximately 114 copies per cell), amplifies in chloramphenicol to the same extent as Col E1, and retains the single Eco RI site. With regard to containment of the plasmid, it is of importance that pVH51 is mobilized by a conjugative plasmid at a much lower frequency than Col E1 (7). Similar low molecular weight derivatives of Col E1–like plasmids that carry antibiotic resistance genes and are extensively used as plasmid cloning vehicles have been constructed (8).

Kanamycin resistant (Km^r) derivatives of plasmids Col E1 and mini-Col E1 have been constructed in vitro by insertion of a 4.5-megadalton kanamycin-resistance fragment into the Eco RI site of Col E1 (4) and mini-Col E1 (7). One of the two Eco RI sites of Col E1-*kan* (designated pML2) has been removed by exonuclease treatment of pML2 after partial digestion with Eco RI (9). This plasmid, designated pCR1 (9), has two properties that make it advantageous for gene cloning. First, although Col E1 and pVH51 lack easily detectable phenotypes that facilitate detection, pCR1 specifies kanamycin resistance, a very effective selective marker. Second, since one Eco RI site has been deleted, DNA can be inserted into the remaining Eco RI site of pCR1 without loss of the Km^r marker. A restriction map of pCR1 is shown in Fig. 1.

In view of the advantages of pCR1 as a cloning vehicle, the effect of this plasmid on the survivability properties of the enfeebled *E. coli* strain χ1776 (10) were examined to determine the suitability of χ1776 (pCR1) as an EK2 host-vector system for gene cloning.

In the absence of exogenous diaminopimelic acid (DAP), a cell wall constituent, the viability of strain χ1776 decreases to a level of at most 10^{-6} of the starting cell concentration (Fig. 2A) as observed by R. Curtiss III and co-workers. Death of χ1776 resulting from lack of DAP also occurs in the presence of another strain, χ1780, that carries the conjugative plasmid R64 drd 11, indicating that at least the strain χ1780 does not rescue χ1776 by cross-feeding (Fig. 2B). The presence of pCR1 in χ1776 does not prevent or alter death due to lack of DAP (Fig. 2C), nor can χ1780 "rescue" pCR1 by mobilization of pCR1 out of χ1776 and into χ1780 (Fig. 2D).

Another important property of strain χ1776 is its sensitivity to bile salts, a characteristic that should reduce the survival of this organism in the digestive tract. Strain χ1776 carrying pCR1 shows no increased resistance to bile salts than χ1776 without pCR1 (Table 1). Indeed, the data suggest that χ1776 (pCR1) is even more sensitive to bile salts than χ1776. These results essentially confirm the containment properties of χ1776 observed by R. Curtiss III and co-workers and demonstrate that pCR1 does not significantly alter these properties.

Table 1. Average plating efficiencies (P) of χ1776 and χ1776 (pCR1) as a function of bile salts concentration. Exponential and early stationary cultures of χ1776 and χ1776 (pCR1) grown at 37°C in L2 broth were concentrated by centrifugation and resuspended in 1.0 ml of L2 broth. Dilutions were made in the same medium and plated onto L2 agar containing 0, 0.15, 0.37, or 0.75 percent Bacto bile salts No. 3. Survival on plates containing bile salts was determined by means of the titer on plates without bile salts. The plating efficiency (P) is log (viable count of χ1776) − log [viable count χ1776 (pCR1)] for each concentration of bile salts. The survival of χ1776 and χ1776 (pCR1) at the different bile salts concentrations ranged from 2.9×10^{-3} to 9×10^{-7} at 0.15 percent, 6.9×10^{-7} to 3.4×10^{-8} at 0.37 percent, and 7.1×10^{-6} to 1.5×10^{-8} at 0.75 percent.

Bile salts (%)	Average plating efficiency ($P \pm$ S.E.)	Experiments (No.)
0.15	1.7 ± 4.3	4
0.37	0.9 ± 0.56	5
0.75	0.8 ± 0.31	5

Finally, the ability of conjugative plasmids to promote the transfer of pCR1 out of χ1776 was tested in triparental matings using (i) donor strains χ1776 (pCR1) or C600 (pCR1) as a control, (ii) nine *E. coli* strains (11) carrying different R (antibiotic resistance) plasmids capable of mobilizing pCR1, and (iii) an *E. coli* recipient. The nine R plasmids tested for mobilization of pCR1 were R64 drd 11, R6K, R16, N-3, R27, R46, R471a, RIP175, and R71a. The R factors mobilized pCR1 from χ1776 at frequencies below 10^{-6} under conditions when the mobilization of pCR1 from the control strain C600 (pCR1) occurred at frequencies of 5×10^{-4} to 10^{-5} after 25 hours of mating. Other investigators have shown that a sex plasmid, the F1 plasmid, can mobilize Col E1 derivatives from strains analogous to C600 at frequencies approximately 100-fold higher than what was observed with these nine R plasmids (12). This difference could be explained if F and its derivatives are more efficient than R plasmids at mobilizing Col E1.

The recovery of pCR1 from χ1776 as covalently closed circular DNA is similar to that found for the C600 strain using sarkosyl (13) or Triton-X100 (14) lysis. However, amplification in the presence of chloramphenicol (4) yielded at best 5 percent of the total DNA as covalently closed circles.

Containment can also be facilitated through the use of special plasmid cloning vehicles as well as enfeebled hosts. For instance, since drug-resistant strains of *E. coli* have a selective advantage for establishment in the gut of animals being starved (15) or treated with antibiotics

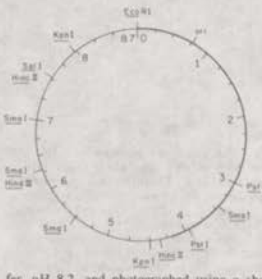


Fig. 1. Restriction map of the Col E1 derivative pCR1. Purified pCR1 DNA was digested at 37°C in buffer consisting of 7 mM tris, pH 7.4, 60 mM NaCl, 7 mM $MgCl_2$, and 7 mM mercaptoethanol for all enzymes except Hpa I [10 mM tris, pH 7.4, 10 mM $MgCl_2$, 6 mM KCl, 1 mM dithiothreitol (DTT)], and Eco RI (100 mM tris, pH 7.4, 50 mM NaCl, 6 mM $MgCl_2$, and 6 mM mercaptoethanol). Reactions were stopped by heating at 65°C for 10 minutes, and 20 μl of bromophenol blue dye containing 25 percent glycerol and 5 percent sodium dodecyl sulfate was added to each reaction mixture. Digests were subjected to electrophoresis in 1.0 percent, 1.2 percent, or 1.4 percent agarose gels at 120 to 140 volts at room temperature for approximately 2 hours. After electrophoresis, gels were stained for 30 minutes with ethidium bromide in tris-borate buffer, pH 8.2, and photographed using a shortwave ultraviolet transilluminator. Eco RI- or Hind III-digested bacteriophage λ DNA and Eco RI–digested pML2 (Col E1-*kan*) were used as molecular weight standards. The enzymes Bam HI, Hpa I, and Bgl II did not cleave pCR1. Hinc II also cleaves the smaller Hinc II fragment twice, producing three fragments of 0.5, 1.0, and 1.3 megadaltons. The relative order of these fragments has not been conclusively determined, but the largest (1.3 megadaltons) is cleaved by Hind III. Hae II and Hae III cleave Col E1 into 6 and 14 fragments, respectively (19). Insertion into the Hind III site inactivates Km^r (D. Figurski and R. Meyer, unpublished results), but insertion into the Sal I site does not (20). Loss of the small Pst I fragment does not inhibit Col E1 replication (A. Otsuka, unpublished results). The divisions represent megadaltons of DNA. The kanamycin determinant is denoted by the lighter section of the map.

(*16*), plasmid cloning vehicles carrying selective markers other than antibiotic resistance may be preferred in certain circumstances. For this purpose, two plasmids that carry genes for tryptophan biosynthesis (pVH151, Col E1-*trpE trpD*; and pVH153, mini-Col E1-*trpE trpD*) were constructed in collaboration with C. Yanofsky. The *trp* fragment (4.7 megadaltons) was obtained by Eco RI digestion of λ-*trp* bacteriophage λED10f(bot) and contains the poED region of the tryptophan operon (*17*). The *trp* fragment was inserted at the Eco RI site of Col E1 and mini-Col E1. At present, experiments are in progress to delete one of the two Eco RI sites in each plasmid to facilitate the insertion of exogenous DNA.

Other plasmid vehicles that may also facilitate containment are those that are disabled by a mutation in the plasmid. One such type, pJC307, a Col E1 plasmid that is unable to replicate at 37°C, has been isolated (*18*). The thermosensitive phenotype of this mutant suggests that Col E1 codes for at least one protein essential for its own replication and has led to our current efforts to isolate amber-suppressible Col E1 mutants that will be dependent upon a specific host genotype for replication and maintenance. The use of such mutant plasmid vectors should increase significantly the degree of biological containment afforded by an enfeebled host strain.

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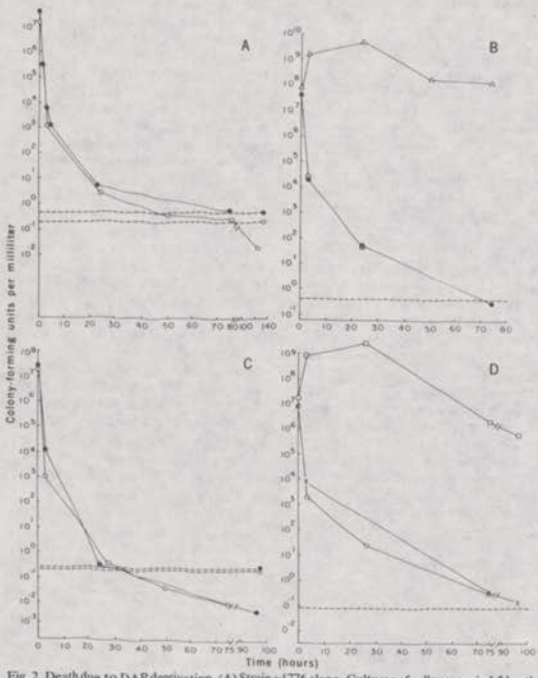


Fig. 2. Death due to DAP deprivation. (A) Strain χ1776 alone. Cultures of cells grown in L2 broth [L broth + diaminopimelic acid (DAP) (200 μg/ml) + thymine (thy) (50 μg/ml)] to exponential or early stationary phase were harvested by centrifugation at 15°C. The pellets were washed and resuspended in 10 ml of BSG buffer (8.5 g of NaCl, 0.3 g of KH_2PO_4, 0.6 g of $NaHPO_4$, and 100 mg of gelatin per liter) and were used to inoculate DAP⁻ medium (L broth containing thy and lacking NaCl), at a cell concentration of 5 ml/liter. Cultures were incubated with shaking at 37°C. Samples were removed at the times indicated and titered on L2 agar. At later times, cells were concentrated by filtration, and colonies were grown on sterile Millipore filters placed on L2 agar. Controls showed that viability was not affected by filtration. The dotted line indicates the level at which the number of colony-forming units have decreased by 10^6 from the original titer. The symbols (○) and (●) represent points from two different experiments. (B) Strain χ1776 in the presence of χ1780. Cultures of both strains were treated as described in (A). Titers were determined with the use of the following media: χ1776, L2 agar plus nalidixic acid (50 μg/ml) (●); χ1780, MacConkey lactose agar (Δ); and χ1776 (R64), L2 agar plus nalidixic acid plus tetracycline-HCl (25 μg/ml) (□). Strain χ1780 [*trx-63 supE42* λ⁻ *his-53 lysA12 T3ʳ xyl-14 arg-65* (R64 drd 11 $Sm^r Tc^s$)] was obtained from R. Curtiss III. (C) Strain χ1776 (pCR1) alone. Cultures were treated and the titers were determined as described in the legend to Fig. 2A. The symbols (○) and (●) represent data from two different experiments. (D) Strain χ1776 (pCR1) in the presence of χ1780. Titers were determined using the following media: χ1776, L2 agar + nalidixic acid (○); χ1780, MacConkey lactose agar (□); χ1776 (pCR1) and χ1780 (pCR1), L2 agar plus kanamycin sulfate (25 μg/ml) (×).

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7 March 1977

References

1. M. Bazaral and D. R. Helinski, *J. Mol. Biol.* **36**, 185 (1968).
2. D. B. Clewell and D. R. Helinski, *J. Bacteriol.* **110**, 1135 (1972).
3. D. B. Clewell, *ibid.*, p. 667.
4. V. Hershfield, H. W. Boyer, C. Yanofsky, M. Lovett, D. R. Helinski, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 3455 (1974).
5. P. Fredericq, *C.R. Soc. Biol.* **148**, 746 (1954).
6. P. Kahn and D. R. Helinski, *J. Bacteriol.* **88**, 1573 (1964).
7. V. Hershfield, H. W. Boyer, L. Chow, D. R. Helinski, *ibid.* **126**, 447 (1976).
8. M. C. Betlach, V. Hershfield, L. Chow, W. Brown, H. M. Goodman, H. W. Boyer, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **35**, 2037 (1976); R. L. Rodriguez, F. Bolivar, H. M. Goodman, H. W. Boyer, M. C. Betlach, in *Molecular Mechanisms in the Control of Gene Action* (Academic Press, New York, 1976), p. 471.
9. C. Covey, D. Richardson, J. Carbon, *Mol. Gen. Genet.* **145**, 155 (1976).
10. The *E. coli* strain χ1776 *F⁻ tonA53 dapD8 minA1 supE42 Δ40 (gal-uvrB) λ⁻ minB2 rfb-2 nalA25 ibyA37 menC65 T3ʳ Δ29 (bioH-asd) cycB2 cycA1 hsdR2* was developed by R. Curtiss III and his colleagues as an enfeebled host for recombinant DNA research.
11. These strains consisted of the nine R plasmids in the same host strain χ1753 (*trx-63 supE42* λ⁻ *his-53 lysA12 T3ʳ xyl-14 arg-65*). All were provided by R. Curtiss III.
12. L. Clark and J. Carbon, *Cell* **9**, 91 (1976).
13. R. Meyer, D. Figurski, D. R. Helinski, *Mol. Gen. Genet.*, in press.
14. L. Katz, D. T. Kingsbury, D. R. Helinski, *J. Bacteriol.* **114**, 577 (1973).
15. M. G. Smith, *Nature (London)* **261**, 348 (1976).
16. P. A. M. Guinee, *Ann. N.Y. Acad. Sci.* **182**, 40 (1971).
17. D. R. Helinski, V. Hershfield, D. Figurski, R. Meyer, in *Tenth Miles Internation Symposium* (in press).
18. J. Collins, S. Yanofsky, D. R. Helinski, in preparation.
19. A. Oka and M. Takanami, *Nature (London)* **264**, 193 (1976).
20. D. Hamer and C. A. Thomas, Jr., *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1537 (1976).
21. We thank D. Figurski, R. Meyer, and E. Remaut for communicating unpublished restriction mapping results and J. Gibson and M. Yanofsky for technical assistance. This work was supported by NIH grant AI-07194 and NSF research grant GB-29492. K.A.A. and V.H. were supported by PHS postdoctoral fellowships 1F32 A105129-01 and GB54149, respectively.

EK2 Derivatives of Bacteriophage Lambda Useful in the Cloning of DNA from Higher Organisms: The λgt*WES* System

Abstract. *A derivative of bacteriophage λ has been modified and tested together with an appropriate host system to meet the criteria of EK2 biologic containment for cloning DNA from higher organisms. In this report certain of the safety features are summarized and some of the tests carried out to confirm the containment properties of the vector are described. The cloning efficiency of this system, together with available gene purification and hybrid screening technology, indicate that it can be used to clone DNA fragments carrying specific, unique mammalian genes.*

Thomas *et al.* (*1*) have constructed a mutant strain of coliphage λ which is especially suited for cloning fragments of foreign DNA. The phage, λgt·λC, carries two Eco RI restriction sites between which Eco RI–generated DNA fragments of 1,000 to 14,000 base pairs (1 to 14 kilobase pairs) can be inserted. Because incorporation of a DNA fragment of approximately 1 kbp or more is required for phage growth, this system also provides a strong positive selection for fragment-bearing recombinants.

Recently we introduced three amber mutations—*W*am403, *E*am1100, and *S*am100—into this phage with the aim of reducing its likelihood of encountering a susceptible host and surviving in nature (*2*). We have further modified the resulting phage, λgt*WES*·λC, by substituting the phenotypically inert Eco RI λ fragment, λB, for λC (Fig. 1). The λC fragment contains the genes for specialized recombination and, according to its EK2 requirements, must be biochemically separated from the two larger λ fragments before in vitro recombination. The new derivative λgt*WES*·λB, can be used in recombination experiments directly (*3*). For testing purposes we have also constructed two model recombinants, one of which contains the genes for galactose metabolism derived from the λp*gal*8 recombinant described in Enquist *et al.* (*2*) (Fig. 1). The other contains a 7.6-kbp Eco RI fragment of *Escherichia coli* DNA.

These phage contain amber mutations in genes corresponding to (i) the major capsid protein of the phage, which is also required for cleavage of replicated DNA (the *E* gene), (ii) a protein needed for the joining of phage heads and tails (the *W* gene), and (iii) a protein required for host lysis (the *S* gene). While the *W* and *E* genes are suppressed by several amber suppressors including *sup*E, the S mutation is not efficiently suppressed by *sup*E and requires a specific suppressor, *sup*F. In lysates, the frequency of wild-type revertants of each of these mutations is approximately 10^{-5} to 10^{-6} (Table 1). Wild-type revertants could not be detected in lysates of phage carrying double or triple mutations. λgt*WES*·λC may be propagated conveniently through lytic culture or as a lysogen since it carries the λ integration-excision system and the temperature-sensitive repressor c1857. λgt*WES*·λB can be prepared as a high-titer stock through lytic infection in liquid culture. Both phage can accept Eco RI fragments of foreign DNA as noted above, but λgt*WES*·λB can also accept Sst I fragments 0.01 to 10 kbp in length (Fig. 2). The presence of these Sst I sites also provides a selection against parental type Eco RI recombinants since the vector may be digested with both Sst I and Eco RI, destroying the parental λB fragment.

Bacteriophage λ has a number of features that recommend it as a safe and easily contained vector. This phage has a fastidious host range and is apparently restricted to growth in a limited number of *E. coli* strains. In fact, we have surveyed more than 300 separately isolated, human hospital strains of *E. coli* and were unable to find any capable of supporting growth of either λ wild-type or λgt*WES* phage (Table 2). In addition, λ and the λgt*WES* derivatives are readily inactivated by passage through the mouse alimentary tract

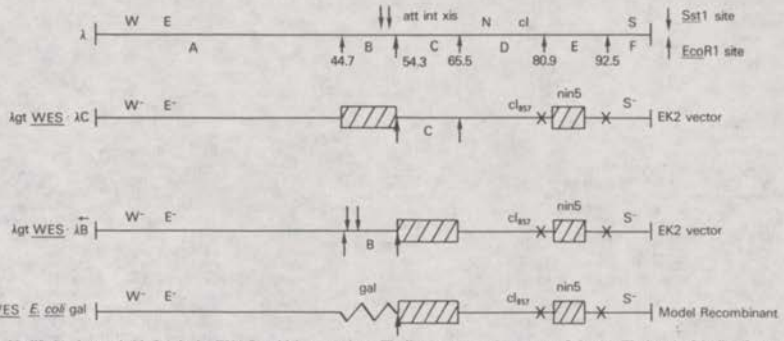


Fig. 1. Modified λ phage suitable for cloning DNA from higher organisms. The lines represent the genome of phage λ. The length of the line drawn represents the full length of the genome of wild-type bacteriophage λ. Letters over each line refer to specific λ genes. Letters under each line refer to Eco RI restriction fragments of λ with each arrow indicating an Eco RI site. The numbers under each arrow represent the position of the site as a percentage of the λ genome. Arrows over each line indicate the position of an Sst I site (note inversion as compared to wild type). Scored boxes represent deleted portions of the λ genome. X represents the point at which an Eco RI site has been eliminated by mutation. The broken line represents the location of *E. coli* DNA containing the galactose operon. Details of the construction of the two vectors have been described by Enquist *et al.* (*2*) and Tiemeier *et al.* (*3*). The model recombinant was constructed by crossing λgt*S*am100·λC with λ*W*am403 *E*am1100 *gal*8 *bio*256 *imm*434 c1ts1 as described by Enquist *et al.* (*2*). The resulting phage eliminated the RI site at 54.3 percent λ and substituted an RI site within and close to the right end of the *gal*8 insertion. The internal Eco RI fragment containing a small portion of the *gal*8 insertion and the λ genes *int*, *xis*, and a portion of the *red* gene were deleted by in vitro recombination, yielding the phage depicted in the diagram. The position of the left end of the *gal*8 substitution is at 44.7 percent on the λ map (*13*), very close to the original λ Eco RI site.

Table 1. Ability of λ derivatives to grow on nonsuppressor-containing hosts. All stock lysates had titers of 1 to 2×10^{10} per milliliter. Where possible, each mutation was tested individually in the strain used in its construction (2).

Derivative	Frequency of amber* revertants
λWam403 *E*am1100	$< 10^{-8}$*
λWam403 *E*am1100 *S*am100 · λC	$< 10^{-8}$*
λ*S*am100 · λC	$\sim 10^{-3}$†
λWam403	$\sim 5 \times 10^{-6}$†

*A 0.01-ml portion of undiluted stock plated on *Escherichia coli* strain 594 (*sup*0). †A 0.01-ml portion of 1000-fold diluted stock plated on *E. coli* strain 594 (*sup*0).

Table 2. Ability of phage λ, safer vector, and model recombinant to grow on environmentally encountered *E. coli* strains. Experiments on hospital samples obtained from the NIH Clinical Center Microbiology Diagnostic Laboratory were tested by plating 0.02-ml droplets of phage containing $\sim 10^8$ plaque-forming units on lawns of each strain, using standard procedures (*1*). λgt*WES* · *E. coli* contains a 7.6 kilobase pair Eco RI fragment of *E. coli* DNA.

Strain source	Number tested	λ	Number of strains permitting plaque formation	
			λgt-*WES* · λC	λgt-*WES* · λC
Waterborne*	> 1000	0	NT	NT
Hospital samples	> 300	0	0	0

*Data supplied by R. Davis.

Table 3. Ability of model recombinant to survive in mouse alimentary tract. Three BALB/c mice were fed drinking water containing 10^9 plaque-forming units of λgt*WES* · *E. coli*, a model recombinant, per milliliter. Approximately 100 ml was consumed during the experiment. Stool samples (~ five droppings) were collected on sterile paper on the days indicated and were suspended in TMG (0.01*M* tris, 0.01*M* $MgSO_4$, 0.01 percent gelatin, *p*H 7.4) containing chloroform. The suspension was tested for plaques on *E. coli* Ymel (a *sup*F host).

Sample	Phage recovered (per milliliter)
Water supply, day 1	$\sim 10^9$
Stool, day 1	0
day 2	0
day 4	0
day 6	0
Water supply, day 7	$\sim 10^9$

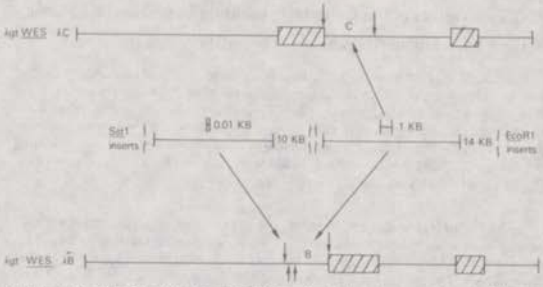


Fig. 2. Lengths of Eco RI and Sst I restriction fragments which may be inserted into λgt*WES*·λC and λgt*WES*·λB. Symbols are as indicated in the legend to Fig. 1. The arrows above the line represent Eco RI sites, and those below the line represent Sst I sites. Abbreviation: *KB*, 1000 base pairs.

Table 4. Safety features of λgt*WES* · λC or λgt*WES* · λB.

1. Three conditional lethal mutations (amber)
   *E*am1100; no phage heads and no cleavage of DNA to form cohesive ends
   *W*am403; no joining of phage heads and tails
   *S*am100; no lysis or phage release
   *sup*F suppresses all three mutations
   sup E does not suppress *S*am 100 efficiently
2. *c*Its857
   Temperature-sensitive repressor; at 37°C, λ*c*Its 857 will not form a stable lysogen
3. *nin*5 deletion
   Removes a transcription stop signal; greatly reduces probability of plasmid formation
4. Recombinants are recombination defective (*red*$^-$)
   λ *red*$^-$ phage grow less well than *red*$^+$ phage, conferring a selective disadvantage
5. Under permissive conditions, recombinants are capable of high-titer growth in small, easily manipulated volumes
6. Restriction barrier
   Phage can be propagated in nonmodifying hosts; unmodified DNA is destroyed on infection of a restricting host

Table 5. Test for the persistent association of cloned fragment with the permissive host used in its propagation. Routine preparative procedure: *E. coli* DP50 [*sup*F, *sup*E, *dap*D8, *lac*Y, Δ (*gal-uvr*B)*thy*A, *mal*A^r, *hsd*S] was grown overnight in tryptone broth supplemented with diaminopimelic acid (DAP, 100 μg/ml) and thymidine (50 μg/ml), and washed and resuspended in an equal volume of 0.01*M* $MgSO_4$. Approximately 1.5×10^8 bacteria were incubated with 10^8 λ *W*am403 *E*am 1100 *gal*$^+$ (*att-red*)$^+$ *c*Its857 *nin*5 *S*am100 (multiplicity of infection = 10^{-2}) for 10 minutes at 32°C in 0.2 ml of 0.01*M* $MgSO_4$, then diluted with 5 ml of L broth (supplemented with DAP and thymidine as above) and incubated at 38°C with vigorous shaking. Lysis occurred after 8 to 9 hours and incubation was continued for 24 hours. The phage titer was determined at the time of lysis (9 hours). Viable bacteria were determined by removing 1-ml portions, diluting and washing with 5 ml of 0.01*M* $MgSO_4$, centrifuging and washing again, and resuspending in 1 ml of 0.01*M* $MgSO_4$. Incubation was at 32°C on minimal-salts plates supplemented with DAP (100 μg/ml), thymidine (40 μg/ml), biotin (0.5 μg/ml), lysine (4 μg/ml), and glucose (0.5 percent). The *gal*$^+$ bacteria were determined from portions of cells treated as just described, except that plating was on medium containing 0.5 percent galactose in place of glucose. In control experiments, supplemented minimal-salts plates were checked for their ability to grow *gal*$^-$ and *gal*$^+$ derivatives of DP50 *sup*F in the presence of glucose or galactose. In the sample immediately after lysis, 4.5×10^9 cells were plated on minimal-galactose plates incubated at 32°C. No *gal*$^+$ colonies were detected. In the sample 24 hours after lysis, 7.4×10^9 cells were plated on minimal-galactose plates. No *gal*$^+$ colonies were detected. The validity of the detection assay was assessed by adding *gal*$^+$ DP50 *sup*F (λ*gal*8 *c*Its857) cells to overgrown cells before spreading on minimal-galactose plates. Recovery of *gal*$^+$ cells was approximately 100 percent.

Time	Phage titer (ml^{-1})	Bacterial titer (ml^{-1})	*gal*$^+$ transductants (ml^{-1})
At lysis	2.3×10^{10}	4.8×10^9	< 1
24 hours after lysis		7.4×10^9	< 10

[Table 3 and Geier, Trigg, and Merril (4)].

While λgt*WES* phage are at an obvious survival disadvantage because of their suppressor requirements, they have a further disadvantage in that fragment-containing phage lack the λ general recombination functions (*red*) required for normal phage growth (5). The yield of such *red*-deficient phage is one-half to one-tenth that of the λ wild type. Nevertheless, sufficient recombinant phage may be prepared from 1 to 2 ml of lysates to permit detailed enzymatic, hybridization, and electron microscopic analyses (6). The specific safety features of the λgt*WES* system are summarized in Table 4 (2). The presence of each genetic feature, including the amber mutations, the temperature-sensitive repressor gene, the *nin*5 deletion, and the absence of the specialized recombination genes, can be determined by simple genetic tests (2, 3).

In addition to other detailed tests required for EK2 certification (7), we have tested the possibility that a cloned fragment could form a persistent association with a bacterial host used in its propagation. The experiments described in Table 5 represent a typical preparative phage growth using the model *gal*$^+$ phage recombinant and the attenuated host strain *E. coli* K12 DP50/*sup*F (8). The *gal*$^+$ genes carried by the model recombinant represent a cloned DNA fragment. Any persistent association with the DP50/*sup*F host (in which the entire galactose operon is deleted) during lytic growth of the λ*WES gal*$^+$ phage will give rise to *gal*$^+$ colonies among the bacterial survivors in the lysate. As shown in Table 5, no *gal*$^+$-containing bacteria were detected among the bacteria surviving either at the time of lysis or 24 hours thereafter.

λgt*WES*·λC was certified as an EK2 vector by the NIH Advisory Committee on Recombinant DNA Research at its January 1976 meeting, but has been superceded by λgt*WES*·λB, which was certified in February 1977. The Advisory Committee has further required that the phage be grown preparatively in the attenuated host strain *E. coli* K12 DP50/*sup*F (8).

We have considerable experience in the use of λgt*WES* derivatives for cloning of partially purified DNA fragments derived from total mouse genomic DNA. We generally obtain about 4500 hybrid phage per microgram of Eco RI DNA fragment (6). This efficiency suggests that any unique sequence purified about 1000-fold could be identified by available screening techniques (9). We recently cloned an Eco RI fragment containing a segment of the mouse ribosomal RNA sequences (6). This fragment occurs about once per 5000 mouse Eco RI fragments and is approximately 200-fold reiterated with respect to unique sequences. McClements and Skalka (10) cloned a similar ribosomal fragment from genomic chicken DNA using the λgt*WES*·λC vector. While the requirements of EK2 biological containment clearly reduce the flexibility of the cloning technology, the λgt*WES* system should permit cloning of unique sequences of genomic DNA from higher organisms. Other attenuated strains of λ, such as those constructed by Blattner and his associates (11) and Donoghue and Sharp (12) should be useful in this respect as well.

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References and Notes

1. M. Thomas, J. R. Cameron, R. W. Davis, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 4579 (1974).
2. L. Enquist, D. Tiemeier, P. Leder, R. Weisberg, N. Sternberg, *Nature* **259**, 596 (1976).
3. D. Tiemeier, L. W. Enquist, P. Leder, *ibid.* **263**, 526 (1976).
4. M. R. Geier, M. E. Trigg, C. R. Merril, *ibid.* **246**, 221 (1976).
5. L. W. Enquist and A. Skalka, *J. Mol. Biol.* **75**, 185 (1973).
6. D. Tiemeier, S. Tilghman, P. Leder, in preparation.
7. P. Leder, L. Enquist, D. Tiemeier, applications to the NIH Advisory Committee on Recombinant DNA Research for EK2 certification of λgt*WES*·λC and λgt*WES*·λB, December 1976 and January 1977.
8. This strain, derived by R. Curtis, D. Pereira, and their associates at the University of Alabama (personal communication) has a number of convenient genetic features that make it useful for testing and reduce its ability to grow outside the laboratory (see Table 5). To propagate the λgt*WES* phages, we have introduced *sup*F into this strain.
9. R. A. Kramer, J. R. Cameron, R. W. Davis, *Cell* **8**, 227 (1976).
10. W. McClements and A. M. Skalka, *Science* **196**, 195 (1977).
11. F. R. Blattner *et al.*, *ibid.*, p. 161.
12. D. J. Donoghue and P. A. Sharp, *Gene*, in press.
13. W. Szybalski, personal communication.
14. We are again most grateful to C. Kunkle for her expert editorial assistance in the preparation of this manuscript.

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Chemical Synthesis of Restriction Enzyme Recognition Sites Useful for Cloning

Abstract. *By a triester chemical synthesis method, three decameric DNA's have been made; these act as substrates for several restriction endonucleases, including Eco RI, Bam I, and Hind III. These homogeneous decamers form duplexes that can be efficiently blunt-end ligated to themselves or to other DNA molecules by the action of T4 DNA ligase and thus are useful tools for molecular cloning experiments.*

Restriction endonucleases recognize specific sequences in double-stranded DNA. Many of these endonucleases cleave their recognition sites to produce blunt ends, while others such as Eco RI, Bam I, and Hind III, leave cohesive or "sticky" ends (1–3). DNA fragments with these cohesive ends can be inserted easily into, and subsequently excised from, a suitable cloning vehicle such as the plasmid pMB9 (4), which has one site each for Eco RI, Bam I, and Hind III.

Unfortunately, cohesive ends are lacking in many DNA fragments of interest, such as randomly cleaved DNA, complementary DNA (cDNA), and many restriction endonuclease fragments. We have recently introduced a new general procedure suitable for cloning almost any DNA molecule (5). This method (Fig. 1) involves the addition by blunt-end ligation of short DNA segments to both ends of the DNA to be cloned. The added segments contain restriction endonuclease cleavage sites, and treatment with the corresponding endonuclease leaves cohesive ends on the subject DNA. This DNA then can be cloned by incorporating it into a plasmid or other vector that has been opened with the same restriction enzyme. The feasibility of this new method was demonstrated by adding an octadeoxyribonucleotide containing the Eco RI recognition sequence to a 21-base-pair duplex bearing the *lac* operator sequence. A clonable, excisable DNA fragment was obtained, and was shown to function as *lac* operator should, both in vitro and in vivo (5).

We report here the chemical synthesis by the improved triester method of Itakura *et al.* (6) of three new decameric "linker" molecules (see Fig. 1), which together contain seven different recognition sites cleavable by 23 different restriction endonucleases including Eco RI, Bam I, and Hind III (2). These linker molecules should add great flexibility to cloning methodology, and also provide substrates for physicochemical studies on restriction enzyme recognition, methylation, and cleavage mechanisms.

The synthetic scheme in Fig. 2 outlines the strategy used in the convergent synthesis of the Bam I decamer by the triester method, using 2,4,5-triisopro-

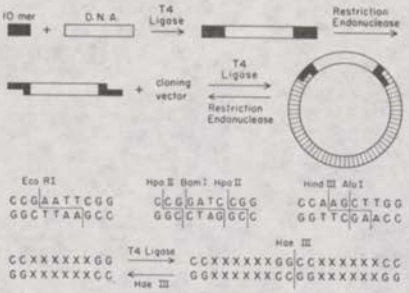


Fig. 1. (Top) A decameric linker (10 mer) bearing a restriction enzyme site is joined by T4 DNA ligase to both ends of the DNA to be cloned, and cohesive ends are then produced by treatment with restriction endonuclease. This "sticky-ended" DNA then can be incorporated into a vector that has been cut open with the same restriction endonuclease. (Bottom) The three chemically synthesized decameric linker molecules investigated in this study. The ends of the decamer sequences are designed so that, after blunt-end ligation with T4 DNA ligase, a site recognized by the Hae III restriction enzyme is produced.

pylbenzenesulfonyl tetrazolide (TPST) as a condensing reagent (*7*). The other two decamers were made similarly. Procedures and reaction conditions are given in the legend of Fig. 2. Two milligrams of fully deblocked Eco RI decamer were obtained, whereas for the Bam I and Hind III decamers the yields were 26 mg and 80 mg, respectively. The lower yield of synthesis of the Eco RI decamer may be attributable to the particular deblocking conditions used for the removal of the β-cyanoethyl group on the 3′ ends. These conditions subsequently were changed for the Hind III and Bam I decamer syntheses (see legend of Fig. 2). The pyridine-ethanol mixture provided a faster, more efficient removal of β-cyanoethyl protecting groups than tetrahydrofuran did. The use of pressure silica gel chromatography for separation of protected oligonucleotides greatly aids the triester synthesis. Chromatography under pressure leads to improved and faster separation (4 hours). It also produces 5 to 10 percent higher yields at each step, resulting in significant increases in yields of the final product. Moreover, use of NH_4OH rather than NaOH in organic solvents for removing protecting groups also increases the yield and purity. The three decamers travel as a single band on 20 percent polyacrylamide–7*M* urea gel electrophoresis, with the same R_f value as bromphenol blue (Fig. 3).

Because the DNA strands synthesized are self-complementary, they form the duplexes shown in Fig. 1. These duplexes are relatively stable, having a T_m of about 48°C in 0.3*M* NaCl, 0.1 m*M* EDTA, 10 m*M* tris-HCl, *p*H 7.5, at a DNA concentration of 50 μg/ml. These duplexes are homogeneous and act as restriction enzyme substrates (Fig. 3). The Eco RI decamer is totally cleaved by its endonuclease to produce the labeled trimer as shown in Fig. 3, lanes 1 and 2. The Bam I decamer is also completely digested by its endonuclease to a labeled trimer and a second band running close to the [γ-^{32}P]ATP marker (lanes 3 to 6). Since this second band does not appear in the decamer without enzyme, it most likely can be ascribed to a nuclease contamination of the enzyme preparation. The Hind III decamer is cleaved to two identical five-base-pair pieces by Alu I, which recognizes adenine-guanine-cytosine-thymine (AGCT), but is not affected by Hsu I endonuclease, which recognizes AAGCTT (lanes 12 to 14). (Hsu I and Hind III recognize the same base sequence.) However, if the decamer is first polymerized with T4 DNA ligase under blunt-end joining conditions (legend of Fig. 3), then the polymer is cleaved by Hsu I endonuclease to pieces of approximately 30 base pairs in length, with no further degradation of these pieces (lanes 15 and 16). Thus it appears that Hsu I, unlike Eco RI, Bam I, or Alu I, requires a DNA strand of 30 base pairs or more as a substrate.

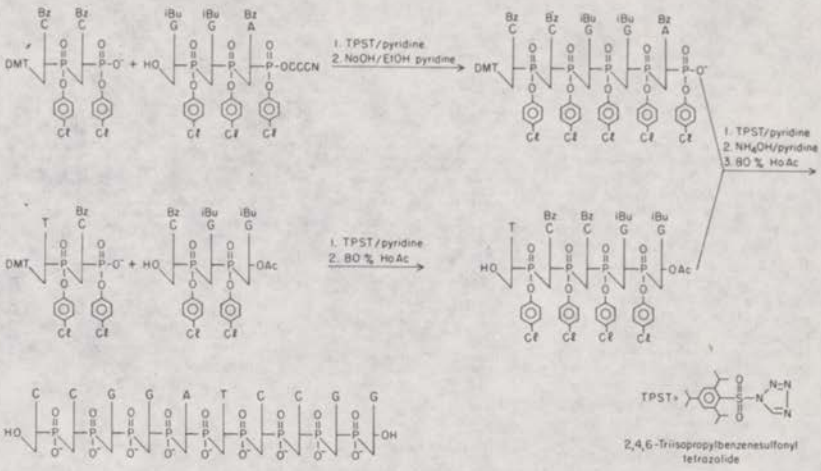
A comparable story is found with the Bam I decamer. It has two Hpa II sites per decamer (Fig. 1). In spite of this, Hpa II will not digest the decamer, apparently because the sites are too near the ends. However, if the decamers are polymerized with the T4 DNA ligase, then the polymer is quantitatively cleaved to smaller fragments by Hpa II endonuclease (lanes 8 to 10). The specific recognition by the Hpa II enzyme of the outer four base pairs of the Bam I decamer after polymerization is a confirmation of the sequence of the ends.

All three of these decameric linker molecules have CC at the 5′ end and GG at the 3′ end. Hence, blunt-end ligation produces a new Hae III restriction endonuclease site (GGCC). Polymers of the three linkers are cleaved back to decamers by Hae III enzyme (lane 19 and data not shown). Recognition of the central portion of each decamer by its proper endonuclease, and recognition of the outer two base pairs by Hae III, confirm the sequences as synthesized. The complete cleavage of the molecules as judged by the appearance of the gels allows one to assess the overall purity in terms of length and sequence homology as 90 percent or better.

For cloning DNA fragments, three methods for joining to the vector are in common use: (i) restriction enzyme "sticky ends" (*8*); (ii) tailing, either with polydeoxyadenylate [poly(dA)] or polydeoxythymidylate [poly(dT)] (*9*) or, more recently, with polydeoxyguanylate and polydeoxycytidylate [(*11*); and A. Otsuka, personal communication]; and (iii) blunt-end ligation by T4 ligase (*12*). This latter method is based on the ability of T4 ligase to rather efficiently join even or blunt-ended DNA duplexes under the appropriate conditions (*13*). All of these methods are useful, but none is completely general.

The three "linkers" described here and the procedure outlined in Fig. 1 should allow almost any DNA fragment to be cloned in an existing vector and also aid in the construction of new cloning vectors. Eco RI, Bam I, or Hind III cohesive ends can be added at will to any DNA bearing 5′-phosphate/3′-hydroxyl flush ends. If not already blunt-ended, most DNA's can be made so, either by repair with DNA polymerase [see (*12*)] or treatment with S1 nuclease (*14*).

Suitable plasmid vectors are known for insertions at all three restriction sites: Eco RI, Bam I, and Hind III. The plasmid pMB9, mentioned earlier, has been described (*4*). The plasmid RSF2124 has an Eco RI site within its colicin gene (*10*), and therefore one can screen for insertion by looking for disruption of colicin production after recombination. The plasmids pBR316 and pBR313 have both ampicillin and tetracycline resistance genes, with a Bam I and Hind III site in the latter



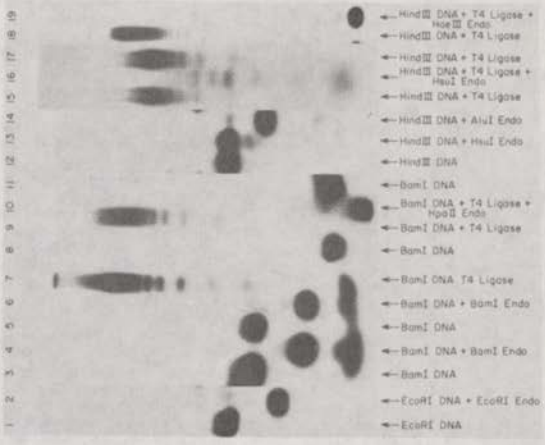


Fig. 2 (above). The convergent triester synthesis scheme used in preparing the Bam I decamer is shown. The other two decamers were made similarly. Most procedures, chemicals, and solvents have been described (6, 7); only new improvements are given here. Purification of the protected polynucleotide fragments was aided by the use of medium-pressure silica gel chromatography. Chromatronix glass columns with Fluid Metric RPG-50 solvent pumps were used to purify the intermediate and decameric products. The 2-inch columns were run at 25 pounds per square inch, 1-inch columns at 100 pounds per square inch, and ½-inch columns were run at 175 pounds per square inch in chloroform, with 5 to 10 percent methanol, depending on the polarity of the molecule being eluted. Removal of the β-cyanoethyl phosphate protecting groups at intermediate stages of the synthesis was carried out in a solution of 0.1*M* NaOH and tetrahydrofuran for preparation of Eco RI decamers, and in a solution of 0.05 *M* NaOH and a mixture of pyridine and ethanol (1 : 1) for the Bam I and Hind III decamers. All reactions were monitored on thin-layer silica gel plates (6). Final deblocking of the decameric products was done in a 1 : 2 mixture of pyridine and 30 percent NH_4OH at room temperature for 2 days, or at 70°C for 3 hours, followed by treatment with 80 percent acetic acid for 15 minutes. The deblocked products then were run on cellulose thin-layer chromatography plates in a mixture of 100 ml H_2O and 60 ml of isobutyric acid for 16 hours. The plates were dried, and the slowest moving band was recovered from the cellulose in 3 percent NH_4OH. Evaporation of solvent gave a white residue, which was dissolved in 70 m*M* phosphate buffer at *p*H 7, heated to 80°C, and then allowed to cool slowly at 4°C. The DNA duplex was then chromatographed on a Sephadex G-75 column (1 by 100 cm) at 4°C, eluted with 0.1*M* ammonium bicarbonate buffer, *p*H 7.5. Abbreviations: DMT, 4,4-dimethoxytrytyl protecting group for the 5′ end; CCCN, β-cyanoethyl protecting group for the 3′ phosphate; Ac, acetate protecting group for the hydroxyl end. For details about TPST, see (7). Fig. 3 (right). Accumulated electrophoretograms showing restriction enzyme reactions and blunt-end ligation. An explanation of each lane is in the text. The DNA's were phosphorylated and ^{32}P-labeled with T4 polynucleotide kinase (5), then treated as indicated. Conditions for T4 DNA ligase (blunt-end ligation) were 66 m*M* tris, *p*H 7.6, 6.6 m*M* $MgCl_2$, 10 m*M* dithiothreitol, 0.4 m*M* adenosine triphosphate (ATP), 1 μ*M* [5′-^{32}P]DNA, approximately 3.0 units of T4 ligase, 27°C, 16 hours, 15-μl volume. Conditions for Eco RI were 100 m*M* tris, *p*H 7.5, 5 m*M* $MgCl_2$, 100 m*M* NaCl, 0.02 percent Triton X100, 1 μg of [5′-^{32}P]DNA, 20°C, 5 hours, 10-μl volume. Conditions for Bam I were 100 m*M* tris, *p*H 7.5, 5 m*M* $MgCl_2$, 1 μg of [5′-^{32}P]DNA, 27°C, 5 hours, 10-μl volume. Conditions for Hsu I, Hpa II, and Hae III were T4 DNA ligase buffer, 1 μ*M* T4 ligated [5′-^{32}P]DNA, 37°C, 4 hours. Alu I: 6.0 m*M* tris, *p*H 8.0, 6.0 m*M* $MgCl_2$, 6.0 m*M* β-mercaptoethanol, 1 μg of [5′-^{32}P]DNA, 27°C, 5 hours, 10-μl volume. The enzymes Eco RI, Bam I, Hsu I, Alu I, Hae III, T4 DNA ligase, and T4 polynucleotide kinase were the gifts of Drs. H. Heyneker and P. J. Greene of the University of California, San Francisco. Hpa II was purchased from New England Bio-Labs. Gel electrophoresis was conducted in 20 percent acrylamide with 1.5 percent bis, 7*M* urea, at 15 volt/cm for various times in 0.05*M* tris-borate, *p*H 8.3.

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(R. Rodriguez, F. Bolivar, H. Goodman, H. Boyer, and M. Betlach, personal communication). Hence, incorporation of DNA into a Bam I site can be monitored by looking for ampicillin resistance and tetracycline sensitivity in these two plasmid strains.

The linkers reported here have been used to clone eukaryotic DNA and complementary DNA (cDNA). As described (*15*), the Eco I decamer has been ligated to repetitive DNA from sea urchin sperm, and the resultant cohesive-ended fragments were cloned in RSF2124 plasmids. These clones will provide a source of repetitive DNA in quantity, helping to determine new features of the eukaryotic genome. The Bam I decamer has been added to cDNA's and cloned to the tetracycline gene of the pH313 plasmid (J. Shine and H. Goodman, personal communication). The decamers have also been shown to be useful for constructing new cloning vehicles by converting one restriction site to another, for example, Hind III to Eco RI (H. Heyneker, personal communication), and Eco RI to Bam I (A. Riggs, unpublished data). Therefore, the chemically synthesized decameric linkers reported here are generally applicable tools for molecular cloning experiments.

The three decameric linkers themselves were constructed with CC . . . . . GG at the ends for stability (T_m = 48°C), resulting in efficient blunt-end ligation, and so that polymerized decamers could be cloned and cut apart again by Hae III restriction enzyme. Cloning would provide a means of obtaining a steady supply of more linkers. However, "polylinker" clones have not yet been obtained, perhaps because of in vivo instabilities. Therefore, triester chemical synthesis may prove easier than cloning for moderate scale production of DNA molecules as small as decamers. At present, 25-mg amounts of greater than 90 percent pure material can be made in a relatively short time.

Note added in proof: The linker synthesis has also been pursued in parallel with this work by Bahl *et al.* (*16*).

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References and Notes

1. J. Hedgpeth, H. M. Goodman, H. W. Boyer, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 3448 (1972).
2. The Eco RI decamer (CCGAATTCGG) is recognized by Eco RI and Eco RI*. The Bam I decamer (CCGGATCCGG) contains the specific sequences for Bam I, Bst I, Bsu NI, Dpn I, Mbo I, Mos I, Hpa II, Hap II, and Mno I. The Hind III decamer (CCAAGCTTGG) contains sequences for Alu I, Bbr I, Chu I, Hinh III, Hind III, and Hsu I. After polymerization, each decamer will contain the sequence GGCC recognized by Hae III, Blo I, Bsu R, Hhg I, Pal I, and Sfa I. For a review of restriction enzymes and their recognition sequences, see R. Roberts, *Critical Rev. Biochem.*, in press.
3. R. Old, K. Murry, G. Roizes, *J. Mol. Biol.* **92**, 331 (1975).
4. R. L. Rodriguez, F. Bolivar, H. M. Goodman, H. W. Boyer, M. Betlach, in *ICH-UCLA Symposia on Molecular and Cellular Biology*, P. Nierlich and W. J. Rutter, Eds. (Academic Press, New York, 1976), vol. 5, pp. 471–477.
5. H. L. Heyneker, J. Shine, H. M. Goodman, H. Boyer, J. Rosenberg, R. E. Dickerson, S. A. Narang, K. Itakura, S. Lin, A. D. Riggs, *Nature (London)* **263**, 748 (1976).
6. K. Itakura, N. Katagiri, C. P. Bahl, R. H. Wightman, S. A. Narang, *J. Am. Chem. Soc.* **97**, 7327 (1975).
7. J. Stawinski, T. Hayuni, S. A. Narang, *Can. J. Chem.* **54**, 670 (1976).
8. A. C. Y. Chang and S. H. Cohen, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 1030 (1974).
9. D. A. Jackson, R. H. Symons, P. Berg, *ibid.* **69**, 2904 (1972).
10. M. So, R. Gill, H. S. Falkow, *Mol. Gen. Genet.* **142**, 239 (1975).
11. F. Rougeon, P. Kourilsky, B. Mach, *Nucleic Acids Res.* **2**, 2365 (1975).
12. K. Backman, M. Ptashne, W. Gilbert, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 4174 (1976).
13. V. Sgaramella, J. H. Van de Sande, H. G. Khorana, *ibid.*, p. 1468.
14. G. S. Ghangas and R. Wu, *J. Biol. Chem.* **250**, 4601 (1975).
15. R. Scheller, T. Thomas, A. Lee, W. Niles, W. Klein, R. Britten, E. Davidson, *Science* **196**, 197 (1977).
16. C. P. Bahl, K. J. Marians, R. Wu, J. Stawinski, S. A. Narang, *Gene* **1**, 81 (1977).
17. We thank J. Rosenberg and W. Gilbert for their stimulating contributions to the development of the linker concept. We also thank A. S. Lee, H. Heyneker, and J. Shine for help in demonstrating some of the enzyme reactions discussed in this report, and L. Shively for technical help. Supported by NIH grants GM-12121 and HD-04420, NSF grants PCM75-05886 and GB-26517, and an NIH predoctoral traineeship to R.H.S. This is contribution No. 5457 from the Norman W. Church Laboratory of Chemical Biology, California Institute of Technology.

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Screening λgt Recombinant Clones by Hybridization to Single Plaques in situ

Abstract. *A rapid, direct method for screening single plaques of* λgt *recombinant phage is described. The method allows at least 10^4 clones to be screened per day and simplifies physical containment of recombinants.*

Recombinant DNA technology has made it possible, in principle, to isolate large quantities of DNA corresponding to any single gene. This isolation can be accomplished in any of three ways: (i) purification of the gene or its transcript before insertion into a vector for cloning (*1*); (ii) genetic selection of the desired gene after "shotgun" (*2*) cloning the genome of the donor organism (*3, 4*); or (iii) the use of complementary RNA or DNA probes to screen clones produced through a shotgun experiment. The first method is limited by the practical difficulty of physically purifying the complete nucleic acid sequence of any gene. Application of the second is limited to genes for which appropriate mutations are available in hosts which can express foreign genetic information. The third method is limited only by the availability of complementary nucleic acid probes and has been the most widely applied.

Screening methods have been developed for both recombinant plasmid (*5*) and bacteriophage (*6, 7*) cloning systems. The most rapid of these, the methods of Grunstein and Kramer, are based on a technology developed by Olivera and Bonhoeffer (*8*) for growing and lysing cells on membrane filters. The speed and simplicity of these methods is limited by the need to pick and spot plaques or colonies from plates onto filters for further growth. We have found that a single plaque of a λgt recombinant phage contains enough phage DNA for detectable hybridization to complementary labeled nucleic acid, and that this DNA can be fixed to a nitrocellulose filter by making direct contact between the plaque and the filter. These findings have allowed us to develop a method for screening recombinant phage which has significant advantages over previous methods: (i) physical containment of recombinant molecules (and organisms carrying them) is made simpler because this method requires fewer manipulations of viable phage, requires the growth and handling of much smaller quantities of recombinant DNA-containing phage and cells, and produces no contaminated materials other than the petri dishes on which the plaques are grown. (ii) At least 2×10^4 plaques can be screened per hour (*9*), which compares with about 100 per hour by the fastest previous method (*7*). (iii) Because up to 2×10^4 plaques can be screened on a single petri dish and nitrocellulose filter, the method requires smaller amounts of materials, including radioactively labeled probe. (iv) Because screening can be done directly from the transfection plate, all the independently constructed phages containing similar or identical inserts can be isolated and individually

characterized. This can give information on the heterogeneity of repeated genes or of genes from different somatic tissues.

We believe that this method will be widely applicable and, therefore, present it here in detail.

Cells (*10*) are infected with phage, plated in L-soft agar on L plates (*11*), and grown for at least 12 hours at 37°C. Large plaques are usually desirable, and 0.1 ml of late log phase cells per plate has been found to give large plaques of many λgt phages. The number of phages which can be plated on a single petri dish depends on the relative growth rates of the desired recombinant and the background phages. After growth, the plates are placed at 4°C for about 15 minutes to harden the agar. Phage and DNA are transferred to a nitrocellulose filter by placing the dry filter (88 mm, Millipore HA or Schleicher and Schuell, BA 85) on the lawn of cells, no air bubbles being allowed to form between the soft agar and the filter. Phages are allowed to adsorb to the filters for 1 to 20 minutes. If duplicate filters are desired, several filters can be adsorbed sequentially for 30 to 60 seconds each, or filters can be stacked and adsorbed at once. If filters are stacked, they must be allowed to adsorb for at least 20 minutes. During adsorption, it is convenient to mark the filters and plates for orientation. After adsorption, the filter is carefully lifted from the plate to avoid removing the soft agar layer which occasionally adheres to the filter. Should this occur, the agar can be removed by gently shaking the filter during the denaturation and neutralization steps (see Fig. 1, plate 2a and replicas b to d, for examples). Both the DNA and phage are denatured and fixed in situ by dipping the filters in 0.1*N* NaOH and 1.5*M* NaCl for 20 seconds; the filters are then neutralized by dipping in 0.2*M* tris, *p*H 7.5, and 2 × SSCP (*12*) for 20 seconds. Filters are blotted and baked at 80°C in a vacuum for 1.5 to 2 hours.

Hybridization to the nitrocellulose replicas is carried out in a siliconized glass petri dish. The ^{32}P-labeled probe (*13*) (10^5 to 10^6 counts per minute per filter) is placed in enough 5 × SSCP and 50 percent formamide to cover all filters. The hybridization mixtures were usually incubated for 12 to 18 hours. Buffer and probe can be recovered and reused for at least 2 weeks. After hybridization, the filters are washed in a large (10 to 15 ml per filter) volume of 5 × SSCP and 50 percent formamide at 42°C for 30 minutes and then for 20 to 30 minutes in 2 × SSCP at room temperature. The filters are then covered with plastic wrapping film and placed against x-ray film for autoradiography. From 24 to 48 hours of exposure is usually sufficient with a homogeneous probe of specific activity of 10^5 to 10^6 count min^{-1} μg^{-1}. X-ray intensifying screens (Kodak X-Omatic Regular) have been used with flash-activated film at −70°C to shorten exposure time.

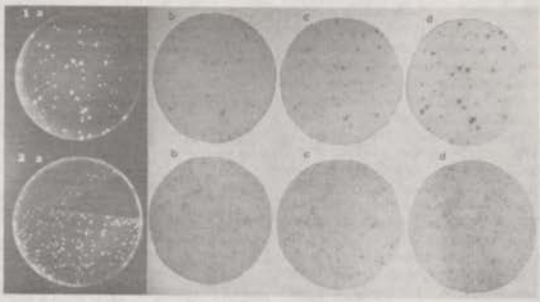


Fig. 1. Plaques 1a and 2a of λgt-Sc1109 phage and autoradiographic replicas (1b to 1d and 2b to 2d). Replicas were produced (as described in the text) by sequential adsorption to filters, beginning with 1d and 2d. The probe was ^{32}P-labeled ribosomal RNA and Chronex 4 x-ray film was exposed for 20 hours for autoradiography. A sector of the top agar from plate 2a was removed with the last filter adsorbed (2b). Note that plaques are visible on the bottom agar and that the autoradiographic replica is not changed in the sector. Plaques 1a and 2a are visualized by the fluorescence of ethidium bromide which is sprayed onto the plates.

The method described above should be generally applicable to all recombinants of λgt phages. In the experiments described below we used the phage λgt-Sc1109 (*7, 14*) in model systems to optimize the method. Phage grown on *E. coli* C600 for less than 9 hours give negligible autoradiographic signals; 12 to 14 hours of growth is optimal. Although T plates generally yield larger plaques, these apparently contain fewer phage particles and less DNA than comparably sized plaques grown on L plates because, at all growth times, nitrocellulose replicas of T plates gave lower signals than those of L plates (*11*).

To determine the maximum number of plaques per plate which can be screened under various relative growth conditions, two experiments were performed. In the first, to model a situation in which the background grows much better than the desired recombinant phage, a small number of λgt-Sc1109 phage were grown together with increasing numbers of λgt2 (*15*) phage. No effect on the signals was apparent up to about 2000 plaque-forming units (pfu) per plate, but the signals were weak and variable with more than 4000 pfu per plate. In the second competition experiment, λgt-Sc1109 was plated with increasing numbers of λgt-λB, which has approximately the same plaque size as the recombinant phage. In this case, sufficient signal was obtained with more than 2×10^4 pfu per plate. A practical rule seems to be that the method will accommodate the greatest number of plaques that does not produce confluent lysis.

Time of phage and DNA adsorption to the nitrocellulose replica filter does not seem to be critical; adsorption times of 1 to 60 minutes have been used and little difference noticed. Very long adsorptions resulted in slightly more diffuse plaque spots on the nitrocellulose replicas and the autoradiograms. Stacks of at least five filters can be adsorbed at one time to produce replicates (for hybridization to several different probes, for example). For most applications, better replicates are produced by placing the filters sequentially on the plate for a brief adsorption to each (Fig. 1).

Dipping the nitrocellulose replica directly into a beaker of denaturing or neutralizing solution does not result in loss of signal or in distortion of the shapes or relative positions of the plaque spots. Denaturation and neutralization times of 0 to 60 seconds all give acceptable results; 15 to 20 seconds of each is optimal, giving an approximately twofold increase in signal over either no denaturation or no neutralization. Baking the filters before hybridization is not necessary, but in this model system the signal was increased approximately twofold by baking for 90 to 120 minutes.

After autoradiography, it is necessary to align the autoradiogram of the nitrocellulose replica with the original plaque

plate to allow the plaques corresponding to positive signals to be picked. The most reliable system we have used for this alignment involves labeling the filters and plates during adsorption by puncturing the filter at three points around its circumference with a hypodermic needle and injecting 0.1 to 0.2 μl of India ink into the agar at each point. The ink diffuses very little during the 2 days normally required for hybridization and autoradiography, and it allows absolute orientation of the filters, autoradiograms, and plates. From a plate with more than 1000 plaques, we normally pick about 20 plaques from the area of each positive signal for rescreening and plaque purification. Once pure clones are obtained, the sizes and restriction spectra of the inserted DNA's can be rapidly determined by the method of Cameron *et al.* (*16*).

The large capacity and speed of this method make practical, we believe, the isolation of eukaryotic unique genes by screening all the recombinants produced through shotgun cloning the entire genome. This should make possible isolation of a large number of genes for which physical or genetic purification schemes are not available.

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References and Notes

1. M. Thomas, R. L. White, R. W. Davis, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2294 (1976); B. E. Noyes and G. R. Stark, *Cell* **5**, 301 (1975); S. G. Clarkson, H. O. Smith, W. Schaffer, K. W. Gross, M. L. Birnstiel, *Nucleic Acids Res.* **3**, 2617 (1976); G. N. Yenikolopov, A. P. Ryskov, T. Nitta, G. P. Georgiev, *ibid.*, p. 2645; S. C. Hardies and R. D. Wells, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3117 (1976); R. Higuchi, G. V. Paddock, R. Wall, W. Salser, *ibid.*, p. 3146.
2. A shotgun experiment is defined (NIH guidelines) as one which involves the production of recombinant DNA's between the vector and total or partially purified DNA from a cellular source.
3. K. Struhl, J. R. Cameron, R. W. Davis, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1471 (1976).
4. L. Clarke and J. Carbon, *Cell* **9**, 91 (1976).
5. L. Kedes, A. Chang, D. Houseman, S. Cohen, *Nature (London)* **255**, 533 (1975); M. Grunstein and D. Hogness, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3961 (1975).
6. K. Jones and K. Murray, *J. Mol. Biol.* **96**, 455 (1975); Yu. V. Ilyin, N. A. Tchurikov, G. P. Georgiev, *Nucleic Acids Res.* **3**, 2115 (1976); B. Sanzey, O. Mercereau, T. Temyrick, P. Kourilsky, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3394 (1976).
7. R. A. Kramer, J. R. Cameron, R. W. Davis, *Cell* **8**, 227 (1976).
8. B. M. Olivera and F. Bonhoeffer, *Nature (London)* **250**, 513 (1974).
9. This calculation is based on the worst case in which only 2000 plaques can be grown on each of 50 plates, the screening of which would require about five working hours over a 3-day period.
10. The usual host was *E. coli* C600 rk^- mk^+. Cells and phage are described by J. R. Cameron, S. M. Panasenko, I. R. Lehman, R. W. Davis, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3416 (1975).
11. The T plates contained 1 percent Bacto-tryptone, 0.5 percent NaCl, 1 percent agar, made to *p*H 7.5 with NaOH. The L plates contained 1 percent Bacto-tryptone, 0.5 percent Bacto yeast extract, 0.5 percent NaCl, 1.25 percent agar, made to *p*H 7.5 with NaOH. Seven percent agar was used for the T-soft and the L-soft agar.
12. Standard saline citrate phosphate buffer (SSCP) is (1×) 120 m*M* NaCl, 15 m*M* sodium citrate, 13 m*M* KH_2PO_4, 1 m*M* EDTA, titrated to *p*H 7.2 with NaOH.
13. In the model experiments described, the probe was total yeast ribosomal RNA (labeled with ^{32}P) prepared by the method of G. M. Rubin, *Methods Cell Biol.* **12**, 45 (1975).
14. λgt-Sc1109 (the gift of R. Kramer) is a recombinant λgt phage which carries two Eco RI fragments of *Saccharomyces cerevisiae* DNA, one of which carries the 5 · 8S and 26S rRNA genes. Approximately 5 percent of this phage's DNA is complementary to rRNA.
15. The λgt2 [λgt1 in Struhl *et al.* (*3*)] and λgt-λB were the gifts of M. Thomas.
16. J. R. Cameron, P. Philippsen, R. W. Davis, *Nucleic Acids Res.*, in press.
17. We thank T. St. John for helpful discussions and L. Horn for help in manuscript preparation. This work was supported by NSF grant PCM 76-02600 and NIH grant GM 021819-03. One of us (W.D.B.) was supported by NIH training grant 5T01-GM 01156 and by a fellowship from the Bush Foundation, St. Paul, Minn.
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Use of Phage Immunity in Molecular Cloning Experiments

Abstract. *Immunity to phage superinfection is a useful selective marker in molecular cloning experiments. Plasmids which have unique sites for several different restriction endonucleases and which specify immunity to bacteriophage λ are described.*

Antibiotic resistance genes carried on plasmids are useful as selective markers in molecular cloning experiments (*1*). We show here that phage λ immunity may be used as an alternative selective marker on plasmids that are carried in *Escherichia coli*. Plasmids bearing the c1 (repressor) gene of bacteriophage λ direct the synthesis of λ repressor and render carrier cells immune to superinfection by λ (*2*).

In a model experiment, we have compared selection for immunity or antibiotic resistance, using a plasmid (pKB158) (*2*) that carries both the λ c1 gene and a gene specifying tetracycline resistance (*tet*). Cells were transformed (*3*) with either no DNA, 1 μg of pKB158 DNA, 1 μg of pKB158 DNA digested with Eco RI, or 1 μg of pKB158 DNA digested with Eco RI and subsequently treated with T4 polynucleotide ligase. Transformants were selected on either tryptone plates plus 20 μg of tetracycline per milliliter or tryptone plates seeded with 10^9 λKH54h_λ and 10^9 λKH54h_{80} (*2*, *4*). The results are given in Table 1. Selection for λ immunity yielded about half as many transformants as did selection for tetracycline resistance. A small background of mucoid bacteria survived the selection for immunity even when no DNA was present in the transformation; however, all (36 of 36 tested) of the nonmucoid λ immune clones carried pKB158, as judged by the fact that they were tetracycline resistant. In summary, clones carrying plasmids which bear the λ c1 gene are easily selected and distinguishable from the small background of mucoid bacteria which survive the selection procedure. A possible disadvantage of the use of phage immunity in molecular cloning experiments is that selection for immunity is not possible with phage-resistant strains (such as χ1776) and is not easily maintained during growth in liquid media with standard *E. coli* strains.

We briefly describe two well-charac-

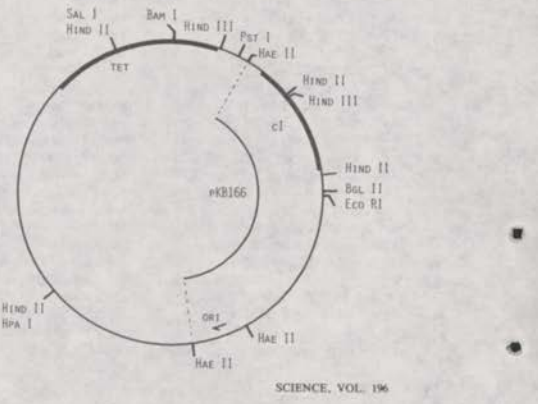


Fig. 1. Structure of pKB158. Map showing the locations of various restriction endonuclease cleavage sites on pKB158. Also shown are genes c1 and *tet* (as heavy lines) and the Col E1 origin of replication *ori* (by an arrow). Only three of the more than ten Hae II sites are indicated. The material contained in pKB166 is indicated by an arc inside the map of pKB158. pKB158 has four Hind II sites, one of which is also recognized by Sal I and another of which is also recognized by Hpa I.

terized plasmids which carry the λ *c*I gene and which may be useful as cloning vectors. Plasmid pKB158, used in the experiments described above, contains unique cleavage sites for the restriction endonucleases Eco RI, Bgl II, Pst I, Bam I, Sal I, and Hpa I (see Fig. 1). Hpa I cleavage of pKB158 produces a flush-ended linear molecule, which may be used to clone flush-ended DNA fragments generated by a variety of restriction endonucleases (*5*). Joining of flush-ended DNA molecules to each other is mediated by high concentrations of T4 polynucleotide ligase (*2*).

We have prepared a smaller derivative of pKB158 which lacks the *tet* gene, in the following manner: pKB158 was digested with Bam I and Hpa I. The larger fragment produced (see Fig. 1) contained the λ *c*I gene and the Col E1 origin of replication. This fragment was partially digested with Hae II, diluted, and treated with T4 polynucleotide ligase. Cells were transformed with this DNA and λ immune transformants were selected as described above. About 5000 immune colonies were washed from their plates with tryptone broth, and a drop of this pool was used as an inoculum for a culture from which plasmid DNA was prepared. The plasmid DNA was separated by polyacrylamide gel electrophoresis and the smallest plasmid present on the gel was eluted and used to transform cells; this plasmid was named pKB166 (see Fig. 1).

Plasmid pKB166 contains only two Hae II fragments (2000 and 350 base pairs long), including the one reported to contain the Col E1 origin of replication (*6*). It is one of the smallest plasmids described to date, about 2400 base pairs long (1.6×10^6 daltons). Of those 2400 base pairs, 1100 are derived from the λ immunity region (*c*I and adjacent sequences) and 1300 are derived from Col E1. Since *E. coli* strains stably maintain pKB166 and are immune to colicin E1 when they harbor pKB166, the 1300 base pairs of pKB166 which are derived from Col E1 [corresponding to the region from 0.79 to 1.00 on the map in (*6*)] contain sufficient genetic information to direct replication and to express immunity to colicin E1.

pKB166 has unique recognition sites for Eco RI, Bgl II, and Hind III, and has only two recognition sites each for Hae II and Hind II. DNA fragments generated by Bam I or Mbo I as well as Bgl II may be cloned in the Bgl II site in pKB166, because all three endonucleases produce identical sticky ends. DNA fragments generated by Hae II may be cloned in partially digested pKB166. DNA fragments cloned in the Hind III or Hind II sites in pKB166 will destroy *c*I or its promoter and eliminate the expression of λ immunity. pKB166 is comparable with the so-called mini Col E1 plasmids (*7*) in that it is extremely small, can be amplified by treatment with chloramphenicol, determines two selectable phenotypes (immunity to phage λ and to colicin E1), and has several restriction endonuclease cleavage sites in which DNA fragments may be cloned.

Table 1. Selection of pKB158-bearing clones by λ immunity or by tetracycline resistance. Cells (5 to 6×10^7) from a transformed culture were spread on selective plates (described in the text) and incubated overnight at 37°C. The number of colonies which grew are tabulated.

DNA	Selection for	
	λ Immunity	Tetracycline resistance
None	6	0
pKB158	248	451
pKB158 + Eco RI	9	1
pKB158 + Eco RI + T4 ligase	28	51

The polypeptides encoded by pKB166 are few and with the exception of λ repressor are small. This we infer from experiments involving a plasmid (pKB266) which is nearly identical to pKB166, differing principally in that pKB266 has *lac* promoter fragments which transcribe *c*I [compare pKB158 and pKB252 in (*2*)]. A minicell-producing strain, χ1274 (*8*), was transformed with pKB266 and minicells were purified as described by Inselberg (*9*). The minicells were labeled with [^{35}S]methionine and extracts of the labeled minicells were analyzed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. The principal (> 95 percent) ^{35}S-labeled polypeptide greater than 8000 daltons present in such extracts was λ repressor (molecular weight, 26,000).

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References and Notes

1. S. N. Cohen, A. C. Y. Chang, H. W. Boyer, R. Helling, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 3240 (1973); C. Covey, D. Richardson, J. Carbon, *Mol. Gen. Genet.* **145**, 155 (1976); R. L. Rodriguez, F. Bolivar, H. Goodman, H. W. Boyer, M. Betlach, in *Molecular Mechanisms in Control of Gene Expression*, D. Nierlich, W. S. Rutter, C. F. Fox, Eds. (Academic Press, New York, 1976), p. 471; M. So, R. Gill, S. Falkow, *Mol. Gen. Genet.* **142**, 239 (1975).
2. K. Backman, M. Ptashne, W. Gilbert, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 4174 (1976).
3. S. N. Cohen, A. C. Y. Chang, L. Hsu, *ibid.* **69**, 2110 (1972).
4. Phage λKH54 [F. Blattner, M. Fiandt, K. K. Hass, P. A. Twose, W. Szybalski, *Virology* **62**, 458 (1974)] has a deletion in its *c*I gene. A deletion mutant is used to select λ immune clones because point mutants revert to *c*I$^+$ at a low frequency and produce lysogens which spuriously survive the selection. The use of two host ranges (χ_s and h_{80}) reduces the background by eliminating clones which are resistant to one of the host ranges employed.
5. K. Backman, unpublished results.
6. A. Oka and M. Takanami, *Nature (London)* **264**, 193 (1976).
7. V. Hershfield, H. W. Boyer, L. Chow, D. R. Helinski, *J. Bacteriol.* **126**, 447 (1976); H. Avni, P. E. Berg, A. Markovitz, *ibid.* **129**, 358 (1977).
8. H. I. Adler, W. D. Fisher, A. Cohen, A. A. Hardigree, *Proc. Natl. Acad. Sci. U.S.A.* **57**, 321 (1967).
9. J. Inselberg, *J. Bacteriol.* **102**, 642 (1970).
10. We wish to thank M. Ptashne, in whose laboratory these experiments were performed, for support and helpful discussions.

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Hybridization in situ of SV40 Plaques: Detection of Recombinant SV40 Virus Carrying Specific Sequences of Nonviral DNA

Abstract. *The detection and recovery of SV40 genomes containing foreign DNA sequences can be facilitated, and the risk of accidental dispersal reduced, by in situ hybridization and radioautography.*

Specific nucleic acid sequences contained in the cells of bacterial colonies can be detected without prior isolation of the cell's DNA by in situ hybridization of radioactive nucleic acid probes (RNA or DNA) to imprints of the bacterial colonies immobilized on nitrocellulose disks (*1*). Similarly, λ phage plaques, formed on lawns of sensitive bacteria, can be screened for the presence of recombinant phages carrying specific DNA segments (*2*). Beside facilitating detection and isolation of bacterial or phage clones carrying recombinant DNA molecules, such methods decrease the risk of dispersion of these organisms by reducing the scale and simplifying the number of mechanical and chemical manipulations generally used to identify organisms carrying specific recombinant DNA molecules.

SV40 DNA has been used as a vector for cloning and propagating foreign DNA segments in animal cells (*3–5*). Where the desired recombinants do not possess a distinguishable phenotype, the recombinant virus genomes are detected by hybridization of the viral DNA isolated from cells infected with each of the putative recombinant virus stocks (*3–5*). But this procedure is cumbersome and time-consuming; furthermore, because the number of plaques that can be screened is limited, the ability to detect

recombinants that occur at low frequency is compromised.

To circumvent this problem we have developed a simple procedure that distinguishes plaques containing SV40 genomes carrying specific segments of nonviral DNA from those caused by infection with SV40 alone. This is achieved by (i) transferring the cell monolayers and their plaques to nitrocellulose disks, (ii) treatment of the imprinted nitrocellulose with alkali to denature and immobilize the cell and viral DNA to the disk, (iii) hybridization with appropriate highly labeled nucleic acid probes, and (iv) radioautography to identify the plaques containing DNA homologous to the probes. Virus contained in the plaques can be recovered from the corresponding region of the agar overlay which was removed from the cell layer prior to the imprinting step. By this procedure λ-SV40 hybrid viral genomes (4) can be detected and recovered even when they are present in an SV40 preparation at a frequency of about 10^{-5}.

Cell and plaque imprints on nitrocellulose. Infection of CV-1P monolayer cultures with SV40 virus or DNA produces plaques that are discernible after staining of the cell monolayer with 0.01 percent neutral red. Before transfer of the cell monolayers and virus plaques to nitrocellulose disks, the agar overlays were removed by reaming the agar–petri dish boundary and gently prying the agar layer into a sterile petri dish. Alignment marks on the nitrocellulose disk, petri plate, and agar overlay permit subsequent recovery of virus from plaques that contain the desired DNA sequences. A simple method for marking plaques on the agar is to lay another nitrocellulose membrane over the agar and record the position of the plaques with a suitable marker prior to removing the agar overlay from the petri dish. The agar, wrapped in plastic wrap, can be stored at 4°C until the radioautograms are developed (about 3 days).

To make an imprint of the cell monolayer and plaque contents suitable for in situ hybridization, a dry nitrocellulose disk (Millipore, 47 mm) was laid carefully on top of the moist, but not wet, cell monolayer and patted down with a rubber policeman. The filter was then moistened by blotting with wet paper toweling (with a solution of 50 m*M* tris at *p*H 7.5 and 0.15*M* NaCl) and peeled off the plate with forceps. No dry spots should remain on the nitrocellulose disk because it may stick to the dish and be torn during removal. Transfer of the cell monolayer to the nitrocellulose is quantitative, and, where the neutral red stain has been applied, the plaques are readily visible on the filter (Fig. 1A). Although faded, the plaques remain visible even after the denaturation and annealing procedure.

Cells and virus adsorbed to the nitrocellulose disk were lysed by placing the disk on a blotter saturated with 0.5*N* NaOH for about a minute after which the disk was dried on a piece of blotting paper for about a minute. This procedure was repeated (once or twice) more, and the membrane was then neutralized by repeating the above procedure with 1.0*M* tris-HCl, *p*H 7.6. Finally, the filters were immersed in a solution of 0.1*M* tris-HCl, *p*H 7.6, and 0.15*M* NaCl, air-dried, and baked for 2 hours at 80°C at reduced pressure.

Hybridization of plaque imprints. The nitrocellulose disks were incubated prior to use in a solution (PM) of triple-strength SSC (saline sodium citrate) containing 0.02 percent each of Ficoll, polyvinylpyrrolidine, and bovine serum albumin for 6 hours at 65°C to reduce the background due to nonspecific DNA binding (6). Then, such pretreated disks were annealed in PM solution (3.0 ml) containing 6 × SSC, salmon sperm DNA (100 μg/ml) and ^{32}P-labeled nick-translated DNA (7) in a chamber constructed from two glass plates (8.5 by 8.5 cm) separated by a rubber gasket. The particular radioactive probes, their specific activities, and the amounts used in the various annealing experiments are indicated below. After annealing, the filters were rinsed three times at 65°C for 1 hour in the following buffers: (i) PM with 6 × SSC; (ii) 0.1*M* phosphate buffer, *p*H 7.0 with 2 × SSC, and (iii) 2 × SSC. Autoradiograms of the nitrocellulose disks were made with Kodak X-Omat XR/5 film and a Kodak X-Omatic in-

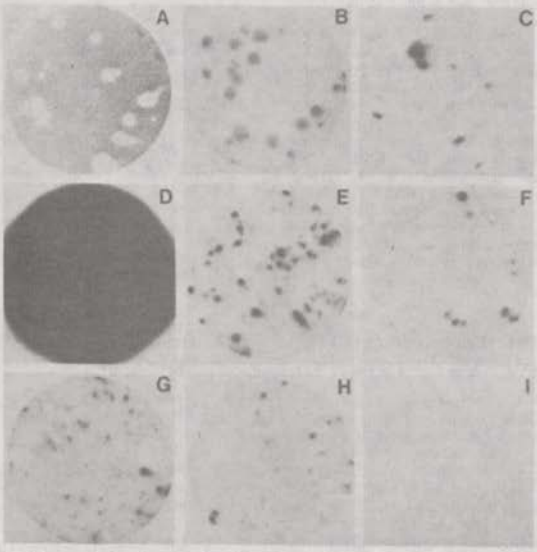


Fig. 1. Images of virus plaques transferred from infected cell monolayers to nitrocellulose disks and hybridized to radioactive probes homologous to the viral genome. (A) A neutral red stained monolayer of SV40 infected CV-1P cells after transfer to a nitrocellulose disk; (B) autoradiogram of a nitrocellulose imprint of SV40 plaques after annealing with ^{32}P-labeled nick-translated SV40 DNA; (C) autoradiogram of a nitrocellulose imprint of λ-SVGT plus tsA58 induced plaques after annealing with ^{32}P-labeled nick-translated λ DNA; (D) autoradiogram of an imprint of a monolayer infected with 10^5 pfu of wild-type SV40 4 days earlier and annealed with labeled SV40 DNA; (E and F) autoradiogram of a monolayer infected with 10^4 pfu of wild-type SV40 plus 50 and 10 pfu, respectively, of a λ-SVGT, 4 days earlier and annealed with labeled λ DNA; (G and H) autoradiogram of monolayer infected with 10^5 pfu of wild-type SV40 plus 50 and 10 pfu, respectively, of λ-SVGT 4 days earlier and annealed with labeled λ DNA; (I) autoradiogram of monolayer infected with 10^5 pfu of wild-type SV40 alone and annealed with labeled λ DNA. Autoradiograms were exposed for 14 or 48 hours after annealing with labeled SV40 or λ DNA, respectively.

tensifying screen to increase the sensitivity and decrease the exposure times.

Figure 1A shows a nitrocellulose disk after transfer of the cell monolayer and its plaques; the infection had occurred about 6 days earlier, and the monolayer had been stained a day before. After hybridization with ^{32}P-labeled nick-translated SV40 DNA (5×10^7 count/min per microgram, 10^5 count/min per disk) and radioautography, the location of the plaques is clearly discernible (Fig. 1B) and coincides with the position of the visible plaques in the corresponding disk. If transfer of the monolayer and plaques to the nitrocellulose disks is delayed beyond 7 days, viral DNA appears to be lost from the plaques (presumably, because of lysis or detachment of the virus-bearing cells from the dish), and the autoradiographed images of the plaques display hollow centers ("halos"). With the same ^{32}P-labeled SV40 DNA probe annealed to nitrocellulose disks imprinted with uninfected cell monolayers, there are no plaques detectable on the radioautogram (not shown). To recover the virus from the plaques, agar plugs were removed from the agar overlay with the use of the radioautographic images to locate the plaques; about 10^4 plaque-forming units (pfu) per plug were recovered after dispersion and removal of the agar.

Detection of recombinant viral genome. An SV40 variant containing a recombinant genome of λ and SV40 DNA (λ-SVGT-1) has been constructed, cloned, and propagated in monkey kidney cell cultures (*4*). Because the λ DNA segment replaces the genes coding for the virion capsid proteins, propagation of the recombinant virions requires a helper virus to supply these missing functions; those functions are provided by coinfection of the cells with tsA58, a mutant of SV40 defective in an early gene, at 41°C. Thus, monkey kidney cell monolayers infected with λ-SVGT-1 and tsA58 DNA's or their virions yield plaques that contain the helper virus and the defective recombinant (*3*, *8*). When such mixedly infected monolayers are transferred to nitrocellulose each plaque registers on the radioautogram after hybridization with either ^{32}P-labeled nick-translated SV40 DNA (Fig. 1B) or λ DNA (Fig. 1C); the latter probe, which had a specific activity of 2×10^7 count/min per microgram, was made with a recombinant plasmid DNA carrying the λ DNA segment included in the λ-SV40 hybrid genome (D. Charney and P. Berg, unpublished). No radioautographic plaques were detected when the labeled λ DNA was annealed to imprints of monolayers infected with SV40 alone (see Fig. 1I).

One of our purposes in developing this method was to simplify the search for a rare recombinant genome. Since the ordinary plaque assay accommodates not more than 100 to 200 plaques per plate, the detectability of specific recombinants would be limited to frequencies of not less than 10^{-3}, although greater sensitivity would be achieved if the number of plates screened is increased. To determine whether specific recombinant genomes could be detected in the presence of a great excess of normal genomes, the following mixed infections were performed.

One series of CV-1P monolayer cultures (5×10^6 cells per culture) was infected with 10^5 pfu of wild-type SV40 plus 50 or 10 pfu of λ-SVGT; the titer of the λ-SVGT was determined independently with excess of tsA58 in the usual way (*8*). Another series of similar CV-1P monolayer cultures was infected with 10^4 pfu of wild-type SV40 plus 50 or 10 pfu of λ-SVGT. Cultures were overlayed with agar and incubated at 37°C; imprints were made 3, 4, 5, and 6 days after infection, and these were subsequently annealed with either ^{32}P-labeled SV40 or λ DNA. No significant hybridization was detected on any of the radioautograms after 3 days of infection (data not shown). Four days after infection, hybridization of each of the nitrocellulose imprints with labeled SV40 DNA yielded intense and uniform blackening of the film (Fig. 1D) indicating that SV40 DNA had replicated throughout the cultures, a not too surprising finding in view of the input multiplicity of wild-type virus. But, at the same time, in the cultures infected with 10^4 pfu of wild-type virus, areas that hybridize with the λ DNA probe could readily be detected (Fig. 1, E and F). Even in the cultures infected with 10^5 pfu of wild-type virus, foci containing λ DNA sequences could be seen (Fig. 1, G and H). After 5 days, the foci that contain λ DNA were larger and easily counted; the titer of the λ-SVGT-1 agreed within 30 percent of the value obtained in the standard way with tsA58 as the helper. Six days after the infection there was extensive degeneration of the cell monolayer, and it was too difficult to make imprints on the nitrocellulose disks.

This experiment suggests that, given the appropriate radioactively labeled probe, which can be RNA or DNA complementary to the sequence being sought, a recombinant SV40 genome can be detected if it is present with a frequency of about 10^{-5} or greater. Very likely by examining a larger number of cultures an even rarer recombinant could be found.

After a region in the culture containing a clone of recombinant genomes is detected, it is also important to recover it for further purification and characterization. When cultures were used that contained only one or two λ-SVGT-1 foci per infected culture (10^6 pfu), the agar layers were cored at the positions indicated by the radioautogram and the extracted material was assayed for the ratio of λ-SVGT to wild-type virus. Whereas the λ-SVGT-1 constituted only about 10^{-4} of the input virus, it was present at 10^{-2} frequency in the recovered virus. Quite likely one or two repetitions of this procedure would yield isolated plaques containing the recombinant and helper virions.

The procedure described here is applicable, in principle, to any animal virus that can be used for molecular cloning, whether it produces plaques or foci of infected cells, provided that a suitably labeled nucleic acid probe for the DNA segment being sought is available. It simplifies the task of detecting rare recombinant genomes but it is especially helpful where the recombinant genome cannot contribute any genetic functions, and, therefore, requires a nondefective virus helper for its propagation—as, for example, in (*3*). This method of screening recombinants reduces the probability of dispersing potentially hazardous recombinants. Instead of having to pick innumerable plaques and to isolate viral DNA from cultures infected with each of them (operations which require considerable handling, centrifugations, and the like), the desired recombinants can be detected and isolated within the more secure confines of a petri plate. Thus, the adoption of in situ hybridization provides "physical containment" over and above that provided by the laboratory design.

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References and Notes

1. M. G. Grunstein and D. S. Hogness, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3961 (1975).
2. R. A. Kramer, J. R. Cameron, R. W. Davis, *Cell* **8**, 227 (1976).
3. D. Ganem, A. L. Nussbaum, D. Davoli, G. C. Fareed, *ibid.* **7**, 349 (1976); A. L. Nussbaum, D. Davoli, D. Ganem, G. C. Fareed, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1068 (1976).
4. S. Goff and P. Berg, *Cell*, in press.
5. D. H. Hamer, D. Davoli, C. A. Thomas, Jr., G. C. Fareed, *ibid.*, in press; D. H. Hamer, G. C. Fareed, C. A. Thomas, Jr., *ibid.*, in press.
6. D. T. Denhardt, *Biochem. Biophys. Res. Commun.* **23**, 641 (1966).
7. P. W. J. Rigby, M. Dieckmann, C. Rhodes, P. Berg, in preparation.
8. J. E. Mertz and P. Berg, *Virology* **62**, 112 (1974).
9. Supported by PHS grants GM 13235-10 and CA 15513; American Cancer Society grant VC 23-D; and (to L.P.V.) a fellowship from the Jane Coffin Childs Memorial Fund for Medical Research.

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Increase in Conjugational Transmission Frequency of Nonconjugative Plasmids

Abstract. *Addition of Eco RI fragment 6 of the* Escherichia coli *sex factor F to pSC101 increases the frequency of its transmission by R1-19 and ColVB. Transmission frequencies of pSC101 and two pSC101 chimeras are also increased after the putative transposition of drug resistance element Tn3 from R1-19. These increases may result from addition of an origin of conjugational transfer to the plasmids.*

One problem in the containment of recombinant DNA is the possibility of transmission of chimeric plasmids to new bacterial strains by conjugation. Although the plasmid vectors used for cloning are not self-transmissible (that is, they are nonconjugative), these vectors can be transmitted to recipient strains during mating if a conjugative plasmid is also present in the donor cell. We refer to this process as mobilization of the nonconjugative plasmid by the conjugative one. The frequency of mobilization is different for each plasmid and varies over a wide range, from as low as 1×10^{-5} per donor for the vector pSC101 (*1*) to as high as 4×10^{-1} per donor for Col EI (*2*). We were interested in the factors that determined the frequency of mobilization of nonconjugative plasmid vectors and chimeras, and whether a plasmid mobilized at low frequency, for example pSC101, could be converted to one mobilized at high frequency.

We have studied the mobilization of a series of chimeric pRS plasmids that were constructed in vitro from a partial Eco RI endonuclease digest of the conjugative plasmid F and a limit digest of the nonconjugative plasmid vector pSC101 (*3*) to see whether attachment of a fragment of F (the *Escherichia coli* sex factor) could allow pSC101 to be mobilized at high frequency. We expected that attachment of the transfer (*tra*) genes, required during mating for the production of pili and transfer-associated DNA metabolism (*4*), would be unlikely to affect the frequency of mobilization since all the *tra* functions would already be provided by any coresident conjugative plasmid. Previous findings from studies on F′ deletions indicated that when a large part or all of the region contained on Eco RI fragment 6 of F was deleted, the F′ became cis-dominant transfer deficient (*5*, *6*), whereas a deletion or other mutation of any of the *tra* genes resulted in a recessive Tra⁻ F′ (*5*, *7*). Since fragment 6 appeared to affect the transfer ability of an F′, we examined the mobilization frequencies of pRS plasmids with and without fragment 6 of F.

Donor strains were constructed by transformation of a *recA*⁻ F⁻ strain of *E. coli* K12 with purified pRS plasmid DNA (*8*), followed by introduction of the mobilizing plasmid by conjugation. Table 1 shows the transmission frequencies for the pRS plasmids with ColVB or R1-19 (*9*) as conjugative mobilizing plasmids and with the *tet*⁺ gene, which determines tetracycline resistance (Tc^R), as the selective marker. We used pSC105, a chimeric plasmid having a DNA fragment which confers resistance to kanamycin (Km^R) inserted into pSC101 at the Eco RI site rather than F fragments (*10*), as a control. Chloramphenicol resistance (Cm^R), determined by the *cat*⁺ gene, was used to detect the transmission of R1-19. For the crosses involving R1-19 the addition of fragment 6 is sufficient for high frequency transmission of *tet*⁺. With ColVB, fragment 6 increases the frequency 100- to 1000-fold, but fragment 3 appears also to be needed for maximum transmission of *tet*⁺.

Previous results (*11*) indicated that low frequency mobilization of pSC101 occurred after a *recA*-independent recombination event between pSC101 and the conjugative plasmid pSC50 (*9*). A recent paper (*12*) demonstrated that this event is the transposition of the transposable element Tn3 from the plasmid pSC50 to pSC101. It is therefore possible that attachment of Eco RI fragment 6 to pSC101 increases the frequency of such a transposition and thereby increases the transmission frequency. On the other hand, it is possible that attachment of fragment 6 provides a site or gene required for a transposition-independent mobilization process, such as the initiation of rolling circle conjugational transfer replication (*4*). Since the evidence for transposition-mediated mobilization was the discovery that the *tet*⁺ gene originally carried by pSC101 was found in transconjugant progeny on either of two new plasmids, each larger than pSC101 (*11*), we looked for such plasmids in the progeny from our crosses. The donors and Tc^R Cm^R progeny from each of the crosses involving R1-19 listed in Table 1 were screened for the presence of plasmid DNA. The donor strains showed two DNA bands which appeared to correspond to the separate nonconjugative and R1-19 plasmids as determined by R_f values (Table 2). The DNA patterns for the progeny, however, fell into two classes: (class I) progeny from donors

Table 1. Transmission frequencies of chimeric plasmids mobilized by ColVB or R1-19. *recA*⁻ donor strains were grown in Luria (L) broth plus tetracycline (15 μg/ml) [plus chloramphenicol (25 μg/ml) for the R1-19 matings], washed one time with L broth, and mated in a 1 : 10 ratio with a *recA*56, F⁻ strain (JC7199) for 1 hour at 37°C. Matings were interrupted by vortexing the mating mixture for 1 minute. Transmission of pRS and pSC plasmids was measured by selecting Tc^R (Pro⁺ TL⁺) progeny. Transmission of R1-19 was measured by Cm^R (Pro⁺ TL⁺) progeny. The numbers of Tc^R progeny and Cm^R progeny from the same donor were compared. ColVB transfer was not measured but >98 percent of the viable donor cells tested produced colicin and therefore carried the ColVB plasmid at the time of mating. Retransmission frequencies were measured as described above except that the recipient was the *recA*56F⁻ strain, JC7214 and selection was for Tc^R (His⁺ Met⁺) or Cm^R (His⁺ Met⁺) progeny. Abbreviations: Tc, tetracycline; Cm, chloramphenicol; Pro, proline; TL, threonine + leucine; His, histidine; Met, methionine; R, resistant; ⁺, proficient; ⁻, deficient.

Chimeric plasmid	Eco RI fragments of F	Tc^R progeny per 100 donors mobilizing plasmid		Tc^R/Cm^R progeny	Retransmission frequency from progency of R1-19 crosses	
		ColVB	R1-19		Tc^R progeny/ 100 donors	Tc^R/Cm^R progeny
pSC101		2×10^{-5}	2×10^{-5}	1×10^{-4}	5	4×10^{-1}
pSC105			3×10^{-2}	9×10^{-4}	3	4×10^{-1}
pRS5	7,5,3,6	2	6×10^{1}	5×10^{-1}		
pRS7	(17,19),2,12	8×10^{-5}				
pRS8	1(17,19),2,12,16,10	5×10^{-4}				
pRS21	5	5×10^{-5}	5×10^{-2}	5×10^{-4}	5×10^{-1}	4×10^{-1}
pRS27	6,15	2×10^{-2}	8×10^{1}	8×10^{-1}	2×10^{-1}	1
pRS30	3,6,15,1	7	6×10^{1}	6×10^{-1}		

that transmitted *tet*$^+$ at high frequency also appeared to show the original plasmid bands; (class II) progeny from donors which transmitted *tet*$^+$ at low frequency showed one band which appeared to correspond to R1-19 and a second band which had a lower mobility (and presumably larger size) than the original nonconjugative plasmid.

The presence of a presumably new plasmid band in progeny from donors carrying pSC101, pSC105, and pRS21, which are mobilized with low frequency, is in accord with the evidence of transposition-mediated mobilization obtained by Kopecko and Cohen (*11*). But since no new band was seen in the donor strains, we suspect that the transposition takes place in only a small number of the donor cells. High frequency mobilization, on the other hand, is characterized by the presence of plasmid bands with approximately the same mobility in both donor and recipient strains as would be expected if the nonconjugative plasmid were transmitted without having first to recombine with the conjugative plasmid. Therefore fragment 6 would appear to function not by increasing the frequency of transposition, but by providing something necessary for transmission which the mobilizing plasmid cannot provide.

To test further the idea that low frequency mobilization is preceded by a transposition event, progeny whose DNA had been analyzed on agarose gels were used as donors in matings to determine the frequency of retransmission of the *tet*$^+$ marker from these strains. If *tet*$^+$ had become associated with a high frequency transmissible plasmid, one would expect tetracycline resistance to be transmitted to a new recipient at high frequency. The results of these crosses are shown in the last two columns of Table 1. Transmission of *tet*$^+$ is 1000-fold higher for the strains carrying derivatives of the low frequency mobilized plasmids pSC101, pSC105, and pRS21 and nearly equals the transmission frequency of *cat*$^+$. Presumably these new plasmids are capable of being mobilized at high frequency. We have not yet determined which antibiotic resistance markers are carried by the new plasmids, but on the basis of the results of Kopecko *et al.* (*12*), we expect that they may show resistance to ampicillin which is carried on the Tn3 element. We are in the process of purifying plasmid DNA from all the progeny strains in order to compare the plasmids with their parents and to characterize them more fully. Our conclusions about the nature of these plasmids must be tentative until the more detailed results are in.

These data indicate that nonconjugative plasmid vectors and chimeras which are mobilized at low frequency can be converted to plasmids capable of high frequency mobilization. Such chimeric plasmids can be created in vitro by the addition to a plasmid vector of a DNA fragment providing a necessary protein or site. In the case of fragment 6 of F, we have hypothesized that the required site is the origin of transfer (*oriT*) of F since F$^-$ plasmids with all or part of fragment 6 deleted have a cis-dominant transfer deficient phenotype (*13*). We have not ruled out the possibility that a gene on fragment 6 codes for a cis-acting protein. Plasmids which can be transmitted at high frequency can also be derived from the chimeric plasmid in vivo even in *recA*$^-$ strains (*11*, *12*). The derivation could involve recombinational fusion of the two plasmids with subsequent recombinational separation in the donor or the progeny (*11*). Alternatively, there might be transposition of an *oriT* site from the conjugative to the nonconjugative plasmid in the donor. Since the recent findings of Kopecko *et al.* (*12*) show the transposition of the Tn3 from the conjugative plasmid pSC50 to pSC101, we think it is possible that Tn3, like fragment 6 of F, may carry an *oriT* which allows high frequency transmission of pSC101.

Note added in proof: We have purified plasmid DNA from the progeny of the R1-19 mobilizations of pSC101, pSC105, pRS21, and pRS27. The plasmids derived from mobilization of pSC101, pSC105, and pRS21 confer resistance to ampicillin and have an insertion in the pSC101 moiety of the plasmid as shown by Eco RI digestion and agarose gel electrophoresis. The plasmid derived from mobilization of pRS27 does not confer resistance to ampicillin and has the original plasmid bands with no DNA insertion.

Table 2. Results of agarose gel electrophoresis of DNA prepared from donor and progeny strains from crosses in which R1-19 was the conjugative plasmid. The DNA was prepared by the method of Meyers *et al.* (*14*) from 30-ml cultures grown in Luria broth containing tetracycline (15 μg/ml) and chloramphenicol (25 μg/ml), and was analyzed on 0.7 percent agarose gels. Linear pVH51 DNA was added to each well as an internal standard for R_f determinations. The R_f values for each plasmid band were calculated as the ratio of the distance from well to the distance from well of pVH51. When the R_f of the band assumed to be R1-19 differed from well to well, the R_f of the second band differed correspondingly in class I results. DNA preparations were also made from strains carrying each plasmid separately, and the following R_f values for each plasmid were obtained: R1-19, 0.29; pRS5, 0.39; pRS27, 0.51; pRS30, 0.40; pSC101, 0.70; pSC105, 0.52; pRS21, 0.50. Several slab gels were run to obtain these results and those shown in the table.

Class	Conjugative plasmid	Non-conjugative plasmid	R_f of donor plasmids	R_f of progeny plasmids	Tentative conclusion
I	R1-19	pRS5	0.28	0.31	Both R1-19
			0.36	0.38	Both pRS5
I	R1-19	pRS27	0.29	0.29	Both R1-19
			0.46	0.45	Both pRS27
I	R1-19	pRS30	0.26	0.31	Both R1-19
			0.33	0.37	Both pRS30
II	R1-19	pSC101	0.31	0.31	Both R1-19
			0.66	0.50	New plasmid in progeny
II	R1-19	pSC105	0.30	0.30	Both R1-19
			0.52	0.46	New plasmid in progeny
II	R1-19	pRS21	0.30	0.30	Both R1-19
			0.54	0.42	New plasmid in progeny

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References and Notes

1. S. N. Cohen and A. C. Y. Chang, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 1293 (1973).
2. D. T. Kingsbury, D. G. Sieckmann, D. R. Helinski, *Genetics* **74**, 1 (1973).
3. R. A. Skurray, H. Nagaishi, A. J. Clark, *Proc. Natl. Acad. Sci U.S.A.* **73**, 64 (1976).
4. N. S. Willetts, *Annu. Rev. Genet.* **6**, 257 (1972).
5. M. S. Guyer and A. J. Clark, *J. Bacteriol.* **125**, 233 (1976).
6. ———, N. Davidson, unpublished results.
7. M. Achtman, N. S. Willetts, A. J. Clark, *J. Bacteriol.* **106**, 529 (1971); N. S. Willetts, *ibid.* **112**, 773 (1972).
8. S. N. Cohen, A. C. T. Chang, L. Hsu, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 2110 (1972).
9. The R1-19 plasmid used here was obtained from D. R. Helinski and does not express resistance to kanamycin. It appears to be similar to the pSC50 plasmid isolated by Kopecko and Cohen (*11*). An R1-19 plasmid obtained from N. S. Willetts expresses resistance to kanamycin and mobilizes all the chimeric plasmids tested at low frequency (10^{-5} to 10^{-6} per 100 donors). The reason for the difference between the mobilization properties of these two R1-19 plasmids has not been determined.
10. S. N. Cohen, A. C. Y. Chang, H. W. Boyer, R. B. Helling, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 3240 (1973).
11. D. J. Kopecko and S. N. Cohen, *ibid.* **72**, 1373 (1975).
12. D. J. Kopecko, J. Brevet, S. N. Cohen, *J. Mol. Biol.* **108**, 333 (1976).
13. A. J. Clark, R. A. Skurray, N. J. Crisona, H. Nagaishi, in *Molecular Mechanisms in the Control of Gene Expression*, D. P. Nierlich, W. J. Rutter, C. F. Fox, Eds. (Academic Press, New York, 1976), p. 565.
14. J. A. Meyers, D. Sanchez, L. P. Elwell, S. Falkow, *J. Bacteriol.* **127**, 1529 (1976).
15. We thank K. Timmis for the pSC105 strain, and R. Skurray and H. Nagaishi for helpful discussions and assistance. Supported by NIH grant AI-05371 and by NIH training grant GM 01389.

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Five Hundredfold Overproduction of DNA Ligase After Induction of a Hybrid Lambda Lysogen Constructed in vitro

Abstract. *A lambda vector that contains the gene for* Escherichia coli *DNA ligase (λgt4-*lop*-11 lig⁺) has been modified to achieve overproduction of this enzyme. The third Eco RI site in the lambda chromosome has been altered by mutation, and the left-hand Eco RI fragment has been shortened. The new vector, λgt4-*lop*-11 lig⁺, forms a stable lysogen which, upon induction, produces a 100-fold increase in DNA ligase activity. Introduction of a phage mutation (S7) that prevents cell lysis results in an even greater increase (500-fold).*

Molecular cloning is a highly effective means to amplify the number of copies of a specific gene. It should, therefore, be expected to produce large increases in the amount of enzyme specified by that gene. In fact, cloning of genes in Col E1–type plasmids, and in bacteriophage λ, has resulted in significantly increased levels of exonuclease I and in enzymes of tryptophan and arabinose metabolism (*1*).

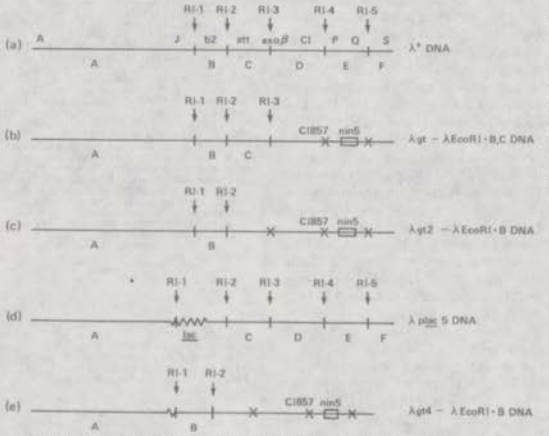
We had reported earlier the construction of a hybrid bacteriophage λ DNA containing the gene for *Escherichia coli* DNA ligase (*2*). The original vector used to construct the λ hybrid was λgt-λB, a strain that had been modified to incorporate endonuclease Eco RI–generated restriction fragments (*3*). In this vector, the inserted DNA replaces the two central Eco RI fragments of λ that are not required for growth. The fragment containing the ligase gene was derived from a mutant strain of *E. coli* carrying a ligase overproducing mutation (*lop*-11). The *lop*-11 mutation itself leads to a fivefold overproduction of ligase (*4*), and infection of *E. coli* with a λgt-*lop*-11 *lig*⁺ hybrid resulted in a tenfold increase in DNA ligase activity over that in extracts of uninfected *lig*⁺ cells (*2*). We now describe modification of this λ DNA molecule to produce a vector which, under the appropriate conditions, generates an amount of DNA ligase that is approximately 5 percent of the total cellular protein of *E. coli*.

The λ Eco RI-B and -C fragments containing the attachment site and part of the gene for λ exonuclease are not required for phage growth. However, since phages lacking the λ *exo* gene (*red*⁻) (for example, λgt-*lop*-11 *lig*⁺) grow poorly, we have constructed a vector, λgt4, in which the λ *exo* gene has been retained. This was accomplished in the following way: A λ vector (λgt2) was prepared in which the third Eco RI site was removed by mutation. Thus, the Eco RI-C fragment containing λ *exo* and the attachment site will persist after cleavage with Eco RI (Fig. 1c). λgt2 was converted to λgt4 by removing the left Eco RI fragment and replacing it with the shorter left Eco RI fragment of λ*plac*5 (Fig. 1d), a *lac* transducing phage (*5*). Eco RI cleavage of the λgt4 DNA at the new Eco RI site (between the *E. coli* and λ DNA segments) results in a new Eco RI-A fragment which is 2.2 kilobase pairs shorter than that from λgt2 and allows insertion of a correspondingly greater length of DNA.

λgt-*lop*-11 *lig*⁺ was cleaved with Eco RI, mixed with Eco RI–cleaved λgt4 DNA, and joined with DNA ligase. λgt4-*lop*-11 *lig*⁺ recombinants carrying the *lig* fragment in the same orientation as λgt-

Table 1. Assay of DNA ligase activity. Cultures (250 ml) of 594 (Su⁻) or C600 carrying the various λ prophages were grown in broth (10 g of Bacto tryptone, 5 g of Bacto yeast extract, and 5 g of NaCl per liter) to an absorbancy at 595 nm of 0.7, shifted to 43°C for 15 minutes, then incubated at 37°C. Cells carrying λgt4 and λgt4-*lop*-11 *lig*⁺ were harvested after 20 minutes; cells carrying λgt4-*lop*-11 *lig*⁺ S7 were harvested after 2 hours. DNA ligase assays were performed as described by Modrich and Lehman (*13*). Protein concentrations were measured by the method of Lowry *et al.* (*14*).

Phage	Activity (unit/ml)	Specific activity (unit/mg)
Uninfected	3.5	0.35
λgt4	3.5	0.45
λgt4-*lop*-11 *lig*⁺	343	31
λgt4-*lop*-11 *lig*⁺ S7	1950	140



λ*plac*5; fragment B can be replaced by foreign DNA. Boxes indicate deletions; jagged lines are *E. coli* DNA.

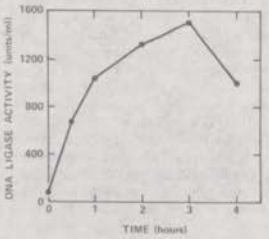


Fig. 1 (left). Construction of λgt4. (a) Eco RI cleavage sites in λ DNA (numbered RI-1 to RI-5 above the line) and cleavage fragments (lettered A to F below the line). (b) In λgt-λ Eco RI-B, and -C DNA, both right-hand Eco RI sites are altered by mutation; fragments B and C can be replaced by foreign DNA. (c) In λgt2-λ Eco RI-B DNA, the Eco RI-3 site is altered by mutation; fragment B can be replaced by foreign DNA. (d) In λ*plac*5 DNA, a new Eco RI-1 site is present in the *E. coli lac* gene substitution. (e) In λgt4-λ Eco RI-B DNA, the Eco RI-3 site is altered by mutation, and the left end is derived from λ*plac*5; fragment B can be replaced by foreign DNA. Boxes indicate deletions; jagged lines are *E. coli* DNA. Fig. 2 (right). Synthesis of DNA ligase after induction of λgt4-*lop*-11 *lig*⁺S7. Lysogens of λgt4-*lop*-11 *lig*⁺S7 in strain 594 (a streptomycin-resistant, nonsuppressing strain of *E. coli*) were grown in broth to an absorbancy at 595 nm of 0.7. The cultures were shifted to 43°C for 15 minutes, then maintained at 37°C for the times indicated. Harvesting of cells and assay procedures have been described (*2*, *13*).

lop-11 *lig*$^+$-1 were then isolated as described (*2*). Since these phages contain the attachment site and *int* and *xis* genes, they can integrate into the host chromosome and can be maintained in the prophage state (*6*). Recombinants containing *lig* can be easily detected since a conditionally lethal ligase mutant, *lig*ts 7 (*7*), will grow at 43°C when λgt4-*lop*-11 *lig*$^+$ is carried as a prophage. A double lysogen of λ$^+$ and λgt4-*lop*-11 *lig*$^+$ was used to prevent the induction of phage growth at high temperature that occurs as a result of the temperature-sensitive mutation (cI857) in the λ repressor carried by the λgt vector. Lysogens containing λgt4 or λgt4-*lop*-11 *lig*$^+$ were induced by a shift to 43°C, and the cell extracts were assayed for DNA ligase activity (Table 1). This modified vector system produced an increase in ligase levels to approximately 100-fold over that in uninfected *lig*$^+$ cells, a significant improvement over the tenfold increase observed with λgt-*lop*-11 *lig*$^+$ (*2*).

λgt4-*lop*-11 *lig*$^+$ was further modified by introducing an amber mutation (S7) in the S gene (which prevents cell lysis) (*8*) by genetic recombination (*9*) between λgt4-*lop*-11 *lig*$^+$ and λcI857 *nin*5 S7. Lysogens containing λgt4-*lop*-11 *lig*$^+$ S7 can be incubated for up to 2.5 hours after induction without significant cell lysis. DNA ligase levels in these cells increased continuously from the time of induction until lysis finally occurred (Fig. 2). Extracts of such cells harvested 2 hours after induction showed DNA ligase levels that exceeded those in uninfected *lig*$^+$ extracts by more than 500-fold. Since there is also overproduction of λ capsid proteins, the increase in specific enzyme activity is somewhat less (400-fold). We estimate that ligase represents approximately 5 percent of the protein in these extracts.

Early work with the lactose repressor carried on a defective λ phage demonstrated the correlation between the number of copies of lactose *i* gene and the amount of repressor present (*10*). Since then, a variety of phages have been used as vehicles for gene amplification. For example, a λ transducing phage that contains the DNA ligase gene has been isolated which, during vegetative growth, produced a 30-fold increase in ligase activity (*11*). The λ vector system that we have constructed in vitro has a number of very attractive features. Cells containing the vector as a prophage are stable, and λ carries no known translocons capable of the *rec*A-independent transfer of DNA segments known to occur with some plasmids (*12*). Since the hybrid DNA is present in only a single copy in the prophage, it should be possible to clone genes in λ which would be lethal to the host in multiple copies. Furthermore, the presence of the cloned gene in a single, unique sequence within the cell lessens the chance of sequence variants arising among a pool of gene copies. Amplification of the gene and, hence, the amount of enzyme coded for by the gene, is achieved, when desired, by induction of the prophage. The amber mutation in the S gene allows a long period of gene amplification and expression before extracts are prepared and, thus, even further enhances overproduction. It is obvious that the enzyme overproduction attained with this system significantly reduces the amount of purification necessary to obtain homogeneous protein and thus should make it substantially easier to prepare the large quantities of pure DNA ligase required for the detailed analysis of its structure and mechanism.

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References and Notes

1. D. Vapnek, N. K. Alton, C. L. Bassett, S. R. Kushner, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3492 (1976); Y. Hershfield, H. W. Boyer, C. Yanofsky, M. A. Lovett, D. R. Helinski, *ibid.* **71**, 3455 (1974); A. S. Hopkins, N. E. Murray, W. J. Brammar, *J. Mol. Biol.* **107**, 549 (1976); L. Clarke and J. Carbon, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 4361 (1975).
2. J. R. Cameron, S. M. Panasenko, I. R. Lehman, R. W. Davis, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3416 (1975).
3. M. Thomas, J. R. Cameron, R. W. Davis, *ibid.* **71**, 4579 (1974).
4. M. M. Gottesman, M. L. Hicks, M. Gellert, *J. Mol. Biol.* **77**, 531 (1973).
5. J. Shapiro, L. MacHattie, L. Eron, G. Ihler, K. Ippen, J. Beckwith, *Nature (London)* **224**, 768 (1969); A. Rambach and P. Tiollais, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 3927 (1974).
6. M. E. Gottesman and M. B. Yarmolinsky, *Cold Spring Harbor Symp. Quant. Biol.* **33**, 735 (1968).
7. C. Pauling and L. Hamm, *Proc. Natl. Acad. Sci. U.S.A.* **60**, 1495 (1968).
8. A. R. Goldberg and M. Howe, *Virology* **38**, 200 (1969).
9. J. S. Parkinson, *Genetics* **59**, 311 (1968).
10. W. Gilbert and B. Müller-Hill, in *The Lactose Operon*, J. R. Beckwith and D. Zipser, Eds. (Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y., 1970), p. 93.
11. M. M. Gottesman, *Virology* **72**, 33 (1976).
12. S. Cohen, *Nature (London)* **263**, 731 (1976).
13. P. Modrich and I. R. Lehman, *J. Biol. Chem.* **245**, 3626 (1970).
14. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *ibid.* **193**, 265 (1951).
15. Supported by National Cancer Institute contract NO1 CP61026 and NCI postdoctoral fellowship CA 09131 (to J.R.C.).

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Interspersion of Short Repetitive Sequences Studied in Cloned Sea Urchin DNA Fragments

Abstract. *The length and spacing of repetitive sequence elements were studied in several cloned sea urchin DNA fragments. Sequence organization in these fragments was found to be of the short interspersed form expected for sea urchin DNA, and for one clone the evidence shows that nearby repeats do not belong to the same repetitive sequence family.*

Interspersion of short repetitive DNA sequences with single copy sequences appears to be a widespread feature of higher animal genomes. DNA sequence organization in many animal genomes, representing a wide range of phylogenetic groups, has now been examined in some detail (*1–8*). In the typical case a majority of the genome consists of short repetitive sequences about 300 nucleotide pairs long interspersed with single copy sequences about 1000 to 3000 nucleotide pairs long. Exceptions to this general pattern of DNA sequence organization have so far been found only in the DNA's of several insects, including *Drosophila melanogaster* (*5*, *6*), the honeybee *Apis mellifera* (*7*), and the midge *Chironomus tentans* (*8*). The genomes of these organisms are relatively small, and they do not contain detectable quantities of short repetitive DNA sequences interspersed with single copy DNA.

Current knowledge of DNA sequence organization derives largely from measurements of binding to hydroxyapatite of partially renatured DNA fragments of various lengths which contain repetitive duplex. The fraction of these fragments included in repetitive duplex regions and in the flanking single-strand nonrepetitive "tails" has been assayed optically, and by measuring the amount of renatured DNA that is resistant to digestion with single-strand specific nuclease. Additional evidence has been obtained by electron microscopy of the renatured DNA structures (*3–5*). These methods have yielded a quantitative insight into the average patterns of sequence organization in animal DNA's. We now describe recent progress in analyzing the specific features of interspersed sequence organization in several cloned fragments of sea urchin DNA. In such fragments, it is possible to determine the spacing and the exact length of the individual repetitive sequence elements, as well as their repetition frequency in the genome. An additional question of significant theoretical interest (*9*), which can be investigated in cloned DNA fragments, is whether neighboring

interspersed repetitive sequences are members of the same or of different repetitive sequence families.

Eco RI fragments of sea urchin DNA which contain both unique and repetitive sequences were cloned in the plasmid pSC101 and in an ampicillin resistant derivative of Col E1, RSF2124 (*10*). The methods used and the general characteristics of the clones obtained have been described. Eight cloned fragments have been examined to various extents in the present work. These range in size from 3300 to 8300 nucleotide pairs. The repetitive sequence content of each fragment was determined by reacting labeled cloned DNA fragments with excess 2000 nucleotide long sea urchin DNA to C_0t 20 (C_0 is the concentration of nucleotides in moles per liter, and t is the time in seconds), and then measuring the fraction of the cloned fragment resistant to digestion with the single-strand specific S1 nuclease. The resistant fraction measures the amount of duplex formed between the repetitive sequences of the cloned DNA fragments and the excess of sea urchin DNA. The result of these analyses are summarized in Table 1. Here it can be seen that seven out of the eight cloned fragments contain repetitive sequences, and that these constitute 6 to 20 percent of the total sea urchin DNA in the cloned inserts. One fragment contains no detectable repetitive sequence. These results are approximately consistent with expectation for random samples of the sea urchin genome. Thus, at least 75 percent of the total DNA of this organism consists of repetitive sequences averaging 300 nucleotides in length interspersed with longer single copy sequences (*2*). Regions displaying this form of sequence organization (*2*) include on the average about 14 percent of the genome as repetitive sequence, and ≥ 61 percent as single copy sequence, or about 300 nucleotides of repetitive sequence per ≥ 1300 nucleotides of single copy DNA. In some cases the fraction of repetitive sequence will be significantly smaller since many single copy sequences are well over 2000 nucleotides long (*2*). Similarly, some interspersed regions where the single copy sequences are only 1000 nucleotides or less (*2*) could have as much as 25 percent repetitive sequences. An interesting question that can be answered by studying cloned sequences is whether relatively short and relatively long single copy sequence elements tend to be contiguous to each other, or whether they tend to be scrambled.

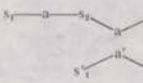
Table 1. Repetitive sequence content of cloned sea urchin DNA fragments. Abbreviation: NTP, nucleotide pairs (*18*).

Clone number*	Fragment size† (NTP)	S1 resistance (%)‡	Σ repetitive sequences (NTP)
CS0870	3300	7	230
CS0895	3700	0	0
CS0849§	5200	20	1040
	6400	6	390
CS0859	5700	15	855
PSC34	7000	13	900
CS0025	8000	12	960
CS0818	8300	18	1440

*The designation CS indicates a sea urchin fragment cloned in plasmid RSF2124. PSC34 indicates a sea urchin fragment cloned in plasmid pSC101. †Estimated from electrophoretic mobility in agarose gels. ‡The S1 resistant duplex content at C_0t 20 was measured by binding to hydroxyapatite. §Clone CS0849 contains two sea urchin fragments, one 5200 nucleotide pairs long, the other 6400 nucleotide pairs long.

To determine the length of the repetitive sequence elements in the cloned fragments, we measured the size of the S1 nuclease resistant DNA duplexes on polyacrylamide gels. Experimental procedures are described briefly in the legends of Figs. 1 and 2. Self-reaction of the cloned sea urchin fragments was avoided by the use of strand-separated cloned fragments. Results for two of the cloned fragments are shown in Fig. 1, A and B. It can be seen that the individual repetitive sequences in these clones, PSC34 and CS0859, are short. Their length is about 300 to 500 nucleotide pairs. Together with the measurements shown in Table 1, these data indicate that there are two or three such sequences in each of the two cloned fragments. The cloned fragments thus appear fairly typical of the interspersed repetitive sequences of the sea urchin genome.

If they are interspersed with single copy sequence, the individual repetitive sequence elements on each fragment could be homologous; for example, s_1-a-s_2-a-s_3, where "s" is single copy sequence and "a" is a given repetitive sequence, or they could be heterologous, for example, s_1-a-s_2-b-s_3, where "a" and "b" belong to different repetitive sequence families. If they are homologous, several different registrations are possible when the DNA is renatured, and a branched or looped renaturation product will sometimes be formed; for example,



If the repeats are heterologous, however, only one registration is possible:

$$s_1\text{-a-}s_2\text{-b-}s_3$$
$$s'_1\text{-a'-}s'_2\text{-b'-}s'_3$$

To distinguish between these alternatives the cloned fragments were renatured and the structures formed were examined in the electron microscope. Out of hundreds of examples of structures of the length of the sea urchin DNA inserts inspected, only long, perfectly paired, nonbranched duplexes were observed. A sample is shown in Fig. 2A. This experiment in it-

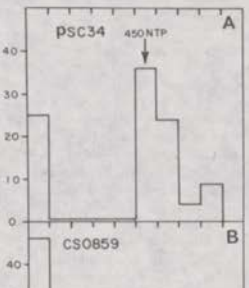


Fig. 1. Size determinations for repetitive sequence elements in clones PSC34 and CS0859. Plasmid DNA's were labeled in vivo with [^{3}H]-thymine. (A) PSC34 supercoils were treated with Eco RI, and the 7000-nucleotide-pair sea urchin fragment was isolated by gel electrophoresis. The (+) and (−) strands of the 7000-nucleotide-pair fragment were prepared by alkaline denaturation of the isolated fragment, followed by electrophoresis in a 1 percent agarose gel (*14*). The separated strands were renatured to C_0t 20 with excess sea urchin DNA 2000 nucleotides in length, and the reaction mixtures were treated with S1 nuclease (*15*). The S1 resistant DNA duplexes were collected on hydroxyapatite columns and sized on a 4 percent polyacrylamide gel with ϕX174 RF restriction fragments as size markers (*16*). DNA at the top of the gel is renatured PSC34 derived from a small percent of cross contamination with the other strand. (B) CS0859 supercoils were treated with Bam HI, which cuts the RSF2124 genome once, but does not cleave this particular sea urchin DNA sequence. The linear molecules of unit length containing both the plasmid and the sea urchin DNA fragment sequence were then strand separated in a poly-ribo(uridylate-guanylate) CsCl gradient (*17*). The separated strands were hybridized to sea urchin DNA, treated with S1 nuclease, and sized on an acrylamide gel as described in (A); *NTP*, nucleotide pairs.

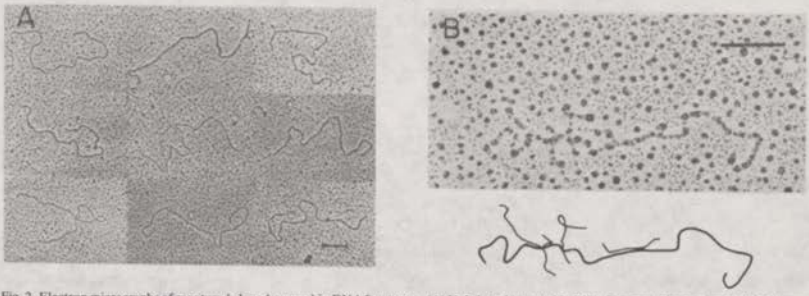


Fig. 2. Electron micrographs of renatured cloned sea urchin DNA fragments. (A) Unit-length sea urchin DNA fragments from plasmid PSC34 were denatured with alkali and renatured. They were then dialyzed into a solution of 57 percent formamide, 0.1*M* tris, 0.01*M* EDTA, *p*H 8.5 (*12*). They were spread for electron microscopy from this medium by a modification (*12*) of the Kleinschmidt procedure. Several renatured PSC34 fragments are shown. The bar represents 1 kilobase pair. (B) A PSC34 molecule is shown after renaturation with excess sea urchin DNA to C_0t 20. A terminal poly(A) tail had been added to the PSC34 fragment as described in text, and most of the excess sea urchin DNA was removed by passing the reaction mixture over an oligo(dT) column. The bar represents 1 kilobase. An interpretation of the complex structure formed is shown below the photograph.

self does not necessarily prove that the adjacent repetitive sequences are heterologous, since it is difficult to exclude the possibility of branch migration. The interpretation given here has been confirmed in an independent manner for clone PSC34 as follows. The sea urchin DNA insert was digested with restriction endonuclease Hha I, yielding nine discrete fragments. When reacted with sea urchin DNA, six of these proved to contain repetitive sequence. None of the Hha I fragments were able to react with each other. Therefore, there is no homology among the repetitive sequences on this fragment. At least for this case, the proximal repeat elements must belong to distinct repetitive sequence families.

The total length of the six fragments that contain repetitive sequence is 3200 nucleotide pairs. However, as Table 1 shows, PSC34 contains only about 900 nucleotide pairs of repetitive sequences. Therefore, 2300 nucleotide pairs of single copy DNA must be included in these fragments. Since the largest two Hha I fragments containing repetitive sequences are 640 and 700 nucleotide pairs, the single copy DNA must be included in more than two fragments. It follows that more than one repetitive sequence element exists on PSC34. This conclusion is consistent with the data of Fig. 1A, where it is shown that the individual repetitive sequence elements are only 300 to 500 nucleotides in length.

To investigate the spacing of the repetitive sequences of the cloned fragments, we developed a technique for visualizing the interspersion pattern in the electron microscope. The 7000-nucleotide sea urchin fragment was purified from the plasmid sequence after Eco RI treatment by gel electrophoresis, and polyadenylate [poly(A)] tracts of a few hundred nucleotides in length were enzymatically attached to the 3′ termini of the fragment by the terminal transferase method (*11*).

The cloned sea urchin DNA fragment was reacted to C_0t 20 with excess sea urchin DNA of average fragment length of 1400 nucleotides, and the partially renatured molecules were then passed over an oligo(dT) (dT, deoxythymidylate) cellulose column. The bound fraction was eluted with a solution of 10 m*M* tris, *p*H 7.4, 5 m*M* NaCl, and 1 m*M* EDTA at room temperature; under these conditions, the repetitive DNA duplexes remained intact. The bound fraction was recycled over another oligo(dT) cellulose column to further reduce the driver DNA background. It was then concentrated and prepared for electron microscopy (*12*). Molecules of the length of the PSC34 fragment were observed in the spreads, and these always displayed branched structures formed by reaction with sea urchin DNA. Shorter DNA driver molecules displaying typical interspersed branched structures (*3*) were also observed. An example of a complex of PSC34 fragment and sea urchin DNA is reproduced in Fig. 2B. There appear to be two duplex regions separated by about 1000 nucleotides. More than 30 such molecules were analyzed, and the structures found in all were consistent with one particular sequence organization. One end of the PSC34 fragment appears as a long (3000 nucleotides) "tail" consisting mainly of single copy DNA, while the other contains interspersed repetitive sequences. In some cases, a short repetitive sequence was observed in the "tail" region of the molecule. Since the driver sea urchin DNA fragments form branched single-stranded structures with the PSC34 fragment, the two families of repetitive sequence represented in clone PSC34 generally appear in the sea urchin genome in an environment of other sequences, almost certainly single copy sequences.

The PSC34 fragment hybridizes with sea urchin oocyte RNA, and thus may contain a transcribed sequence that may be a structural gene responsible for the synthesis of a maternal RNA (T. Thomas and R. Angerer, unpublished results). Although still in progress, our studies demonstrate short period repetitive sequence interspersion in cloned DNA fragments. This, to our knowledge, has not previously been observed since most studies so far published have been carried out on *Drosophila* DNA, which lacks short period interspersion (*13*). The sequence organization observed in these fragments is approximately in accord with average predictions made from physical measurements on the total DNA.

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References and Notes

1. E. H. Davidson, B. R. Hough, C. S. Amenson, R. J. Britten, *J. Mol. Biol.* **77**, 1 (1973); E. H. Davidson, G. A. Galau, R. C. Angerer, R. J. Britten, *Chromosoma* **51**, 253 (1975); R. C. Angerer, E. H. Davidson, R. J. Britten, *Cell* **6**, 29 (1975).
2. D. E. Graham *et al.*, *Cell* **1**, 127 (1974).
3. M. E. Chamberlin, R. J. Britten, E. H. Davidson, *J. Mol. Biol.* **96**, 317 (1975).
4. P. L. Deininger and C. W. Schmid, *ibid.* **106**, 773 (1976).
5. J. E. Manning, C. W. Schmid, N. Davidson, *Cell* **4**, 141 (1975).
6. W. R. Crain, F. C. Eden, W. R. Pearson, E. H. Davidson, R. J. Britten, *Chromosoma* **56**, 309 (1976).
7. W. R. Crain, E. H. Davidson, R. J. Britten, *ibid.* **59**, 1 (1976).

8. R. Wells, H. D. Royer, C. P. Hollenberg, *Mol. Gen. Genet.* **147**, 45 (1976).
9. E. H. Davidson and R. J. Britten, *Q. Rev. Biol.* **48**, 565 (1973); E. H. Davidson, W. H. Klein, R. J. Britten, *Dev. Biol.* **55**, 69 (1977).
10. M. So, R. Gill, S. Falkow, *Mol. Gen. Genet.* **142**, 239 (1975). Our work was done in compliance with the NIH guidelines for recombinant DNA research.
11. R. Roychoudhurg, E. Jay, R. Wu, *Nucleic Acids Res.* **3**, 101 (1976).
12. R. W. Davis, M. Simon, N. Davidson, *Methods Enzymol.* **21D**, 413 (1971).
13. D. M. Glover, R. L. White, D. J. Finnegan, D. S. Hogness, *Cell* **5**, 149 (1975).
14. G. S. Hayward, *Virology* **49**, 342 (1972).
15. R. J. Britten, D. E. Graham, F. C. Eden, D. M. Painchaud, E. H. Davidson, *J. Mol. Evol.* **9**, 1 (1976).
16. A. S. Lee and R. L. Sinsheimer, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 2882 (1974).
17. W. Barnes, unpublished observations.
18. Five of these clones were previously studied by W. R. Crain in this laboratory. The results of both studies are in close agreement.
19. Supported by NIH grants HD 05753 and GM 20927, and by NSF grant BMS 75-07359. A.S.L. has been supported by a fellowship from the American Cancer Society, California Division, J-289.

7 February 1977

Nucleotide Sequences from a Rabbit Alpha Globin Gene Inserted in a Chimeric Plasmid

Abstract. *Rabbit alpha globin gene copies have been made, using reverse transcriptase and DNA polymerase I, and cloned in bacterial plasmids. Plasmid pHb72 has been shown to contain the alpha gene sequence by restriction enzyme analysis and nucleotide sequencing studies, and therefore has been approved for propagation under P2 plus EK1 conditions by the National Recombinant DNA Committee.*

Reverse transcriptase has been used to synthesize complementary DNA (cDNA) copies of a variety of polyadenylated messenger RNA's (mRNA's). Several years ago, we discovered that reverse transcriptase leaves a short double-stranded hairpin structure when it terminates the synthesis of such cDNA's. This structure provides an efficient primer for second-strand synthesis by DNA polymerase I (*1*) so that it is possible to synthesize a double-stranded DNA copy of the mRNA sequence. This, and similar observations (*2*), prompted us as well as others to use the self-priming characteristics of cDNA's made with reverse transcriptase to develop general methods for cloning sequences copied from mammalian mRNA's in bacterial plasmids (*3–5*). Other methods have been developed for cloning mRNA sequences that did not utilize the self-priming nature of cDNA's synthesized by reverse transcriptase (*6*). In some of these cases, however, the use of complicated steps to force priming artificially at the desired location resulted in cloning efficiencies one to two orders of magnitude lower than that which we reported.

We have described the characterization of rabbit beta globin clones that we constructed using the cDNA technique (*3*, *7*). Our analysis included the determination of a 169-nucleotide sequence coding for the beta globin amino acid sequences from cysteine 93 through the carboxyl terminus of the structural gene (*7*, *8*). In the course of screening a series of 36 plasmids (pHb51 through pHb86) for the presence and the orientation of beta globin gene sequences, we identified nine plasmids that appeared to contain alpha globin gene sequences. Here we report the preliminary characterization of these plasmids.

Digestion with the two restriction endonucleases Eco RI and Bam HI provides a convenient method for screening beta globin gene sequences. A fragment 70 nucleotides in length is liberated from plasmids which carry that portion of the beta globin gene which codes for amino acid residues 98 through 122. In addition, it is possible to determine rapidly the orientation of beta globin gene sequences within these plasmids by comparing the fragments liberated by digestion with Bam HI with those liberated with both Bam HI and Eco RI. Since there is a Bam HI site within the beta globin gene and another within the plasmid gene for tetracycline resistance, Bam HI digestion liberates a fragment in the size range 600 to 1100 base pairs. If the orientation of the beta gene insertion is such that the Eco RI site is between the two Bam HI sites, then the corresponding fragment from a Bam HI plus Eco RI digestion will be 70 base pairs shorter. With the reverse orientation the Eco RI digestion does not affect this fragment.

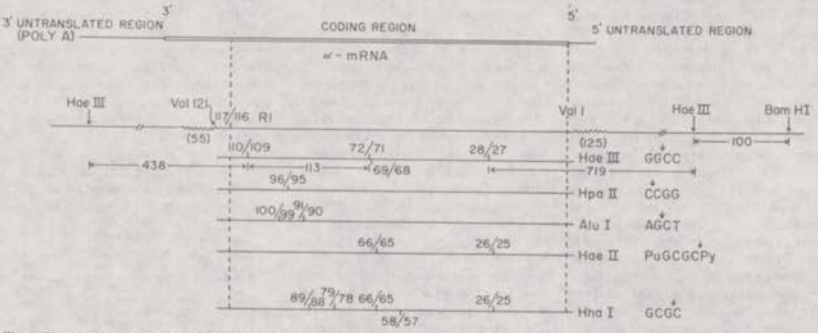


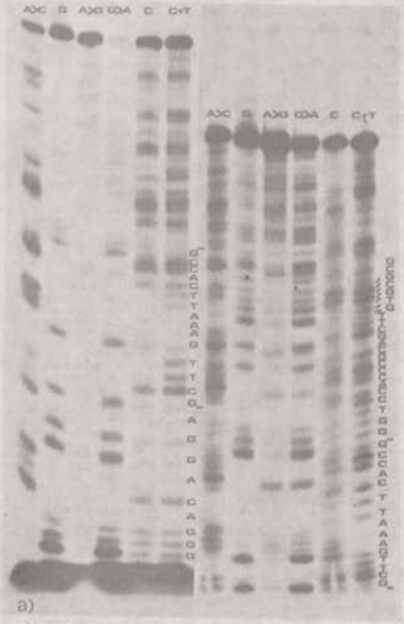
Fig. 1. Plasmid pHb72 restriction fragment map. The number pairs above each line indicate amino acid correspondence for sequences bearing confirmed sites for the restriction endonucleases cited. The number pairs under each line indicate amino acid correspondence for sequences bearing possible sites for the enzymes cited. Single numbers between sites indicate the number of nucleotides between sites. The inserted sequences in pHb72 extend from the codon for valine (Val) 121 to approximately 12 nucleotides beyond the initiation codon. A wavy line indicates poly(dA - dT) homopolymer. The lengths shown (numbers in parentheses) for these homopolymer regions are approximate and were estimated from ladder sequencing gels (data not shown). The arrangement of the fragments in the map was determined by a combination of conventional techniques and correlation of sequencing results with the known amino acid sequence (*10*).

Of the 13 plasmids assayed in this manner, nine of the inserts were oriented with the 3' end (mRNA sense) nearest the external pMB9 Bam HI site. Four were oriented in the opposite direction. This is an interesting contrast to the results of Maniatis *et al.* (*4*), who found that of 19 beta globin clones examined, all were oriented with the 3' end nearest the external Bam HI site.

In the course of such studies it was observed that plasmid pHb72 contained an Eco RI site but no Bam HI site within the plasmid insert. Such a result might be expected for a plasmid containing a small portion of the beta globin gene sequence that terminated between the Eco RI and Bam HI sites. That this was not the case was suggested by RNA-DNA hybridization tests with radioactive globin mRNA and by measurements of the size of the plasmid, which implied the presence of a large insert. Alternatively, the result suggested that plasmid pHb72 might carry an alpha globin gene sequence. The presence of an Eco RI site is consistent with an alpha globin gene insert, since conventional sequence analysis of the globin mRNA's had already demonstrated that a single Eco RI site occurs in both the alpha and beta globin structural genes (*9*). In order to test this hypothesis, an Hae III restriction endonuclease digestion of plasmid pHb72 was carried out and the nucleotide sequence of one of the insert fragments was determined and compared with the known amino acid sequence of rabbit alpha globin (*10*).

To determine the number and size of the Hae III fragments that contain sequences from the gene insert, digests of pHb72 and the parent plasmid, pMB9, were electrophoresed in parallel on a 6 percent polyacrylamide gel. As expected, the pHb72 restriction pattern lacked an 850-base-pair pMB9 fragment containing the Eco RI site where the gene insertion was made. Three distinct new bands, whose sizes were estimated as 719, 438, and 113 base pairs, were observed, and their arrangement in the plasmid was ultimately determined to be as shown in Fig. 1. A band of unusual intensity suggested the presence of an additional "hidden" band about 120 base pairs in size, which comigrated with a pMB9 band. The existence of this band was subsequently confirmed by recovering it by Hae III digestion of the appropriate Alu I fragment.

The smallest of the insert bands was eluted from the acrylamide gel so that the "ladder" sequencing technique of Maxam and Gilbert (*11*) could be used to determine its nucleotide sequence. The fragment recovered from digestion of 100 μg of pHb72 was treated with bacterial alkaline phosphatase. After this enzyme was inactivated by repeated phenol extractions, bacteriophage T4 polynucleotide kinase was used to transfer labeled phosphorus from [γ-^{32}P]adenosine triphosphate to the 5' termini of the fragment. The complementary strands were then separated by denaturation in 0.3*N* NaOH (for 30 minutes at 37°C) and electrophoresis on a tris-phosphate (0.036*M* tris, 0.03*M* NaH_2PO_4, *p*H 7.7) 4 percent polyacrylamide gel (bis-acrylamide: ac-



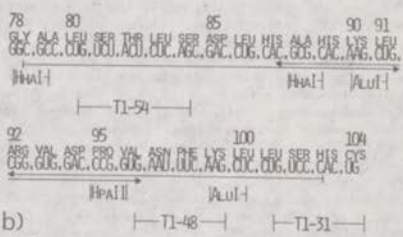


Fig. 2. (a) Autoradiograph of a ladder sequencing gel and the 47-nucleotide sequence derived from it. The 113-base-pair restriction fragment labeled at its Hae III terminal 5'-phosphate was strand-separated (see text). The fast strand was divided into six fractions, and each fraction was chemically cleaved at one of the four bases. The products were resolved by electrophoresis on a 20 percent acrylamide denaturing gel. The six tracks on the right are the same as the six on the left, except that they were electrophoresed into the gel for several hours longer. The base or bases cleaved as well as the relative amounts of cleavage in each reaction are indicated by the heavy letters at the top of each track. The reaction conditions used for each cleavage are given in (*11*) under the following descriptive titles: A > C, "alternative strong adenine, weak cytosine cleavage"; G, "alternative guanine reaction"; A > G, "strong adenine, weak guanine cleavage"; G > A, "strong guanine weak adenine cleavage"; C, "cleavage at cytosine"; and C + T, "cleavage at thymine and cytosine." Brackets show the region of 15-nucleotide overlap for the two experiments. Electrophoresis is from top to bottom. The heavy radioactive band at the bottom is the result of a trivial technical difficulty, a pileup of nucleotides due to a salt-front effect on the resolving gel. This artifact, which caused us to lose the first 21 nucleotides of sequence data at the 5' end of the fragment, can be avoided by running the gel beforehand for 4 hours at 400 volts. (b) A 77-nucleotide sequence derived by combining the ladder sequencing data with earlier sets of data that contributed to the sequence. The T1 fragment numbers refer to sequences reported in previous publications (*9*). The arrows show the directions and lengths of sequences that could be read from a ladder sequencing gel. Arrows running left to right were read from the gel as shown. Arrows running right to left indicate that the complementary sequence was read from the gel. The data were obtained from an Hae III fragment with the labeled 5' ends 21 nucleotides in each direction beyond the portion shown here. Hha I, Alu I, and Hpa II sites exist in the sequence where indicated.

rylamide, 1:29 (*11*, *12*). As estimated from autoradiograph intensity, about 80 percent of the material loaded on the gel was recovered as separated single strands. The rapidly and slowly migrating bands were eluted and divided into portions to be subjected to partial digestions specific for each of the four deoxynucleotides. In addition to the four standard reactions, we have found it useful to employ the "alternate A" and "alternate G" reactions developed by Maxam and Gilbert. The conditions of the reactions used have been described (*7*, *11*).

The partial cleavage products were electrophoresed side by side on a tris-borate 20 percent polyacrylamide 7*M* urea gel. Figure 2a shows the results obtained for the rapidly migrating strand. The radioactive products from a particular cleavage all have common ^{32}P-labeled 5′ ends and 3′ ends that terminate at the nucleotide or nucleotides cleaved in the specific reaction. Separation according to size on the polyacrylamide gel enables the nucleotide sequence to be read directly from the autoradiograph, as indicated in Fig. 2a. This sequence is the complement of the mRNA sequence shown in Fig. 2b and corresponds exactly to the mRNA sequence coding for amino acid residues 87 to 103, as shown by the arrow pointing left under the sequence.

Since the sequence analyses presented here and the ladder sequence analyses we published earlier for the beta globin gene (*7*) correspond to known amino acid sequences, they provide an ideal opportunity to test the accuracy of the new sequencing approach developed by Maxam and Gilbert. Further tests of the accuracy are provided by comparison with the data we have obtained by more conventional sequencing techniques. In general, the accuracy of the method seems to be very good. In some cases a particular nucleotide may be difficult to read. The most troublesome example in Fig. 2a is the third nucleotide from the bottom of the left-hand set of gel tracks, that is, the third G in the sequence GGGAC (*8*). This corresponds to GUCCC in the complementary (message) strand. The only possible interpretations of the data at this point were G (bands in the G and G > A tracks) and C (bands of equal intensity in the C and C + T tracks). Since G residues frequently give bands of varying intensity in the C and C + T tracks, but C residues never give bands in the G track, it was felt that G was the only possible assignment. That this is the correct interpretation is shown by the fact that none of the other possibilities is consistent with the amino acid residue found at this position in the protein.

In addition to the data shown in Fig. 2a, we have obtained analogous sequencing data (not shown) from the complementary strand. From these it was possible to determine the sequence indicated by the arrow pointing right in Fig. 2b. In the large region where the arrows overlap, the nucleotide sequence has been determined entirely independently from the two sets of data, which are in complete agreement. Other checks on the accuracy of the sequence are provided by the ribonuclease T1 fragment sequences T1-54, T1-48, and T1-31 indicated in Fig. 2b. Again the correspondence is exact, indicating the accuracy of the sequencing technique as well as the fidelity of the maintenance of the cloned sequence in the Rec A$^+$ host *Escherichia coli* C600. This particular sequence is remarkable in some respects, especially in having a content of 73 percent G plus C. The presence of an Hpa II site at amino acid residues 95-96 as well as another in a sequence reported earlier by Proudfoot and Brownlee (*13*) from the 3′ untranslated region is also noteworthy. Since there are no such sites within the rabbit beta globin gene, digestion with Hpa II provides a convenient criterion for detecting plasmids carrying alpha globin sequences. Detailed discussion of the alpha globin sequences will be reserved for a subsequent report.

The National Institutes of Health guidelines for recombinant DNA research (*14*) specify that "when a cloned DNA recombinant has been rigorously characterized and there is sufficient evidence that it is free of harmful genes, then experiments involving this recombinant DNA can be carried out under P1 + EK1 conditions if the inserted DNA is from a species that exchanges genes with *E. coli* and under P2 + EK1 conditions if not." A footnote to the guidelines indicates that it is necessary to obtain approval from the NIH for the use of these reduced containment levels, such approval being "contingent upon data concerning: (a) the absence of potentially harmful genes (e.g., sequences contained in indigenous tumor viruses or which code for toxic substances), (b) the relation between the recovered and desired segments (e.g., hybridization and restriction endonuclease fragmentation analysis where applicable), and (c) maintenance of the biological properties of the vector."

We have submitted data as specified above for the rabbit alpha globin plasmid pHb72, and on this basis the National Recombinant DNA Committee has approved its growth under P2 plus EK1 conditions. The rationale suggested in our petition is briefly summarized as follows. The method of joining the gene copy and plasmid, homopolymer tailing, ensures that only one gene sequence can be inserted per plasmid. This was confirmed by measurements of the size of plasmid pHb72. When the gene copy is made from a monocistronic mRNA by using the cDNA cloning technique, it can be inferred with confidence that a globin gene copy cannot also contain other potentially harmful genes. Consequently, demonstration that the plasmid contains a desired globin gene sequence inserted by the cDNA cloning technique can help rule out the possibility that potentially harmful genes are present. The sequencing and restriction analyses presented here provide strong proof of the presence of the alpha globin sequence, which was corroborated by RNA-DNA hybridization data. Finally, the required proof of the "maintenance of the biological properties of the vector" includes demonstration of the maintenance of the tetracycline resistance gene, the ability of the plasmid to amplify in chloramphenicol, and comparison of pHb72 with the pMB9 parent plasmid by restriction fragmentation analysis with Hae III and other restriction endonucleases.

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References and Notes

1. W. Salser, *Annu. Rev. Biochem.* **43**, 923 (1974); see pp. 948–949.
2. A. Efstratiadis *et al.*, *Cell* **7**, 279 (1976).
3. R. Higuchi, G. V. Paddock, R. Wall, W. Salser, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **35**, 1369 (1976); *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3146 (1976); G. V. Paddock, R. Higuchi, R. Wall, W. Salser, *Molecular Mechanisms in the Control of Gene Expression*, D. P. Nierlich, W. J. Rutter, C. F. Fox, Eds. (Academic Press, New York, 1976), p. 535; W. Salser *et al.*, *Prog. Nucleic Acid Res. Mol. Biol.* **19**, 177 (1976). The cloning and all work with live cells carrying globin sequences were carried out under P3 conditions under Asilomar guidelines before EK2 systems were certified. Similar experiments are now being carried out under P3 conditions with the newly certified EK2 systems, as is now specified.
4. T. Maniatis, S. G. Kee, A. Efstratiadis, F. C. Kafatos, *Cell* **8**, 163 (1976).
5. B. W. O'Malley, S. L. C. Woo, J. J. Monahan, L. McReynolds, S. E. Harris, M. J. Tsai, S. Y. Tsai, A. R. Means, in *Molecular Mechanisms in the Control of Gene Expression*, D. P. Nierlich, W. J. Rutter, C. F. Fox, Eds. (Academic Press, New York, 1976), p. 309; F. Rougeon and B. Mach, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3418 (1976).
6. F. Rougeon, P. Kourilsky, B. Mach, *Nucleic Acids Res.* **2**, 2365 (1975); T. H. Rabbits, *Nature (London)* **260**, 221 (1976); K. O. Wood and J. C. Lee, *Nucleic Acids Res.* **3**, 1961 (1976).
7. J. Browne, G. V. Paddock, A. Liu, P. Clarke, H. Heindell, W. Salser, *Science* **195**, 389 (1977).
8. Abbreviations for the amino acid residues are Ala, alanine; Arg, arginine; Asn, asparagine; Asp, aspartic acid; Cys, cysteine; Glu, glutamic acid; Gln, glutamine; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; and Val, valine. Other abbreviations are A, adenine; C, cytosine; U, uracil; G, guanine; and T, thymine.
9. W. Salser *et al.*, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **35**, 23 (1976); G. V. Paddock, R. Poon, H.

Heindell, J. Isaacson, W. Salser, *J. Biol. Chem.*: in press.
10. M. O. Dayhoff, *Atlas of Protein Sequence and Structure* (National Biomedical Research Foundation, Silver Spring, Md., 1972), vol. 5.
11. A. M. Maxam and W. Gilbert, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 560 (1977).
12. G. S. Hayward, *Virology* **49**, 342 (1972).
13. N. J. Proudfoot and G. G. Brownlee, *Nature (London)* **263**, 211 (1976).
14. Department of Health, Education and Welfare, *Fed. Regist.* **41** (No. 131), 27917 (1976).
15. We thank R. Higuchi for his help during the early stages of this work and A. Maxam and W. Gilbert for supplying details of their technique in advance of publication. This research was supported in part by PHS grants GM 18586 and CA 15940 and by grant VC-240 from the American Cancer Society. G.V.P. was supported by a Helen Hay Whitney Fellowship. A.L. and H.C.H. were supported by National Cancer Institute predoctoral training grant 783825-32923-7.

7 February 1977

Analysis of Chicken Ribosomal RNA Genes and Construction of Lambda Hybrids Containing Gene Fragments

Abstract. *The ribosomal RNA genes (ribosomal DNA) of chicken are present in approximately 200 copies and are cleaved into two fragments of molecular weight 5 × 10^6 and 12 to 14 × 10^6 by restriction endonuclease Eco RI. Recombinant phages have been constructed in vitro by joining the smaller fragment of ribosomal DNA and the outer arms of DNA from the vector λgtWES·λC. In one of the recombinants, the coding strand of the cloned fragment is in the proper orientation for transcription with λ "early" genes; in the other two the orientation is reversed, with the coding strand in the proper position for transcription with λ "late" genes.*

Cloning of ribosomal RNA (rRNA) genes, which are present in multiple copies and specify a readily available RNA probe, can provide a convenient system for detailed analysis of eukaryotic DNA (*1*). In addition to yielding hybrid DNA which includes genes that are interesting in themselves, these experiments provide an opportunity to develop methods potentially useful for the cloning of unique sequence genes (*2*). We have employed the certified EK2 vector λgt*WES*·λC (*3*) to clone and characterize a portion of the ribosomal gene from chickens (*Gallus domesticus*).

Ribosomal RNA genes represent 0.02 to 0.12 percent of chicken DNA (*4*, *5*) and are clustered. If these genes are in tandem, as are ribosomal DNA (rDNA) sequences in other eukaryotic chromosomes (*6–8*), then cleavage with restriction endonuclease should yield a number of identical fragments. Figure 1 shows results from experiments in which chicken DNA was digested with Eco RI and the resulting fragments analyzed for the presence of DNA homologous to chicken rRNA. A mixture of 18*S* and 28*S* ^{3}H-labeled chicken rRNA hybridized to fragments in two regions of the gel (Fig. 1, b and c). By comparison with parallel gels containing Eco RI digests of ^{3}H-labeled λ DNA (*9*), we estimate the molecular weights of the two rDNA fragments to be 5 × 10^6 and 12 to 14 × 10^6. The sharp band in the 5 × 10^6 dalton region and the absence of hybridization to DNA between the two bands indicated that Eco RI digestion was complete and that there were at least two sites for Eco RI in the rDNA. Other experiments (not included) showed that the smaller fragment contained sequences homologous with both 18*S* and 28*S* rRNA, whereas the larger fragment contained only 18*S* sequences. These results are similar to those found for rDNA in the amphibian *Xenopus laevis* (*10*) and in several mammals (*8*) and support the suggestion by Southern (*8*) that these two sites of Eco RI cleavage in the rDNA have been conserved since the divergence of amphibians and mammals. The sizes of the avian rDNA Eco RI fragments are closer to those of mammals (mouse, 5.2 × 10^6 and 14 × 10^6 daltons; rabbit, 6.0 × 10^6 and 12 × 10^6 daltons) than those of amphibians (*X. laevis*, 3.0 × 10^6 and 4 to 6 × 10^6 daltons) (*8*, *10*). Our data suggest that the structure of the chicken rDNA is simi-

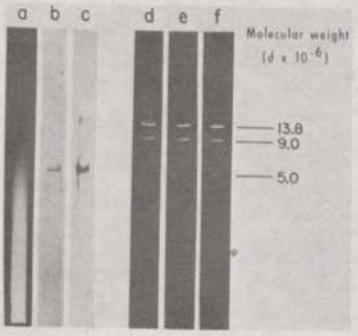


Fig. 1. Agarose gel analysis of chicken DNA and recombinant phage DNA. High-molecular-weight DNA was prepared from erythrocytes of Spafas (Norwich, Conn.) gs⁻ chickens by a method similar to that of Chambon and co-workers (*18*). The DNA was incubated in 0.1*M* tris (*p*H 7.4), 0.05*M* NaCl, 0.005*M* $MgCl_2$, and 0.001*M* *p*-chloromercuribenzoate for 10 minutes at 37°C. Eco RI was added (2 units per microgram of DNA) and the mixture was incubated for 10 minutes. A second portion of Eco RI was then added and incubation was continued for 10 minutes. The reaction was stopped by adding one-tenth volume 10 percent sodium dodecyl sulfate and heating to 65°C for 2 to 5 minutes. Digested DNA was concentrated by ethanol precipitation before electrophoresis; such treatment causes some loss of large fragments. A 10- to 20-μg portion of the concentrated DNA was subjected to electrophoresis on 0.7 percent agarose disc gels (0.5 by 12 cm) in buffer containing 0.04*M* tris, 0.005*M* sodium acetate, and 0.001*M* EDTA (the *p*H was adjusted to 7.9 with acetic acid). Gels were stained for 1 hour or more in ethidium bromide (0.5 μg/ml). The DNA was transferred to cellulose nitrate membranes by the method of Southern (*8*). The 18*S* and 28*S* rRNA probes (specific activity = 4 × 10^8 cpm/μg) were prepared from chicken fibroblasts labeled with [^{3}H]uridine. Each probe was shown to be homogeneous by polyacrylamide gel electrophoresis. The concentration of probe RNA in the hybridization mixture was approximately 0.5 μg/ml. Hybridization was detected by fluorography (*8*, *19*). The stoichiometry of the recombinant DNA Eco RI fragments was determined from densitometer tracings of a photographic negative of ethidium bromide–stained gels. The area under each peak was determined by cutting out and weighing that peak. Molecular weights were taken to be 13.8 × 10^6 and 9.0 × 10^6 for the vector arms and 5.0 × 10^6 for the inserted fragment. Normalizing to the smaller vector fragment, the ratios of fragments (from largest to smallest) are: λGd1, 0.98 : 1.00 : 0.76; λGd2, 1.00 : 1.00 : 0.91; and λGd3, 1.08 : 1.00 : 0.86. Lambda Gd1 DNA, uniformly labeled with ^{32}P, was digested with Eco RI and subjected to electrophoresis on 0.7 percent agarose gels. The radioactivity in each fragment was determined by cutting out the ethidium bromide–stained bands and counting them in 5 ml of Aquasol. The counts were (from largest to smallest) 297, 154, and 80 cpm. Normalizing to the smaller vector arm, the ratio of fragments is 1.25 : 1.00 : 0.94. (a) Ethidium bromide–stained gel containing Eco RI fragments of unfractionated chicken DNA. (b) Fluorogram of Southern transfer containing Eco RI fragments of unfractionated chicken DNA hybridized with 18*S* plus 28*S* ^{3}H-labeled rRNA. In this transfer, hybridization to the 5 × 10^6 dalton fragment is apparent but hybridization to the 12 to 14 × 10^6 dalton fragment is not. (c) Fluorogram as in (b) except that before digestion with Eco RI, DNA was enriched three- to fivefold for ribosomal sequences by selecting material from the heavy side of DNA banded in a CsCl equilibrium gradient. In this case hybridization to both fragments is evident. (d to f) Ethidium bromide–stained gels of Eco RI–digested DNA from (d) λGd1, (e) λGd2, and (f) λGd3.

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lar to that determined for *X. laevis* (*7*, *10*). By analogy with *Xenopus*, we suppose that each unit of the tandem array consists of an 18S region and a 28S region separated by a transcribed spacer with each transcribed segment separated by a nontranscribed spacer (Fig. 2). The minimal estimated molecular weight of the complete chicken rDNA unit is 17×10^6. The hybridization data suggest that Eco RI cleaves the rDNA once within the 18S region and once near or at the junction of the 28S region and the nontranscribed spacer. Thus, the small Eco RI fragment contains part of the 18S region, the transcribed spacer, and most or all of the 28S region. The large Eco RI fragment probably contains some nontranscribed spacer, a transcribed spacer, and part of the 18S region. Ribosomal RNA hybridized to the small fragment in a sharp and well-defined band, but the region of hybridization to the large fragment was not always sharp. This may mean, as in the case of *X. laevis* (*11*), that the large fragment is actually a collection of fragments of varying lengths reflecting heterogeneity in the length of the nontranscribed spacers. Furthermore, this nontranscribed spacer region could contain additional Eco RI sites, generating fragments which would not be detected by hybridization to rRNA.

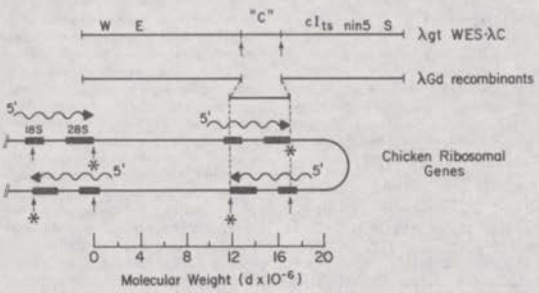


Fig. 2. Schematic showing probable structural relationships between λ vector phage DNA, λGd recombinant phage DNA's, and chicken ribosomal RNA genes. Vertical arrows indicate sites for cleaving by restriction endonuclease Eco RI. Asterisks indicate that additional Eco RI sites could occur within the nontranscribed spacer, generating fragments invisible by our assay (*20*). These fragments would be flanked by the two gene-containing fragments at the position of the asterisks. Horizontal (wavy) arrows represent RNA transcripts with the 5' end assigned by analogy with findings for *X. laevis* (*21*).

For cloning, the 5×10^6 dalton fragment was purified by cutting out the appropriate region of a preparative gel of the Eco RI–digested chicken DNA. We extracted DNA (between 5 and 10 percent of the starting digest) from the gel segment with phenol, and subsequent hybridization analysis (*8*) confirmed that this fraction contained rRNA gene fragments and that they had been isolated free of at least 90 percent of other chicken DNA. The long arms of the Eco RI–digested EK2 vector phage DNA (see Fig. 2) were first separated from the λC fragment so that plaque formation was dependent on the addition of the chicken DNA fragments (*12*). Of approximately 200 recombinant phages tested, three were capable of hybridizing to 18S plus 28S chicken rRNA. The three recombinants λGd1, λGd2, and λGd3 were selected, plaque-purified, and grown on a special RecA host strain (see legend to Table 1), and all operations with the recombinants were carried out under P3 containment. Phage stocks were rechecked by hybridization in situ to either 18S or 28S RNA and found to be positive. Results of filter hybridization to DNA purified from two of the hybrids are presented in Table 1.

The DNA from each recombinant was digested with Eco RI and subjected to agarose gel analysis (Fig. 1, d to f). In each case three fragments were obtained; the two larger fragments (13.8×10^6 and 9.0×10^6 daltons) corresponded in size to the left and right arms of the vector (*9*, *13*). The small fragments ran at the position of the 5×10^6 dalton rDNA fragments. Hybridization with DNA from such gels verified that the small fragment in each clone contained DNA homologous with the 18S and 28S rRNA sequences. The stoichiometry of the Eco RI fragments of each recombinant was determined from densitometer tracings of a photographic negative of the stained gels and verified by measurements of the isotope content of each fragment from a similar analysis of uniformly labeled (^{32}P) λGd1 DNA. The result showed (Fig. 1d and Fig. 1 legend) that the three Eco RI fragments are each present in unit amount in all the recombi-

Table 1. Hybridization of 18S and 28S ^{3}H-labeled rRNA to recombinant phage DNA. Denatured DNA was fixed to 20-mm nitrocellulose filters (*22*). The filters were cut in half and hybridized together with either 18S or 28S ^{3}H-labeled rRNA in 1 ml at concentrations indicated in Fig. 1. Hybridization (in DNA excess) and subsequent washing and ribonuclease treatment to remove nonspecifically bound RNA were as described by Southern (*8*). For the construction of recombinants, Eco RI end fragments of vector phage DNA were purified away from the C fragment (see Fig. 2) by a two-step procedure involving preparative sucrose gradient sedimentation followed by preparative agarose gel electrophoresis of the rapidly sedimenting (longest) two fragments. In a standard transfection assay with Ca^{2+}-shocked *Escherichia coli* (*23*), this preparation yielded only one plaque-forming unit per microgram of end fragment in the absence of any addition, as opposed to 407 in the presence of added C fragment (therefore the minimal purity of end fragments was 99.8 percent). The in vitro recombination mixture (0.1 ml) contained 4.5 μg of λ vector end fragments (heated and rapidly cooled before use to disjoin molecular ends), 1.3 μg of 4 to 6×10^6 dalton chicken Eco RI fragments (isolated from a preparative gel similar to that shown in Fig. 1), 25 μg of *E. coli* transfer RNA, and 3.4 units of T4 ligase (*24*) in 0.05*M* tris (pH 7.4), 0.01*M* $MgCl_2$, 1.2×10^{-4}*M* adenosine triphosphate, 0.01*M* dithiothreitol, and 0.06 percent Povite bovine albumin (Biotest-Serum Institut GmbH, Frankfurt am Main). Incubation for 90 minutes at 16°C was followed by addition of 0.8 ml of 0.1*M* tris (pH 7.1) and 1.6 ml Ca^{2+}-shocked *E. coli* [strain 803, *rec A*, *r*$_K^-$ *m*$_K^-$, *su* II, *su* III (No. ED 8767 from the collection of N. Murray)]. After incubation on ice for 5 minutes and at 45°C for 2 minutes, the mixture was added to soft agar (15 ml) and distributed onto five Trypticase (Baltimore Biological Laboratories) agar plates. Phage from the resulting 212 plaques were screened by in situ hybridization (*2*) with purified 18S plus 28S chicken rRNA. Plaque purification and subsequent growth of hybrids was by standard phage techniques adapted to P3 containment conditions (*25*).

DNA	Duplicate tests	^{3}H hybridized (cpm)	
		18S	28S
λGd2 (0.5 μg of λGd2 DNA + 2.5 μg of λ DNA)	1	5599	6870
	2	5422	5763
λGd3 (0.5 μg of λGd3 DNA + 2.5 μg of λ DNA)	1	8069	5924
	2	6987	6947
Control (3.0 μg of λ DNA)	1	200	182
	2	360	109

nants. We verified the absence of the λC fragment (see Fig. 2) in all three recombinants by confirming the lack of *int* and *xis* gene function in the red-plaque test of Enquist and Weisberg (*14*). In addition, plating tests showed that all three recombinants failed to grow ($< 2 \times 10^{-10}$) on a *sup*0 host bacterium. These results indicated that the safety features included in the outer arms of the vector phage DNA had been conserved. These data are all consistent with simple insertion of the 5×10^6 dalton chicken rDNA fragment between the purified left and right arms of the vector phage DNA.

We determined the direction of insertion of the rDNA fragment in each recombinant by separating the left and right strands of the hybrid phage DNA by electrophoresis (*15*) and then hybridizing with 18S plus 28S rRNA (*8*). For λGd1 and λGd3 the rRNA hybridized with the right strand; for λGd2 hybridization was with the left. Thus, transcription of the rRNA sequences should be under control of the early λ phage promoter in λGd2 and of the late phage promoter in λGd1 and λGd3. Figure 2 is a model which indicates the probable structural relationships between the vector phage DNA, recombinant DNA, and chicken rRNA genes.

The recombinants reported here are being used to study the processing of precursor RNA to mature 18S and 28S rRNA. Cloning of the fragment of molecular weight 12 to 14×10^6 will provide a DNA segment with alternating transcribed and nontranscribed regions, which should serve as a useful model for understanding the organization and regulation of other eukaryotic genes.

Cloning the rRNA gene has provided insight into the requirements for cloning other genes, including those present in a single copy. The fraction of chicken DNA used to construct these recombinants was approximately 5 to 10 percent of the total genome. Three out of about 200 (1.5 percent) of the recombinants contained the 5×10^6 dalton rDNA fragment, which suggests that this fragment makes up about 0.075 to 0.15 percent of the total chicken genome. Knowing the amount of DNA per cell [2.4×10^{-6} μg (*16*)] and the minimal molecular weight of the rDNA unit (17×10^6), we estimate from our data that there are between 215 and 430 copies of the rRNA genes per chicken genome. These values are in line with the data of Sinclair and Brown (*4*), which suggest that there are approximately 175 to 350 copies of the rRNA genes, and with the estimate of Baluda and co-workers (*17*) that there are 210 to 218 copies of the 28S gene. Therefore, in our experiments, the production of a specific recombinant was proportional to the fraction of DNA of interest present in the whole genome. This indicates that the cloning of a single-copy gene will require approximately a 200-fold amplification of the methods of detection or gene purification employed in this study.

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References and Notes

1. J. Morrow, S. Cohen, A. Chang, H. Boyer, H. Goodman, R. Helling, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 1743 (1974).
2. R. A. Kramer, J. R. Cameron, R. W. Davis, *Cell* **8**, 227 (1976).
3. L. Enquist, D. Tiemeier, P. Leder, R. Weisberg, N. Sternberg, *Nature (London)* **259**, 596 (1976).
4. J. Sinclair and D. Brown, *Biochemistry* **10**, 2761 (1971).
5. I. Merits, W. Schulze, L. Overby, *Arch. Biochem. Biophys.* **115**, 197 (1966).
6. P. Wensink and D. Brown, *J. Mol. Biol.* **60**, 235 (1971).
7. P. Wellauer and I. Dawid, *ibid.* **89**, 379 (1974).
8. E. Southern, *ibid.* **98**, 503 (1975).
9. R. Helling, H. Goodman, H. Boyer, *J. Virol.* **14**, 1235 (1974).
10. P. Wellauer *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 2823 (1974).
11. P. Wellauer, I. Dawid, D. Brown, R. Reeder, *J. Mol. Biol.* **105**, 461 (1976).
12. M. Thomas, J. Cameron, R. Davis, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 4579 (1974).
13. D. Tiemeier, L. Enquist, P. Leder, *Nature (London)* **263**, 526 (1976).
14. L. Enquist and R. Weisberg, *Virology* **72**, 147 (1976).
15. G. Hayward, *ibid.* **49**, 342 (1972).
16. M. Baluda, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 576 (1972).
17. M. Shoyab, P. Markham, M. Baluda, *J. Virol.* **14**, 225 (1974).
18. M. Gross-Bellard, P. Dudet, P. Chambon, *Eur. J. Biochem.* **36**, 32 (1973).
19. R. Laskey and A. Mills, *ibid.* **56**, 335 (1975).
20. N. Arthelein and E. M. Southern, *Cell*, in press.
21. I. Dawid and P. Wellauer, *ibid.* **8**, 443 (1976).
22. D. Gillespie and S. Spiegelman, *J. Mol. Biol.* **12**, 829 (1965).
23. J. R. Cameron, S. M. Panasenko, I. R. Lehman, R. W. Davis, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3416 (1975).
24. The T4 ligase was a gift of C. Harvey, Hoffmann-La Roche.
25. National Institutes of Health guidelines for recombinant DNA research, *Fed. Regist.* **41** (No. 131), 27917 (1976). This project was approved by an Institutional Biohazards Committee constituted and functioning as stipulated in these NIH guidelines.

3 January 1977

Clones of Individual Repetitive Sequences from Sea Urchin DNA Constructed with Synthetic Eco RI Sites

Abstract. *Interspersed repetitive sequences were isolated from sea urchin DNA by renaturing to low C_0t followed by treatment with nuclease S1. Synthetic Eco RI sites were ligated onto the repetitive sequence elements, which were then inserted at the Eco RI site of plasmid RSF2124 and cloned. The repetitive sequences can be excised from the plasmid with Eco RI for further study.*

To facilitate studies of individual repetitive DNA sequence families, we have constructed recombinant DNA plasmids containing repetitive DNA from *Strongylocentrotus purpuratus* (*1*). Our object was to obtain cloned sequences terminated at the ends of interspersed repetitive sequence elements (*2*), rather than where restriction enzyme sites happen to fall. This was accomplished through the use of synthetically prepared Eco RI restriction sites that were ligated to the repetitive DNA fragments. The product of the ligation reactions was cloned to provide a source of plasmids from which individual repetitive DNA sequences could be reisolated in relatively large amounts.

Repetitive DNA duplex was prepared as follows (details may be found in the legend to Fig. 1). Sea urchin DNA sheared to a single-strand weight mean length of about 2000 nucleotides was renatured to C_0t 40 (C_0 is the initial concentration of nucleotides in moles per liter, and t is the time in seconds) and then treated with single-strand specific S1 nuclease. Previous studies (*2*, *3*) have shown that by this point most repetitive sequences in the sea urchin genome are fully renatured. The nuclease digestion removes the nonrepetitive regions flanking the interspersed repetitive sequences, since these remain single-stranded at C_0t 40 (*2*). The resistant DNA sequence consists almost entirely of repetitive duplexes. We have shown earlier that a majority of the S1 nuclease resistant duplexes are 300 to 400 nucleotides in length, and that this is the characteristic length of interspersed repeats in the sea urchin genome (*2*, *3*). The resistant repetitive duplexes are expected to have 3′-hydroxyl and 5′-phosphoryl termini (*4*).

A symmetrical decamer containing the Eco RI restriction site was synthesized by a triester chemical synthesis method (*5*). The structure of this decamer is shown in Fig. 1. The 5′-hydroxyl termini of the Eco RI decamers were labeled with ^{32}P, using T4 polynucleotide kinase. The decamer was covalently linked to the repetitive sea urchin DNA duplexes by blunt-end ligation with T4 DNA ligase (*6*). The expected products of the blunt-end ligation are the repetitive sea urchin DNA fragments bearing covalently linked Eco RI decamers, plus polymerized Eco RI decamers. The ligated mixture was next digested with Eco RI endonuclease. Eco RI treatment should yield the repeti-

tive sea urchin DNA with covalently linked Eco RI sticky ends labeled with ^{32}P, plus Eco RI cleavage products of polymerized and monomeric decamer. Sea urchin DNA containing covalently attached ^{32}P-labeled Eco RI cohesive ends was separated from the other cleavage products by gel filtration. From the amount of ^{32}P associated with the sea urchin DNA in the exclusion peak, the specific activity of the Eco RI decamer, and the DNA mass recovered, we calculated that 30 percent of the ends of the sea urchin DNA molecules were covalently linked to an Eco RI site. Thus about 10 percent of the repetitive DNA duplexes could be expected to carry Eco RI sites on both ends. This preparation was reacted with Eco RI–cleaved DNA isolated from the plasmid RSF2124, with T4 DNA ligase included to seal the duplexes formed from the Eco RI cohesive ends. The procedures used to prepare the repetitive DNA and to construct the clones is illustrated in Fig. 1.

RSF2124 is a colicinogenic (E1) plasmid carrying a locus for ampicillin resistance (*amp*^{R}) and a single Eco RI site (*7*). Transfection of *Escherichia coli* strain C600 with the recombinant plasmids was accomplished essentially according to Cohen *et al.* (*8*). Transformation efficiency was from 10^{-7} to 10^{-6} Amp^R colonies per molecule of plasmid DNA. The single Eco RI site in the RSF2124 genome lies within the colicin structural gene or in a control region for colicin E1 production (*7*). Insertion of a segment of DNA into the plasmid genome with the use of the Eco RI cohesive ends thus results in dysfunction of the colicin E1 locus. This property was exploited to assay the Amp^R colonies for insertion of the sea urchin DNA fragments into the plasmid genome. A double-layer technique (*9*) was used to detect colicin-producing clones, as described in the legend to Fig. 1. The frequency of Amp^R clones which were *col*$^-$ was about 10 percent.

Insertion of the Eco RI–sea urchin DNA fragments in the RSF2124 genome should introduce a second Eco RI site into the hybrid plasmid genome. Eco RI cleavage of hybrid plasmid DNA should thus result in unit length linear plasmid DNA molecules plus repetitive DNA fragments. We examined a number of *amp*^{R}*col*$^-$ clones and found that more than 65 percent contain short DNA duplexes which can be excised from the plasmid genome with Eco RI. We consider this a minimum estimate because of occasional difficulties encountered in detecting small inserted fragments representing 3 percent or less of the DNA mass. In other experiments in which long-

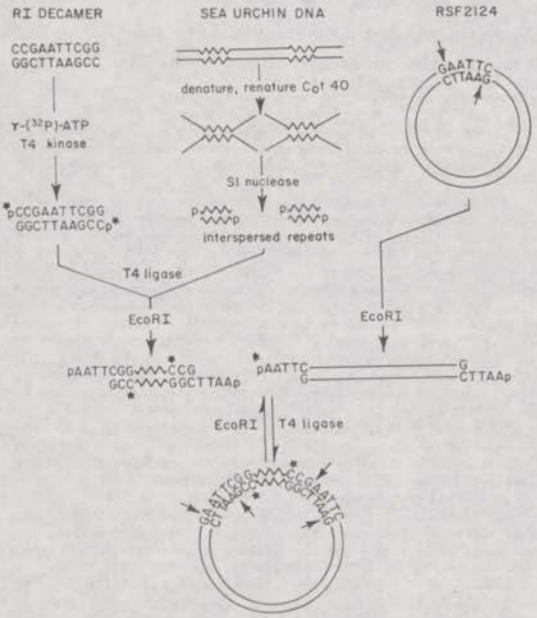


Fig. 1. Enzymatic steps in the construction of recombinant plasmids containing repetitive sea urchin DNA. Repetitive sea urchin DNA (double wavy lines), Eco RI decamer (shown in its entirety), and RSF2124 DNA (double straight lines) were ligated and cloned as follows. The vertical arrows (↑↓) indicate sites of Eco RI cleavage. The star (★) indicates the ^{32}P label at the 5′ termini. Repetitive sea urchin DNA was isolated by renaturing 2000-nucleotide long sperm DNA to C_0t 40, followed by digestion of the single-strand tails with 200 μl of S1 nuclease per milligram of DNA at 37°C for 45 minutes (*3*). Ten nanomoles of Eco RI decamer were 5′ phosphorylated with 7 units of T4 polynucleotide kinase and 50 nanomoles of [γ-^{32}P]ATP (adenosine triphosphate) for 1 hour at 37°C in 0.05*M* tris-HCl (*p*H 9.0), 0.01*M* $MgCl_2$, 0.005*M* dithiothreitol (DTT). A 60-fold excess of ^{32}P-labeled Eco RI decamer (16 μg) was ligated for 17 hours at room temperature to the repetitive DNA fragments (8 μg) with 10 μl of T4 DNA ligase in 0.04 ml, 66 m*M* tris-HCl (*p*H 7.6), 6.6 m*M* $MgCl_2$, 10 m*M* DTT, 0.01 percent NP40, and 0.4 m*M* ATP. This reaction mixture was adjusted with 100 m*M* tris-HCl (*p*H 7.6), 50 m*M* NaCl, 10 m*M* $MgCl_2$, and 0.02 percent NP40 and digested with 15 μl Eco RI endonuclease for 3 hours at 37°C. The Eco RI digest was deproteinized with an equal volume of $CHCl_3$ and isoamyl alcohol (24 : 1). The cleavage products were resolved on a Sephadex G-200 column. Supercoil RSF2124 plasmid DNA, cleaved with Eco RI essentially as described above, was mixed with a 40-fold molar excess of G-200–excluded sea urchin DNA in 66 m*M* tris-HCl (*p*H 7.6), 20 m*M* NaCl, 1 m*M* EDTA, 10 m*M* $MgCl_2$, 10 m*M* DTT, and 66 μ*M* ATP in a total volume of 30 μl. One unit of T4 DNA ligase (Miles Laboratories) was added, and the reaction mixtures were incubated at 15°C for 18 hours. Five microliters of this reaction mixture, diluted 200-fold with 0.05*M* $CaCl_2$, was used to transform *E. coli* strain C600 (RecA$^+$r$^-$m$^-$) to ampicillin resistance essentially according to Cohen *et al.* (*8*). Transformed cells were plated on agar plates containing ampicillin (15 μg/ml). Amp^R colonies were assayed for colicin production by a double-overlay method (*9*). Colonies were transferred to duplicate plates and grown overnight. One plate was retained as a master, and the cells on the second plate were killed with $CHCl_3$. The latter plates were overlaid with top agar containing 1×10^8 colicin-sensitive bacteria (*E. coli* HB101). The plates were incubated overnight and then scored for the absence of colicin production (*col*$^-$). Col$^+$ colonies are surrounded by a clear halo, indicating lack of bacterial growth, while col$^-$ colonies are overgrown by the colicin-sensitive cells. Col$^-$ colonies were stored in stab cultures and assayed for the presence of an inserted sea urchin fragment.

er sea urchin DNA fragments derived from an Eco RI digest of sea urchin DNA were inserted into the same plasmid vector, about 80 percent of the *amp*^{R}*col*$^-$ clones were found to contain sea urchin DNA.

The lengths of the inserted repetitive DNA fragments were estimated by gel electrophoresis, with the use of an Hae III digest of the parent plasmid as a size standard. The lengths of the inserted DNA sequences from 20 clones have now been measured. Of these, 17 are less than 1000 nucleotides. The mode value of their length distribution is 300 to 400 base pairs. Two inserts are in the range of 1000 to 1200 nucleotides, and one is about 2000 nucleotides. Although the sample is small, this distribution is in excellent agreement with the distribution of fragment lengths in the original nuclease S1 digest and with the range of repetitive sequence lengths in the sea urchin genome. More than 90 percent of the repetitive sequence elements in this genome are 300 to 400 nucleotides long, with less than a few percent $\geq$ 2000 nucleotides. Our data thus suggest that the population of repetitive sea urchin DNA duplexes inserted into the RSF2124 vector is reasonably representative of the repetitive DNA sequences in the sea urchin genome.

To verify that the DNA sequences inserted into the plasmid genome in fact consist of repetitive sea urchin DNA, the cloned inserts were reassociated with sheared sea urchin DNA. The following procedure was used. The cloned DNA fragments were excised with Eco RI and labeled at the 5′ termini with ^{32}P by the T4 kinase method (*10*) (Fig. 2). The DNA was renatured after labeling and placed on 3 percent agarose gels along with an Hae III digest of RSF2124. An example is shown in Fig. 2A, which illustrates the preparation of the repetitive DNA insert from clone CS2108. After renaturation, the labeled DNA insert from this clone migrates to a position corresponding to a 190 base pair fragment, where it can be detected either by ethidium bromide staining or by autoradiography. The labeled DNA was eluted from the gel and reassociated with excess sheared sea urchin DNA approximately 500 nucleotides in length. The fraction of DNA reassociated was determined by hydroxyapatite chromatography and is plotted as a function of the driver DNA C_0t in Fig. 2B. The self-reaction of the labeled CS2108 fragment was monitored in separate reactions (not shown). At the tracer concentration used, the rate of the self-reaction was less than 10 percent of the driver reaction and thus does not affect the results. A least-squares solution to the kinetic data reproduced in Fig. 2B yields a rate constant of $3.16 \times 10^{-2} M^{-1}$ sec^{-1} for the reassociation of the CS2108 fragment with sea urchin DNA. The rate constant for the reassociation of single-copy sea urchin DNA of similar fragment length with whole DNA is $1.13 \times 10^{-2} M^{-1}$ sec^{-1}. Thus clone CS2108 contains a DNA sequence which is repeated approximately 25 times per haploid genome. It is unlikely that the reiteration frequency of the CS2108 sequence could differ from this number by more than a factor of two or three, taking into account the limited kinetic data and uncertainties in correcting the observed rate constants for minor length differences and the effect of base pair mismatch.

The repetitive sea urchin DNA clones constructed by the blunt-end ligation

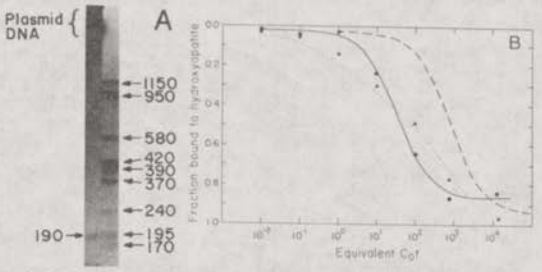


Fig. 2. Analysis of a recombinant plasmid clone constructed by blunt-end ligation. (A) Detection and end labeling of inserted sea urchin fragment. *Amp*^{R}*col*$^-$ clone CS2108 was grown in 40 ml of L broth plus ampicillin (15 μg/ml) to 4×10^8 to 6×10^8 cells per milliliter, and the plasmid content was amplified by incubation overnight in chloramphenicol (200 μg/ml). The cells were harvested by centrifugation and washed with a solution of 10 m*M* tris-HCl (*p*H 7.4) and 1 m*M* EDTA. They were again centrifuged and resuspended in 3 ml of 25 percent sucrose, 50 m*M* tris-HCl (*p*H 8.0), and 40 m*M* EDTA at 0°C. Lysozyme (5 mg; Sigma) was added, and the solution was maintained at 0°C for 5 minutes. Then 0.5 ml of 0.5*M* EDTA and 0.375*M* EGTA (*p*H 8.0) was added for 5 minutes at 0°C. Four ml of Brij lysing buffer [1 percent Brij 58, 0.4 percent sodium deoxycholate, 0.063*M* EDTA, 0.05*M* tris-HCl (*p*H 8.0)] was added and mixed vigorously. Chromosomal DNA was removed from the lysate by centrifugation at 50,000 rev/min for 45 minutes (Beckman/Spinco 50 Ti rotor). Superhelical plasmid DNA was isolated from the cleared supernatant by CsCl–ethidium bromide density gradient centrifugation (ethidium bromide at 250 μg/ml; ρ_0 = 1.669 g/cm^3) in a 50 Ti rotor at 39,000 rev/min. Superhelical DNA was removed from the gradient by side puncture. Ethidium bromide was removed by extraction with isopropanol. Plasmid DNA was dialyzed against 100 m*M* tris-HCl (*p*H 7.5), 100 m*M* NaCl, 5 m*M* $MgCl_2$ at 4°C. Recombinant plasmid DNA (15 μg) was digested with 2 μl of Eco RI at 37°C for 30 minutes. This digest was concentrated by flash evaporation to 0.5 ml and passed over a 0.5-ml Chelex 100 (BioRad) column. The DNA was precipitated at −70°C for 2 hours with 1 volume of isopropanol. The precipitate was collected by centrifugation at 10,000*g* for 30 minutes. The dried precipitate was resuspended in 10 m*M* tris-HCl (*p*H 8.0) and reacted with 1 μl of bacterial alkaline phosphatase (Worthington) at 37°C for 30 minutes. Ten micrograms of superhelical plasmid DNA carrier and 1 volume of 0.3*M* sodium acetate (*p*H 6.8) was added. This solution was extracted twice with a mixture of 80 percent phenol (*p*H 8.0) and 20 percent chloroform: isoamyl alcohol (24 : 1), and once with ether, then precipitated as described above. Labeling at 5′ termini was done according to Maxam and Gilbert (*10*) with [γ-^{32}P]ATP, specific activity 1200 c/mmole (Winston Salser's laboratory, University of California, Los Angeles). Kinase reactions were deproteinized as described above, and precipitated with 2.5 volumes of 100 percent ethanol at −70°C as described above. The dried precipitate was resuspended in a volume of 200 μl of 0.1 *M* NaCl, 0.05*M* tris-HCl (*p*H 7.2). The concentration of the end-labeled CS2108 DNA fragment was determined by its rate of renaturation, and from this the specific activity was calculated to be about 1×10^6 to 2×10^6 count/min per microgram. A portion of the labeled DNA was heat denatured and renatured at 60°C overnight. The renatured DNA fragment was loaded on a 3 percent agarose gel and subjected to electrophoresis (gel 1) along with an Hae III digest of RSF2124 DNA (gel 2) at 60 volts for 3.5 hours. Gels were stained with ethidium bromide (1 μg/ml). Lengths of the Hae III fragments of RSF2124 are indicated in the figure. (B) Reassociation of end-labeled Eco RI fragment from a blunt-end repeat clone with excess sea urchin DNA. The 190 base pair end-labeled CS2108 fragment from (A) was eluted from the gel by dissolving in 5*M* $NaClO_4$ and binding the DNA to hydroxyapatite in 0.12*M* phosphate buffer (PB), followed by thermal elution of the ^{32}P-labeled DNA. This DNA was reassociated with excess 500 nucleotide long (5×10^5 mass ratio) sheared sea urchin DNA in 0.12*M* PB at 55°C. The fraction ^{32}P-labeled DNA (●) and driver DNA (▲) reassociated was determined by hydroxyapatite chromatography in 0.12*M* PB at 55°C. The solid line is the least-squares solution for the reaction of ^{32}P-labeled DNA driven by total sea urchin DNA. The rate constant determined for this reaction is $3.16 \times 10^{-2} M^{-1}$ sec^{-1}. The dashed line illustrates the reassociation of nonrepetitive sea urchin DNA with whole DNA (data not shown). The rate constant for this reaction is $1.13 \times 10^{-2} M^{-1}$ sec^{-1}. The dotted line shows the renaturation of total sea urchin DNA (■).

method described here provide a source from which we are able to obtain relatively large quantities of individual repetitive DNA sequences. Such sequences can be used to isolate all the related members of given repetitive sequence families from the genome. The method described can, of course, be used for cloning any DNA sequence without the requirement that it be terminated by specific restriction enzyme sites (6), and without the addition of substantial homopolymer sequences to the cloned fragment.

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References and Notes

1. This work was done in compliance with the NIH Guidelines for Recombinant DNA Research.
2. D. E. Graham, B. R. Neufeld, E. H. Davidson, R. J. Britten, *Cell* **1**, 127 (1974).
3. F. C. Eden, D. E. Graham, E. H. Davidson, R. J. Britten, *Nucleic Acids Res.*, in press.
4. T. Ando, *Biochim. Biophys. Acta* **114**, 158 (1966).
5. R. H. Scheller, R. E. Dickerson, H. W. Boyer, A. D. Riggs, K. Itakura, *Science* **196**, 177 (1977).
6. V. Sgaramella, J. H. Van de Sande, H. G. Khorana, *Proc. Natl. Acad. Sci. U.S.A.* **67**, 1468 (1970); H. L. Heyneker, J. Shine, H. M. Goodman, H. W. Boyer, J. Rosenberg, R. E. Dickerson, S. A. Narang, K. Itakura, S-Y. Lin, A. D. Riggs, *Nature (London)* **263**, 748 (1976); C. P. Bohl *et al.*, *Gene* **1**, 81 (1977).
7. M. So, R. Gill, S. Falkow, *Mol. Gen. Genet.* **142**, 239 (1975).
8. S. N. Cohen, C. Y. Chang, L. Hsu, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 2110 (1972).
9. P. Fredericq, *Annu. Rev. Microbiol.* **11**, 7 (1957).
10. A. M. Maxam and W. Gilbert, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 560 (1977).
11. T4 polynucleotide kinase, T4 DNA ligase, and Eco RI endonuclease were gifts of P. Green, H. Heyneker, and H. Boyer. Supported by NIH grants HD-05753 and GM-20927 and by NSF grant BMS 75-07359. R.H.S. is supported by an NIH predoctoral training grant, T.L.T. by an NIH postdoctoral fellowship; A.S.L. by fellowship 1-289 and W.H.K. by Lievre fellowship 1-340, both from the American Cancer Society, California Division.

Human Globin Messenger RNA: Importance of Cloning for Structural Analysis

Abstract. *The sequence of most of the human beta globin messenger RNA and large sections of the alpha globin messenger RNA has been determined. Partly because of genetic polymorphism, it was necessary to clone globin complementary DNA in order to extend the analysis. Purified human fetal globin messenger RNA was isolated and used as a template by reverse transcriptase to produce duplex complementary DNA molecules. These molecules were linked in vitro to plasmid DNA by use of T4 ligase in the presence of* Escherichia coli *Pol 1. Several colonies transformed by these molecules have been shown to hybridize with labeled human globin complementary RNA.*

By use of various RNA and DNA sequencing methods, we recently completed the nucleotide sequence of almost the entire translated and 3′ untranslated region of human beta globin messenger RNA (mRNA) (*1–3*). We are currently sequencing both the 3′ untranslated and the translated sequence of human alpha globin mRNA. To complete the analysis we found it necessary to proceed by use of gene cloning procedures, because base sequence analysis of an individual's globin mRNA may show the existence of genetic polymorphism. With the presence of heterogeneities, one cannot join specific base sequences in different regions. Cloning techniques permit one to obtain the large amounts of purified single-species DNA necessary for sequencing analysis.

Most of the mutations resulting in single amino acid substitutions that have been studied in human hemoglobin (Hb) can be accounted for by a single base change in the DNA. However, in beta globin mRNA's there are mutations that cannot be accounted for by such single base substitutions in a unique normal "ancestor" sequence. There are three amino acid codons in the beta globin mRNA for which protein or nucleic acid studies provide evidence for genetic polymorphism. One occurs at amino acid position 20. Normal globin has valine at this position [codon GUN (*4*)]. From RNA sequencing procedures described elsewhere (*5*), we have determined the nucleotide sequence of at least some of the molecules to be GUG (*1*). Amino acid substitutions for amino acid 20 in the Hb molecule have been found in the general population. One such substitution, Hb Olympia, has methionine (AUG) at position 20. Another, Hb Strasbourg, has aspartate (GAY) (*6*).

Table 1. Results of transformation experiments.

DNA species	Transformants after cycloserine selection
No DNA	0
Native PBR313 DNA	0
PBR313 DNA + Hb cDNA	0
PBR313 DNA + Hb cDNA + ligase	60

A second example of potential polymorphism in beta globin mRNA comes from amino acid substitution data at amino acid 67. Normal hemoglobin mRNA codes for valine (GUN); however, Hb Bristol mRNA codes for aspartate (GAY) and Hb M Milwaukee mRNA codes for glutamate (GAR). The third position of the codon for amino acid 67 could therefore be U or C as well as A or G. In our sequencing data, we find only GUG (*1–3*).

The third example of polymorphism in beta globin mRNA exists at codon position 50 (ACN), which codes for threonine. Here cDNA sequencing analysis from one patient indicated the codon to be ACU, whereas complementary RNA (cRNA) analyses of mRNA from another patient (*3*) were consistent with ACA. The amino acid substitution in Hb Edmonton ($Thr^{50} \rightarrow Lys$) suggests the presence of a purine (A or G) as the third base of this codon. These data also support the presence of genetic polymorphism in the third nucleotide position.

Since the alpha chain is known to be coded by duplicate loci per haploid set of chromosomes in a substantial portion of the population (*6*), one would expect more polymorphism in alpha mRNA than in beta mRNA. One such example is present in the untranslated region near the 3′ terminus. Partial venom diesterase digestion of a cDNA fragment from this region showed an interchange between two bases in approximately half the molecules (Fig. 1). We derived the following oligonucleotide sequence from these tracks: either CCCCUCCU or CCCUCCCU. The interchanged nucleotides are residues 60 and 61 beyond the termination codon (unpublished results). This heterogeneity has been found in at least two patients.

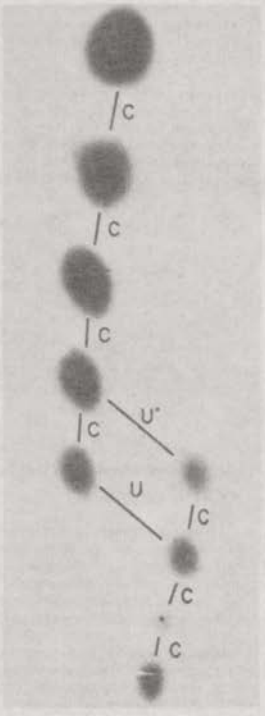
In addition to resolving the problems created by polymorphism, cloning procedures would make practical a number of other sequencing projects. For example, the 5′ terminal residues are not well represented in our current cDNA preparations and are therefore difficult to sequence. Cloning is also necessary to obtain sufficient amounts of purified minor hemoglobin species such as HbF (γ chain) and HbA_2 (δ chain), and for the deficient β mRNA in β thalassemia.

With the issuance of the Asilomar guidelines at the end of the voluntary moratorium, we decided to clone highly purified fetal globin cDNA, using procedures similar to those used successfully by others to clone rabbit globin cDNA (*7*, *8*) RNA of fetal red blood cells, from a newborn infant undergoing exchange transfusion, was purified by sucrose gradient centrifugation followed by oligo(dT) cellulose column chromatography and gel electrophoresis. Globin mRNA purified by this technique contains no detectable RNA species other than 10S globin mRNA when analyzed by polyacrylamide gel electrophoresis and nucleotide sequencing techniques (*2*).

Fetal human globin mRNA purified as described above was used as template for the synthesis of a synthetic DNA copy of the globin genes by a two-step procedure involving viral reverse transcriptase: (i) synthesis of a single-stranded cDNA copy of the mRNA in the presence of actinomycin D (*9*) (Fig. 2A); and (ii) after alkaline hydrolysis of the mRNA and isolation of the cDNA by Sephadex column chromatography, synthesis of a second strand complementary to the cDNA in a self-primed reaction (*8*, *10*), using essentially the same conditions as those used in the first synthetic step but without actinomycin D, oligo(dT), or mRNA (Fig. 2B). The second step resulted in the conversion of approximately 30 percent of the "full length" single-stranded cDNA into DNA with an electrophoretic mobility significantly lower than that of the initial product and consistent with a "hairpin" DNA molecule of the size of a double-stranded transcript of the globin mRNA (Fig. 2B) (*10*).

The resistance of the double-stranded cDNA obtained in step (ii) to degradation by S1 nuclease was 66 percent. Because of the evidence that such a cDNA is a hairpin structure (*8*, *10*), the double-stranded cDNA was then digested with S1 nuclease to degrade the hairpin and provide an "open" duplex DNA molecule suitable for joining to a plasmid. After digestion with S1 nuclease and polyacrylamide gel electrophoresis in the presence of 98 percent formamide, the cDNA preparation was found to migrate as a prominent peak with a mobility approximately equal to that of the original single-stranded cDNA; a number of less prominent and more rapidly migrating peaks were also present (data not shown).

For insertion of the fetal globin cDNA (HbF cDNA) into plasmid DNA, the DNA of plasmid pMB9 or pBR313 was cleaved with the restriction endonuclease



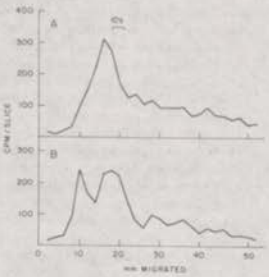


Fig. 1 (left). Autoradiographs of two-dimensional fractionation of partial snake venom phosphodiesterase (SVP) digest of a fragment of Hb cDNA. The fragment labeled at the 5′ terminus was digested with SVP (*3*) and fractionated by electrophoresis from left to right at pH 3.5 and homochromatography from the bottom upward as described in (*3*). The oligonucleotide map shows the possibility of two sequences: CCCUCCC and CCCCUCC. Fig. 2 (above). Polyacrylamide gel electrophoresis of human globin cDNA used in cloning experiments. Polyacrylamide gel electrophoresis was carried out in the presence of 98 percent formamide as previously described (*9*). The disc gels were sliced at 2-mm intervals, and the radioactivity of each slice was determined as described (*9*). The bracket indicates the position of 10S globin mRNA run on a parallel gel and detected by staining with methylene blue. (A) Single-stranded cDNA initially synthesized from globin mRNA in the presence of [^{3}H]deoxycytidine triphosphate and actinomycin D. (B) Double-stranded cDNA obtained by incubating the single-stranded [^{3}H]cDNA in (A) with avian myeloblastosis virus DNA polymerase, using only nonradioactive deoxynucleotide triphosphates in a self-primed reaction without the addition of mRNA, oligo(dT), or actinomycin D; *CPM*, counts per minute.

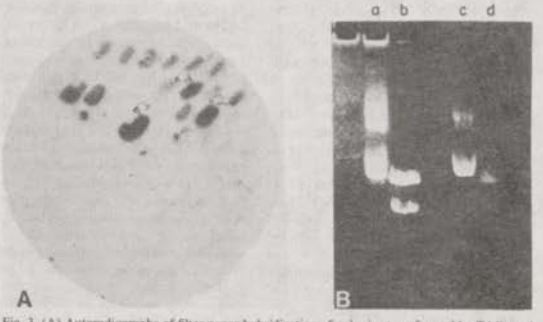


Fig. 3. (A) Autoradiographs of filter paper hybridization of colonies transformed by T4-ligated plasmid–HbF cDNA. In situ hybridization was performed with ^{32}P-labeled cRNA as described in the text. Dark areas represent transformed colonies hybridizing to Hb cRNA. (B) Electrophoresis of potential hybrid DNA recombinants. Columns a and c represent plasmid DNA (form I and form II) isolated from transformed colonies; columns b and d, pBR313 plasmid DNA (form I and form II).

Eco RI or Bam, respectively. The linear plasmid DNA was mixed with double-stranded HbF cDNA and the mixture was treated with S1 nuclease, followed by T4 ligase and *Escherichia coli* DNA polymerase I. The ligated DNA's were used to transform *E. coli* strain HB101. We then looked for colonies containing hybrid DNA molecules by selecting for antibiotic resistance (pMB9) or, as in the case of pBR313, using the cycloserine selection procedure developed by Bolivar *et al.* (*11*), followed by in situ hybridization. Table 1 shows the results of such a transformation. No transformants were obtained when *E. coli* cells were transformed with closed circular pBR313 DNA. Neither were transformants obtained when cells were incubated with plasmid DNA converted to linear form by enzymatic cleavage with Bam and digested with S1 nuclease. However, the mixture of plasmid and HbF cDNA which had been treated with T4 ligase gave rise to 60 transformants.

Hemoglobin mRNA from patients with sickle cell anemia was used to synthesize Hb cDNA. This cDNA was then used as a template for the production of ^{32}P-labeled sickle cell cRNA for use as a hybridization probe. Transformed colonies were lysed, fixed onto nitrocellulose filters (*12*), and hybridized with the ^{32}P-labeled cRNA. After hybridization, filters were audioradiographed. Repeated hybridizations showed that several colonies hybridized to the ^{32}P-labeled Hb cRNA (Fig. 3A).

Plasmid DNA was isolated from two colonies that hybridized to [^{32}P]cRNA. These plasmid DNA's were electrophoresed on a 0.7 percent agarose gel with pBR313 DNA (Fig. 3B). On this gel, plasmid DNA's from transformed colonies showed slower mobilities. This indicates plasmids of larger molecular weight, as one would expect after insertion of Hb cDNA into the plasmid.

With the issuance of the National Institutes of Health guidelines, further characterization of these colonies and plasmid DNA's was postponed until the certification of a EK2 host vector system and the official certification of a P3 facility. However, this research was conducted in a limited-access laboratory with negative pressure. All microbiology was done in that laboratory, in a special biocontainment hood meeting the specifications of the NIH guidelines.

During the period when this research was conducted, the laboratory was monitored twice weekly for containment of the host bacteria. At no time was any evidence found for escape of the bacteria into the laboratory environment. Fecal samples were taken throughout the study and found to be free of resistance markers used in this study.

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References and Notes

1. C. A. Marotta, B. G. Forget, M. Cohen-Solal, J. T. Wilson, S. M. Weissman, *J. Biol. Chem.*, in press.
2. M. Cohen-Solal, B. G. Forget, W. Prensky, C. A. Marotta, S. M. Weissman, *ibid.*, in press.
3. C. A. Marotta, J. T. Wilson, B. G. Forget, S. M. Weissman, *ibid.*, in press.
4. For base residue assignments, N signifies any one of the common deoxynucleotide triphosphates, R a purine residue, and Y a pyrimidine. Other abbreviations used are G, guanine; U, uridine; A, adenine; and C, cytosine.
5. B. G. Forget, C. A. Marotta, S. M. Weissman, I. M. Verma, R. P. McCaffery, D. Baltimore, *Ann. N.Y. Acad. Sci.* **241**, 290 (1974); C. A. Marotta, B. G. Forget, S. M. Weissman, I. M. Verma, R. P. McCaffrey, D. Baltimore, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 2300 (1974); B. DuBuy and S. M. Weissman, *J. Biol. Chem.* **246**, 747 (1971); B. G. Forget and S. M. Weissman, *ibid.* **244**, 3148 (1969); C. A. Marotta, F. Varricchio, I. Smith, S. M. Weissman, M. L. Sogin, N. R. Pace, *ibid.* **251**, 3122 (1976); G. G. Brownlee, *Determination of Sequences in RNA* (Elsevier, New York, 1972).
6. H. F. Bunn, B. G. Forget, H. M. Ranney, *Human Hemoglobin* (Saunders, Philadelphia, 1977).
7. T. Maniatis, S. G. Kee, A. Efstratiadis, F. C. Kafatos, *Cell* **10**, 163 (1976); T. H. Rabbits, *Nature (London)* **260**, 221 (1976); F. Rougeon, P. Kourilsky, B. Mach, *Nucleic Acid Res.* **2**, 2365 (1976); K. O. Wood and J. C. Lee, *ibid.* **3**, 1961 (1976).
8. R. Higuchi, G. U. Paddock, R. Wall, W. Salser, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3146 (1976).
9. B. G. Forget *et al.*, *Cell* **7**, 323 (1976).
10. F. C. Kafatos, A. Efstratiadis, A. M. Maxam, T. Maniatis, *ibid.*, p. 279.
11. F. Bolivar *et al.*, in preparation.
12. M. Grunstein and D. S. Hogness, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3961 (1975).
13. We thank R. Milardo for technical assistance and J. Allen and B. Holloman for their helpful advice.

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Cloned Ribosomal RNA Genes from Chloroplasts of *Euglena gracilis*

Abstract. *Fragments of* Euglena *chloroplast DNA generated by endonuclease R· Eco RI were separated by agarose-gel electrophoresis into 24 distinct bands. At least five fragments contain sequences complementary to chloroplast ribosomal RNA. Most of the Eco RI fragments have been cloned in a plasmid of* Escherichia coli. *Three of the cloned fragments were shown to contain chloroplast ribosomal RNA sequences by DNA-RNA hybridization.*

Chloroplasts from the unicellular alga *Euglena gracilis* contain multiple copies of a circular, double-stranded DNA molecule (Chl-DNA) whose molecular weight is about 92×10^6 (*1*). These chloroplasts also contain ribosomes with sedimentation values similar to those of prokaryotic ribosomes (*2*, *3*), and hybridization of chloroplast ribosomal RNA (Chl-rRNA) to Chl-DNA shows that Chl-DNA contains about two to three rRNA cistrons per chloroplast genome (*3*, *4*). The existence of DNA and a protein-synthesizing apparatus in chloroplasts poses some of the most intriguing problems of modern cell biology. How did these organelles arise, and what are their genetic capabilities? How does the nuclear genome interact with the cytoplasmic genome to regulate the biogenesis of the organelle and its energy-producing reactions? Recombinant DNA methodology provides an approach to answer these questions, for it enables us to dissect the chloroplast genome and to determine the genes contained on specific segments.

As a first step toward understanding the organization of chloroplast genes and their functions, we have cloned most of the chloroplast DNA as discrete fragments inserted in a set of RSF2124 plasmids of *Escherichia coli*. These fragments were generated by treatment of Chl-DNA with the restriction endonuclease Eco RI (*5*) and were identified by agarose-gel electrophoresis, which separates DNA molecules according to size (*6*). The purpose of this report is to show that molecular cloning permits unambiguous identification of unique Chl-DNA fragments containing Chl-rRNA genes.

Electrophoresis of Eco RI–digested Chl-DNA on agarose gels yields 24 distinct bands (Fig. 1a). We were able to separate several bands of similar mobility which had not been resolved (*4*, *7*). In particular, bands D and E can be separated, and therefore were given individual letter designations. However, bands I, M, and W each contain two DNA fragments so nearly identical in size that we were unable to separate them. These bands are designated I_1, I_2, and so forth. The staining intensity of these bands indicates that each contains more than one

Table 1. Hybridization of plasmid DNa with chloroplast rRNA. Preparation of ^{125}I-labeled rRNA and its hybridization to DNA on filters was performed as described (*13*). Hybridization values are the averages of duplicate filters and are corrected for adsorption of rRNA to blank filters. Duplicate filters in the hybridization vials were placed between blank filters. A zero value here indicates that the amount of radioactivity on the test filters fell within the range of that bound to the blank filters. Other values represent 1.3 to 9.3 times as much radioactivity bound to test filters as to blank filters. Experiments were repeated with different preparations of ^{125}I-labeled rRNA, and specific activities ranged from 9.6×10^8 to 1.6×10^9 count/min per microgram. Band N DNA has not been cloned, and therefore it is not represented.

Plasmid	Chloroplast DNA band	DNA per filter (μg)	[^{125}I]rRNA hybridized per filter (ng)	Plasmid	Chloroplast DNA band	DNA per filter (μg)	[^{125}I]rRNA hybridized per filter (ng)
pML10	J	0.41	0	pML22	I + O	0.32	1.58
		0.82	0	pML15	L + O	0.72	1.8
		1.9	0	pML14	O + S + (V or W_1 or W_2)	0.40	0.75
pML19	K	0.30	0.82	pML18	O + (T or U) + X	0.29	1.26
		0.60	3.85	pML35	P	1.9	0
pML31	K	0.27	1.03	pML17	P + R + W(W_1 or W_2)	0.45	1.23
		0.54	1.28			1.35	1.84
pML21	L	0.33	0			5.7	4.79
		0.66	0	pML20	S + (T or U)	0.16	0
		1.1	0			0.31	0
pML23	M	2.3	0	pML26	T or U	0.63	0
pML25	O	0.19	1.35	pML28	W(W_1 or W_2)	0.18	0
pML12	O	0.52	3.5			0.35	0
				pML34	W(W_1 or W_2) + X	1.23	0

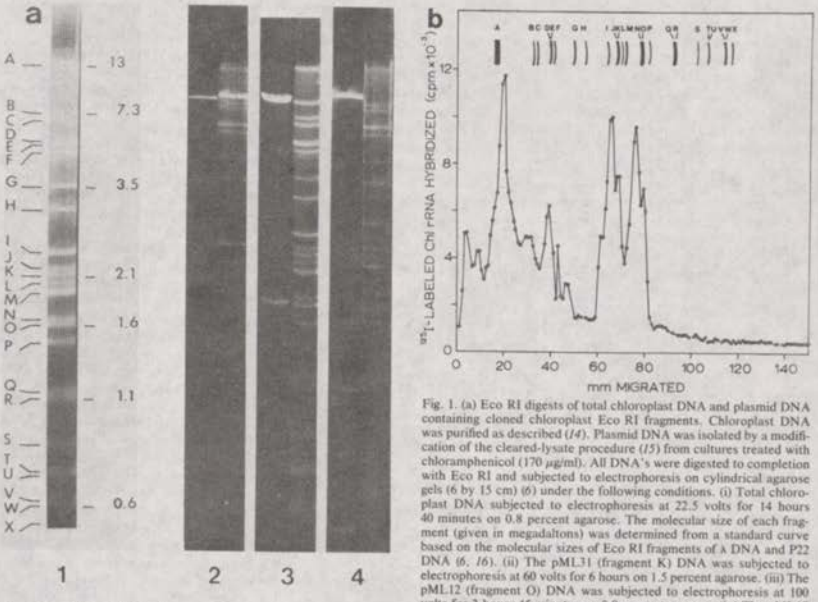


Fig. 1. (a) Eco RI digests of total chloroplast DNA and plasmid DNA containing cloned chloroplast Eco RI fragments. Chloroplast DNA was purified as described (*14*). Plasmid DNA was isolated by a modification of the cleared-lysate procedure (*15*) from cultures treated with chloramphenicol (170 μg/ml). All DNA's were digested to completion with Eco RI and subjected to electrophoresis on cylindrical agarose gels (6 by 15 cm) (*6*) under the following conditions. (i) Total chloroplast DNA subjected to electrophoresis at 22.5 volts for 14 hours 40 minutes on 0.8 percent agarose. The molecular size of each fragment (given in megadaltons) was determined from a standard curve based on the molecular sizes of Eco RI fragments of λ DNA and P22 DNA (*6*, *16*). (ii) The pML31 (fragment K) DNA was subjected to electrophoresis at 60 volts for 6 hours on 1.5 percent agarose. (iii) The pML12 (fragment O) DNA was subjected to electrophoresis at 100 volts for 2 hours 45 minutes on 0.8 percent agarose. (iv) The pML17 (fragments P, R, and W) DNA was subjected to electrophoresis as for gel 2. In gels 2 and 3, the left gel contains plasmid DNA alone and the right gel contains plasmid plus chloroplast DNA. In gel 4, the left gel contains only plasmid DNA and the right gel, only chloroplast DNA. (b) Hybridization of chloroplast ^{125}I-labeled rRNA with Eco RI fragments of chloroplast DNA. Eco RI–digested chloroplast DNA (5 μg) was subjected to electrophoresis on an 0.8 percent agarose slab gel (*6*). DNA fragments from the agarose gels were transferred to a Millipore membrane filter strip (0.45 μm HAWP) by the procedure of Southern (*8*). The Millipore strip containing the DNA fragments was hybridized in 3 ml of solution containing 10 μg of ^{125}I-labeled Chl-rRNA in 2 × SSC plus 0.1 percent sodium dodecyl sulfate (SSC is 0.15*M* sodium chloride and 0.015*M* sodium citrate, *p*H 7.0). After hybridization and ribonuclease treatment, strips were cut into 1-mm pieces and counted in a scintillation counter. Abbreviation: *cpm*, counts per minute.

fragment. Densitometry measurements confirmed this (not shown). In addition, band A probably contains two fragments. Stutz *et al.* were able to resolve two bands of DNA in the molecular weight range of 13×10^6 to 15×10^6 (*3*). On this basis, the sum of the molecular weights of the identified Eco RI fragments accounts for 97 percent of the molecular weight of the intact Chl-DNA.

The DNA fragments were transferred from a slab gel to a membrane filter strip by the procedure of Southern (*8*) and hybridized with ^{125}I-labeled Chl-rRNA. The strips were cut into 1-mm sections, and the radioactivity associated with each section was determined. Ribosomal RNA hybridizes with the DNA of bands A; with D, E, or F; and with several bands in the set J through P (Fig. 1b). By autoradiography of the hybridized filter strips, the radioactive hybrids can be located more precisely in specific DNA bands (*4, 8*). Using this procedure, Stutz *et al.* concluded that bands equivalent to A, D or E, K or L, and O or P contained DNA that hybridizes with rRNA (*4*). It was not possible to determine more precisely which DNA fragments contained partial or complete rRNA genes, because many of the DNA fragments are similar in size (and hence poorly separated on gels). Therefore, we tested DNA from plasmids containing unique fragments of Chl-DNA. In order to demonstrate the utility and sensitivity of the method, we concentrated on the DNA fragments in bands J through P.

Eco RI–generated Chl-DNA fragments were randomly inserted into the single Eco RI site of plasmid RSF2124 (*9*), a derivative of Co1 E1, which contains a gene conferring resistance to ampicillin. The *E. coli* K12 strain M94 (*thr leu thi hsd*) was transformed with the ligated DNA preparation (*10*). Ampicillin-resistant transformants that contained added DNA were identified by their failure to produce colicin E1 (*11*). The specific fragment (or fragments) of Chl-DNA present in a particular plasmid was determined by electrophoresis of the Eco RI–digested plasmid DNA (for example, see Fig. 1a).

Purified DNA from recombinant plasmids containing Eco RI–generated fragments J through P was immobilized on nitrocellulose filters. The bound DNA was hybridized with ^{125}I-labeled Chl-rRNA to test for the presence of rRNA genes (rDNA). The results are shown in Table 1. Chloroplast rRNA hybridized with DNA from plasmids pML19 and pML31, which contain band K DNA, but did not hybridize with DNA from plasmids pML10, pML21, or pML23, which contain Eco RI fragments J, L, or M(M_1 or M_2), respectively. The rRNA also hybridized with DNA from every plasmid that contains fragment O. Plasmid pML17 DNA contains three fragments of Chl-DNA: P, R, and W. The rRNA bound to pML17 DNA, but not to pML35 DNA which contains fragment P alone. Therefore, rDNA must be present in fragment R or W(W_1 or W_2) DNA. Neither of these fragments appeared to hybridize when Eco RI–digested whole Chl-DNA was hybridized with Chl-rRNA (Fig. 1b). This may be due to the decreased efficiency of hybridization of DNA fragments of molecular weight less than 10^6 (*12*), which are not retained well by the nitrocellulose filter. The hybridization observed with the cloned pML17 DNA was not an artifact resulting from the use of smaller molecular weight DNA segments, because plasmids pML26, pML28, pML20, and pML34 also containing smaller molecular weight DNA did not bind rRNA (Table 1).

Two types of experiments were performed to verify the specificity of the hybrids between Chl-rRNA and Chl-DNA. First, competition experiments with each DNA fragment binding rRNA showed that the binding of label from ^{125}I-labeled rRNA was reduced from 50 to 86 percent by the presence of a tenfold excess of unlabeled rRNA, indicating that both compete for the same DNA binding site. (In some cases, saturating levels of rRNA were not achieved, thus competition was not always complete.) Second, we determined the thermal stability of the three DNA-rRNA hybrids (Fig. 2). Each of the hybrids melted (T_m) over a narrow temperature range, a feature characteristic of true RNA-DNA hybrids, and the T_m's, in 0.1 × SSC (saline, sodium citrate), are consistent with those expected for rRNA-DNA hybrids (*3, 13, 14*). The small but reproducible differences in T_m observed with different plasmid DNA hybrids probably reflects differences in the nucleotide sequences of rDNA in a particular hybrid.

In these experiments, we have identified three Eco RI fragments of *Euglena* chloroplast DNA that contain portions of the genes for chloroplast rRNA, by linking these fragments to the plasmid RSF2124 and testing the resulting plasmid DNA's for hybridization with Chl-rRNA. Chloroplast rRNA contains two major molecular species of 1.1×10^6 and 0.55×10^6 daltons (*3*). In our experiments, total rRNA containing both species was used, and, consequently, hybridization to a given fragment may re-

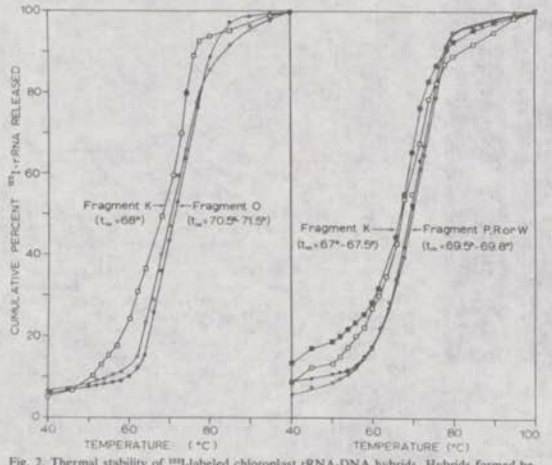


Fig. 2. Thermal stability of ^{125}I-labeled chloroplast rRNA-DNA hybrids. Hybrids formed between ^{125}I-labeled rRNA and plasmid DNA were thermally dissociated in 0.1 × SSC (*13*). Fragment K came from pML19; fragment O, from pML12; and fragments P, R, and W, from pML17. The calculated T_m (in 0.1 × SSC) for a DNA-DNA hybrid with a G + C content of 52 percent is 75.2°C (*17*). The observed T_m's for the rRNA-DNA hybrid for fragment O are 4° to 5°C lower, in good agreement with the previously reported decreased stability of the corresponding RNA-DNA hybrids (*18*).

flect the presence of cistrons for either the 23S or the 16S rRNA, or both. We also found in hybridization studies with an Eco RI digest of total Chl-DNA that at least two other fragments (A and D or E) of Chl-DNA contain rRNA sequences. These fragments can now be used to construct a physical map of the chloroplast genome and to locate precisely the position and orientation of the rRNA cistrons. Furthermore, other genes, such as those for chloroplast transfer RNA cistrons, can be identified by similar hybridization experiments.

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References and Notes

1. J. E. Manning and O. C. Richards, *Biochim. Biophys. Acta* **259**, 285 (1972); N. Slavik and C. L. Hershberger, *FEBS. Lett.* **52**, 171 (1975).
2. S. D. Schwartzbach, G. Freysinnet, J. A. Schiff, *Plant Physiol.* **53**, 533 (1974).; D. Cohen and J. A. Schiff, *Arch. Biochem. Biophys.* **177**, 201 (1976).
3. D. J. Graot and R. Haselkorn, *Biochim. Biophys. Acta* **447**, 82 (1976).
4. E. Stutz, E. J. Crouse, L. Graf, B. Jenni, H. Kopecka, in *Proceedings of Genetics and Biogenesis of Chloroplasts and Mitochondria*, T. Bucher, Ed. (North-Holland, Amsterdam, in press).
5. J. Hedgepeth, H. M. Goodman, H. W. Boyer, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 3448 (1972).
6. R. B. Helling, H. M. Goodman, H. W. Boyer, *J. Virol.* **14**, 1235 (1974).
7. R. B. Hallick, C. Lipper, O. C. Richards, W. J. Rutter, *Biochemistry* **15**, 3039 (1976).
8. E. M. Southern, *J. Mol. Biol.* **98**, 503 (1975).
9. S. N. Cohen, A. C. Y. Chang, H. W. Boyer, R. B. Helling, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 3240 (1973); M. So, R. Gill, S. Falkow, *Mol. Gen. Genet.* **142**, 234 (1975).
10. E. M. Lederberg and S. N. Cohen, *J. Bacteriol.* **119**, 1072 (1974).
11. V. Hershfield, H. W. Boyer, C. Yanofsky, M. A. Lovett, D. R. Helinski, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 3455 (1974).
12. M. Melli and J. O. Bishop, *Biochem. J.* **120**, 225 (1970).
13. S. D. Schwartzbach, L. I. Hecker, W. E. Barnett, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1984 (1976).
14. L. Stolarsky, N. Slavik, C. L. Hershberger, *Biochim. Biophys. Acta* **335**, 30 (1973).
15. P. Guerry, D. J. LeBlanc, S. Falkow, *J. Bacteriol.* **116**, 1064 (1973).
16. R. Dean and E. N. Jackson, unpublished work.
17. M. Mandel and J. Marmur, *Methods Enzymol.* **12B**, 195 (1968).
18. D. E. Kohne, *Biophys. J.* **8**, 1104 (1968); E. H. Davidson and B. R. Hough, *J. Mol. Biol.* **56**, 491 (1971); R. A. Firtel and J. Bonner, *ibid.* **66**, 339 (1972).
19. All experiments involving recombinant DNA molecules were carried out under P2-level containment conditions, as specified in "Guidelines for research involving recombinant DNA molecules" [*Fed. Regist.* **41** (No. 131), 27913 (1967)].
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Cloning of Yeast Transfer RNA Genes in *Escherichia coli*

Abstract. *Four thousand* Escherichia coli *clones containing yeast DNA inserted into the plasmid pBR313 have been isolated. Of these, 175 clones were identified as carrying yeast transfer RNA genes. The initial analysis of the inserted transfer RNA genes via the colony hybridization technique with individual radioactive transfer RNA species is reported. The data indicate that yeast transfer RNA genes are not highly clustered, although some clustering exists. In addition, it was observed that the reiteration number of different transfer RNA genes may vary extensively.*

In order to study the principles of gene organization and expression in yeast we have undertaken a project to isolate and characterize yeast transfer RNA (tRNA) genes. There are several reasons for choosing yeast tRNA genes as a system for studying gene organization and expression. The gene products have been well characterized; most yeast tRNA's have been purified, and many sequenced (*1*). As in *Escherichia coli*, nonsense suppressors have been isolated (*2*), and it has been demonstrated that these suppressor genes are the structural genes of tRNA (*3*, *4*). Thus, there is extensive information available on the genes and gene products for this class of molecules. Recombinant DNA technology now supplies us with a method for isolating the tRNA genes in pure form. We report here our initial efforts to collect and catalog the yeast tRNA genes.

The first step was to obtain an adequate number of *E. coli* clones carrying random fragments of yeast DNA (the "shotgun experiment"). These clones were then screened by hybridization to identify those containing yeast tRNA genes. This

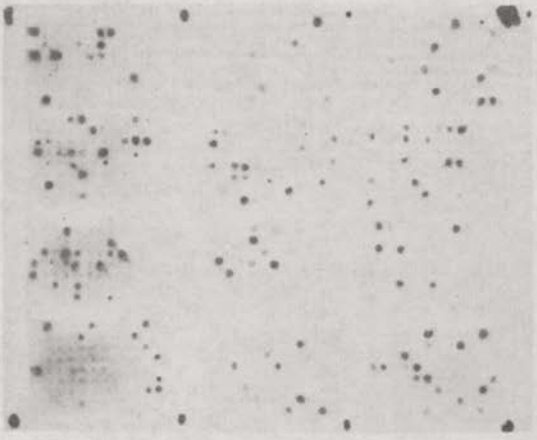


Fig. 1. Screening for recombinant clones carrying yeast tRNA genes. One thousand one hundred and forty clones carrying endo R Hind III restricted fragments of the yeast genome inserted into pBR313 were transferred to 12 filters (95 clones per filter) and assayed for yeast tRNA genes by hybridization with in vivo ^{32}P-labeled 4S RNA. Labeling and extraction of stable RNA's were performed according to Rubin (*12*). The RNA was subsequently separated on a 10 percent polyacrylamide gel in 4*M* urea. The positions of 4S, 5S, and 5.8S RNA were revealed by autoradiography. Appropriate bands were cut out, and the RNA's were eluted in 0.3*M* NaCl and purified by DEAE-cellulose column chromatography. We have modified the colony hybridization procedure developed by Grunstein and Hogness (*11*). The candidate clones were replica-plated in an 8 by 12 cm matrix onto 15-cm (diameter) petri plates (95 clones per dish). After an overnight growth period, a sheet of Whatman 540 filter paper was pressed onto the plate and removed (most of the bacteria adhere to the filter). The filter was then treated with 0.5*M* NaOH, neutralized, dried, and used in the hybridization test. We have routinely used 10^5 to 10^6 count/min of purified labeled RNA per filter. However, when total 4S RNA was used the hybridization solution contained 10^6 to 10^7 count/min per filter. Hybridization was for 12 to 18 hours at 37°C in a solution of 50 percent formamide, 0.6*M* NaCl, 0.06*M* sodium citrate, *p*H 7.0, and 0.5 percent sodium dodecyl sulfate. The filters were then washed, treated with ribonuclease (optional), and autoradiographed.

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subcollection of clones was then further screened with pure tRNA species to identify clones carrying particular tRNA genes.

The vehicle used in these experiments was the plasmid pBR313 (5). This plasmid is a derivative of Col E1 carrying antibiotic resistance genes for tetracycline (tet^r) and ampicillin (amp^r). Foreign DNA can be inserted by ligation into this plasmid (6) at a number of single restriction sites. In our experiments we used endo R Hind III (7) restriction endonuclease to cleave the plasmid DNA. This enzyme cleaves the circular plasmid DNA at a single site in the tet^r gene (5). Yeast DNA was purified from *Saccharomyces cerevisiae* strain X2180 1A (8) and cleaved with endo R Hind III. The yeast DNA fragments were annealed and ligated to Hind III cleaved pBR313 DNA to produce a random collection of hybrid DNA molecules (6). These recombinant plasmids were introduced (9) into *E. coli*

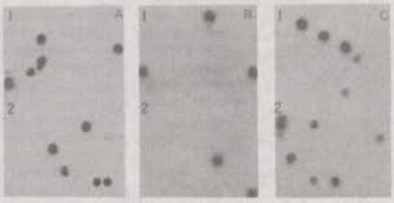


Fig. 2. Screening for specific tRNA genes among 4S-containing clones. The 175 clones that were shown to hybridize with ^{32}P-labeled 4S RNA (see Fig. 1) were tested for their ability to anneal different purified tRNA species. The in vivo labeled 4S RNA's were separated by two-dimensional polyacrylamide gel electrophoresis (3). Individual spots were selected by autoradiography, eluted, and used in our hybridization screening. The hybridization to three individual tRNA samples (T1, panel A; T2, panel B; T3, panel C) is shown here. T1, T2, and T3 were later identified by their ribonuclease T1 two-dimensional oligonucleotide maps as $tRNA_3^{Leu}$ (14), $tRNA_{UUA}^{Leu}$ (15), and $tRNA_2^{Tyr}$ (16), respectively.

Table 1. Catalog of the 4S clones. All 175 clones containing 4S genes obtained by a series of 32 independent transformations are listed. The identity of the various purified tRNA species found to hybridize with each clone is indicated. Screening for the various tRNA species is described in Fig. 2, with the use of either individual 4S spots obtained by two-dimensional separation of in vivo labeled tRNA (3) or by incubating purified tRNA species with bacteriophage T4 polynucleotide kinase and [γ-^{32}P]ATP (13). These tRNA's were the gifts from several laboratories (Table 2). In several cases, the identity of the in vivo labeled tRNA samples eluted from two-dimensional gels could be ascertained by their ribonuclease T1 patterns or by the coincidence of their hybridization patterns to the 175 clones with a known purified tRNA species. The spots T1, T2, and T3 and R8, R10, and R11 were shown by oligonucleotide mapping, respectively, to be tRNA's specific for Leu_3, Leu_{UUA}, Ser_2, Ala, Tyr, and Phe; T9 was not analyzed but did cohybridize with the same clones as $tRNA^{Leu}$. Abbreviations for the tRNA species are as follows: Asp, aspartic acid; Arg, arginine; Gly, glycine; Leu, leucine; Phe, phenylalanine; Ser, serine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; subscripts indicate individual tRNA species.

Clone	tRNA species	Clone	tRNA species	Clone	tRNA species	Clone	tRNA species	Clone	tRNA species
1-i	Thr	7-j	$T2(Leu_{UUA})$	l		n		d	
		m	Gly	n		q	Val_2	k	
2-b	Val_2			o	$T3(Ser_2)$	s		o	
c	Asp	8-a	Thr	p		u	Arg_3, Tyr, Asp		
e	Thr	c	Thr	q	T8			25-l	
f	Thr	e	$T2(Leu_{UUA})$, T9	s	Phe, R8(Ala)	19-b		p	Val_2
j		f	Asp			f	Arg_3, Asp		
k	Leu_3	j		12-a		g	T9	26-d	R8(Ala)
m	Leu_3	k		d	Leu_3	i	T15	f	Val_2
p	Thr	l		e	Asp	k	T15	h	T9
r	Val_2	n		o		m	Leu_3	27-a	
		p	Asp	p		n	T8	b	$T3(Ser_2)$
3-b		q		q	Asp	r		d	
d	Gly, T9	s	T8			t		g	
h		u	Leu_3	13-a				j	
i	Val_2			14-c	T9	20-b	Trp	28-b	
p	R8 (Ala)	9-d	T9	d		f	T9	c	T9
q	T15	e	T9	e	T15	g		e	
		g	$T3(Ser_2)$	g		m	Trp	i	Asp
4-a	Leu_3	k	T15	k	R8(Ala)	n	$T3(Ser_2)$		
d		l				o		29-e	
c		n		15-a				i	T15
g				c		21-d	Arg_3, Asp	l	Val_2, T16
i		10-b		d		k		n	Arg_3
j		e		g	$T2(Leu_{UUA})$	n	Arg_3, Asp	p	
k	R8 (Ala)	f		h	T9	22-c		q	$T2(Leu_{UUA})$
l		g				f		r	Arg_3, T9
o		h	Tyr	16-h		g			
		i		17-a		h		30-e	
5-a	Asp	l		c	Leu_3	i		g	T15
e	Phe	m	Arg_3, Asp	d				h	Gly
f	Gly	p	Arg_3	f	Asp	23-a	T9	i	R6
i	R8 (Ala), T16	q	$T2(Leu_{UUA})$	h	T9	f	Leu_3	l	T9
j				p	T9	g			
		11-d	Thr			h	Gly	31-l	Asp
6-a	Leu_3	f	Leu_3	18-c		k	Tyr		
c		g	$T3(Ser_2)$	f		l		32-e	
g		h		g				f	T16
h	Tyr	j		k		24-b	Asp	g	T16
l	Gly	k		l		c	T15	h	T16

strain C600 SF8 (*10*) by transformation (*6*). After addition of the DNA the transformation mixture was divided into a number of separate growth tubes in order to minimize the chances of obtaining sibling clones. The transformation mixtures were plated on ampicillin medium to select for clones carrying the plasmid. From 32 independent transformations, 12,000 ampicillin-resistant clones were isolated. Four thousand of these clones were tetracycline sensitive, indicating that foreign DNA had been inserted into the *tet*^{r} gene.

This collection of 4000 clones containing yeast DNA was screened by the colony hybridization technique [(*11*) and legend to Fig. 1] to detect those containing sequences complementary to 4*S*, 5*S*, or 5.8*S* RNA. In these experiments the RNA probes were labeled in vivo by growing yeast in medium containing [^{32}P]orthophosphate (*12*). The labeled RNA was purified by polyacrylamide gel electrophoresis (*3*). One hundred and seventy-five clones hybridized specifically with 4*S* RNA, 122 hybridized with 5*S* RNA, and 121 with 5.8*S* RNA. The 5*S* and 5.8*S* clones also hybridized with 4*S* RNA, but this hybridization was due to the presence of contaminating ribosomal RNA sequences in our 4*S* RNA and was not due to tRNA. Figure 1 illustrates the detection of clones hybridizing with 4*S* RNA.

The collection of 175 clones hybridizing to 4*S* RNA was then screened with purified tRNA's. This process is not yet complete, but the results obtained with 18 different species of tRNA give us a preview of the form the final catalog will take. Prior to the final screening process each clone was purified and retested. Two grids were formed containing the entire 4*S* collection (Fig. 2).

Pure yeast tRNA's were obtained in two ways. The tRNA was labeled with ^{32}P in vivo (*12*), and the individual species were purified by two-dimensional polyacrylamide gel electrophoresis (*3*). The purity and, in some cases, the identity of these tRNA's was ascertained by mapping the T1 ribonuclease products of each species. In this way 12 pure species of yeast tRNA have been isolated.

In addition, we have been fortunate in obtaining a number of pure yeast tRNA's from our colleagues (Table 2). These unlabeled tRNA's can be made radioactive by incubation with [γ-^{32}P]ATP and bacteriophage T4 polynucleotide kinase (*13*). In this way we have obtained ten pure tRNA species. In several cases these proved to be identical to members of the first set so that in all we have screened our set of 4*S* clones with 18 different species of pure yeast tRNA. The acceptor activity of six of these is unknown. Figure 2 gives an example of the detection of 4*S* clones hybridizing to a pure species of tRNA. In this case the tRNA's were purified by two-dimensional polyacrylamide gel electrophoresis (*3*). The results with three pure species of tRNA are shown. These were called T1, T2, and T3, but further analysis showed that they were, respectively, $tRNA_a^{Leu}$ (*14*), $tRNA_{UCA}^{Leu}$ (*15*), and $tRNA_a^{Ser}$ (*16*). Figure 2 shows that there are ten clones hybridizing with $tRNA_a^{Leu}$ (panel A), 5 clones hybridizing with $tRNA_{UCA}^{Leu}$ (panel B), and 11 clones hybridizing with $tRNA_a^{Ser}$ (panel C). In panel C there are five dark spots and six light ones. We assume that the light spots are due to a contaminating RNA species in the $tRNA_a^{Ser}$ preparation, but there are several other possible explanations that must be ruled out.

The results of hybridization tests with all 18 pure tRNA probes are given in Tables 1 and 2. Of the 175 clones in the 4*S* collection, 97 hybridize to at least one of the tRNA's. Ten of the clones hybridize to two tRNA's and one clone to three different tRNA species (18u, Table 1). Note that a possible cluster of Arg_b and Asp tRNA genes occurs four times (Table 2).

These results suggest some tentative conclusions regarding the organization of tRNA genes in the yeast genome. The average molecular size of an endo R Hind III fragment is about 2.5×10^6 daltons. Thus each fragment represents $^1/_{1000}$ of the yeast haploid genome (about 10^{10} daltons) (*17*). We have isolated 4000 clones at random. Hybridization studies have shown that there are 360 tRNA genes per yeast haploid genome (*18*). If these genes are widely spaced we would have expected to isolate by chance about 360 clones containing one tRNA species each (although in our collection the chance of obtaining a particular tRNA gene is $1\text{-}e^{-1}$ or 0.63). On the other hand, if tRNA genes are tightly clustered we would have detected far fewer 4*S* clones. Our results suggest that the tRNA genes in yeast are not tightly clustered. If our collection of 175 clones contains 360 tRNA genes, on the average, there will be two genes per clone. The possibility that yeast tRNA genes are widely spaced had already been suggested from the genetic mapping of tyrosine inserting suppressors. These suppressors are unlinked and often map on separate chromosomes (*19*). Clarkson and collaborators (*20*) have studied the organization of tRNA genes in

Table 2. Frequency of occurrence of the different tRNA genes. The data reported under Table 1 are summarized: column 1 lists the total number of clones hybridizing to each tRNA. Column 2 represents the minimum number of independently obtained clones for each tRNA, computed by subtracting any possible siblings. Also listed are the clones that react with more than one of the tRNA's tested, and their frequency of occurrence. We gratefully acknowledge the various donors of the different purified tRNA species as indicated for each tRNA. Abbreviations for the tRNA species are as follows: Ala, alanine; Asp, aspartic acid; Arg, arginine; Gly, glycine; Leu, leucine; Phe, phenylalanine; Ser, serine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; Val, valine.

Item	Number of clones found	Minimum independent number
A. tRNA species		
Ala(R8) (*21*) (G. Keith)	6	6
Asp (*22*) (G. Keith; P. Bolton and D. Kearns)	15	12
Arg_b (*23*) (J. Weissenbach and G. Keith)	8	7
Gly (P. Bolton and D. Kearns)	6	6
Leu_{UCA}(T2) (*15*)	5	5
Leu_a (*14*) (S. H. Chang; G. Pixa and G. Keith)	10	9
Phe(R11) (*24*) (B. Reid)	2	2
Ser_a(T3) (*16*)	5	4
Thr (J. Weissenbach and G. Keith)	7	4
Trp (*25*) (G. Keith; B. Reid)	2	1
Tyr(R10) (*26*) (B. Reid; P. Bolton and D. Kearns)	4	4
Val_b (*27*) (S. Montasser and G. Keith)	7	6
R6	2	2
T8	3	3
T9	14	12
T15	8	7
T16	5	3
B. Possible clusters of two or more tRNA genes		
Gly, T9	1	1
Ala(R8), T16	1	1
Leu_{UCA}(T2), T9	1	1
Ala(R8), Phe	1	1
Arg_b, Asp	4	3
Arg_b, Asp, Tyr(R10)	1	1
Arg_b, T9	1	1
Val_b, T16	1	1

Xenopus. Their data suggest a model in which identical tRNA genes are arranged in tandem, interspersed by spacer DNA. Our results show a different principal of organization in yeast.

Our data also suggest that the frequency of occurrence of the different tRNA genes on the yeast genome may vary extensively from one tRNA to another. Table 2 shows that, whereas nine clones hybridize with $tRNA_3^{Leu}$, only two hybridize with $tRNA^{Phe}$.

It is obviously of importance to analyze and compare the DNA from various of our clones. In doing so, we may be able to answer the following questions:

1) For a set of clones carrying genes for a particular tRNA, what is the conserved sequence—is it confined to the structural gene or does it contain auxiliary information?

2) Do iso- or heteroclusters of tRNA genes exist, and if so what is their organization and mode of transcription?

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References and Notes

1. A. Rich and U. L. RajBhandary, *Annu. Rev. Biochem.* **45**, 805 (1976); B. G. Barrell and B. F. C. Clark, *Handbook of Nucleic Acid Sequences* (Joynson-Bruvvers, Oxford, 1974).
2. D. C. Hawthorne and U. Leupold, *Microbiol. Immun.* **64**, 1 (1974).
3. P. W. Piper, M. Wasserstein, F. Engbaek, K. Kaltoft, J. E. Celis, J. Zeuthen, S. Liebman, F. Sherman, *Nature (London)* **262**, 737 (1976).
4. M. R. Capecchi, S. H. Hughes, G. M. Wahl, *Cell* **6**, 269 (1975); M. C. Brandriss, J. W. Stewart, F. Sherman, D. Botstein, *J. Mol. Biol.* **102**, 467 (1976); R. F. Gesteland, M. Wolfner, P. Grisafi, G. Fink, D. Botstein, J. R. Roth, *Cell* **7**, 381 (1976).
5. F. Bolivar, R. L. Rodriguez, M. C. Betlach, H. W. Boyer, in preparation.
6. J. Velten, K. Pukada, J. Abelson, *Gene* **1**, 93 (1976).
7. H. O. Smith, *Methods Mol. Biol.* **7**, 71 (1975).
8. This strain, originally from Dr. Mortimer, was described by W. Dontze, V. MacKay, T. R. Manney [*Science* **168**, 1472 (1970)].
9. This work was done under P2 conditions according to the guidelines as defined by the National Institutes of Health.
10. K. Struhl, J. R. Cameron, R. W. Davis, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1471 (1976).
11. M. Grunstein and D. S. Hogness, *ibid.* **72**, 3961 (1975).
12. G. M. Rubin, *Methods Cell Biol.* **12**, 45 (1975).
13. A. M. Maxam and W. Gilbert, *Proc. Natl Acad. Sci. U.S.A.* **74**, 560 (1977).
14. S. H. Chang, S. Kuo, E. Hawkins, N. R. Miller, *Biochem. Biophys. Res. Comman.* **51**, 951 (1973).
15. P. W. Piper and M. Wasserstein, in preparation.
16. H. G. Zachau, D. Dutting, H. Feldmann, *Angew. Chem. Int. Ed. Engl.* **5**, 422 (1966).
17. M. M. Bhargava and H. O. Halverson, *J. Cell Biol.* **49**, 423 (1971).
18. E. Schweizer, C. MacKechnie, H. O. Halvorson, *J. Mol. Biol.* **40**, 261 (1969); H. Feldman, *Nucleic Acids Res.* **3**, 2379 (1976).
19. R. A. Gilmore, J. W. Stewart, F. Sherman, *J. Mol. Biol.* **61**, 157 (1971).
20. S. G. Clarkson and M. L. Birnstiel, *Cold Spring Harbor Symp. Quant. Biol.* **38**, 451 (1973).
21. R. W. Holley, J. Apgar, G. A. Everett, J. T. Madison, M. Marquisee, S. H. Merrill, J. R. Penswick, A. Zamir, *Science* **147**, 1462 (1965).
22. J. Gangloff, G. Keith, J. P. Ebel, G. Dirheimer, *Biochim. Biophys. Acta* **259**, 198 (1972).
23. B. Kuntzel, J. Weissenbach, G. Dirheimer, *FEBS Lett.* **25**, 189 (1972).
24. U. L. RajBhandary, S. H. Chang, A. Stuart, R. D. Faulkner, R. M. Hoskinson, H. G. Khorana, *Proc. Natl. Acad. Sci. U.S.A.* **57**, 751 (1967).
25. G. Keith, A. Roy, J. P. Ebel, G. Dirheimer, *FEBS Lett.* **17**, 306 (1971).
26. J. T. Madison, G. A. Everett, H. Kung, *Science* **153**, 531 (1966).
27. V. D. Axelrod, V. M. Kryukov, S. N. Isaenko, A. A. Buyev, *FEBS Lett.* **45**, 333 (1974).
28. We would like to thank our colleagues listed in Table 2 for sending us numerous pure species of tRNA. Drs. P. Piper and H. Feldman supplied us with valuable information used in identifying yeast tRNA fingerprints. We thank Dr. Peter Gerdeschek for his continuous interest in our work. Supported by NCI grant CA 10984.

24 February 1977

Excision and Recombination of Adenovirus DNA Fragments in *Escherichia coli*

A hybrid between the bacteriophage λ genome and the adenovirus type 2 (Ad2) Eco RI-B DNA fragment has been constructed in vitro (*1*). The Ad2-Eco RI-B fragment was inserted as monomers in two directions in the clones analyzed; but in some cases we observed multimers of the inserted fragment in the λ phage vector (*1*). We now report that during growth in *Escherichia coli* recombination may occur within multiple adenovirus DNA fragments of the λ vector.

The vector used, obtained from λ2Pam (*1*, *2*) contains all the essential genes of the phage and has a 26 percent deletion in the middle of the genome. The adenovirus Eco RI-B DNA fragment was purified by electrophoresis in polyacrylamide slab gels. Since the Eco RI endonuclease cleavage sites are symmetrical, a DNA fragment can be inserted in two directions. The resulting two hybrids containing the Ad2-Eco RI-B DNA fragment can be distinguished since there is a

Table 1. Plaque morphology of λ-Ad2-Eco RI-B hybrids plated on different strains.

Clone	C600 $r_k^+ m_k^+$	C600 $r_k^+ m_k^+$ Rec $B^- C^-$	C600 $r_k^+ m_k^+$ Rec A^-
λ-Ad2-Eco RI-B4			
Monomer	Normal	Minute	Normal
Trimer	Normal	Normal	Normal
λ-Ad2-Eco RI-B7			
Monomer	Normal	Minute	Normal
Trimer	Normal	Normal	Normal
λ-Ad2-Eco RI-B1			
Monomer	Normal	Minute	Normal
Trimer	Normal	Normal	Normal

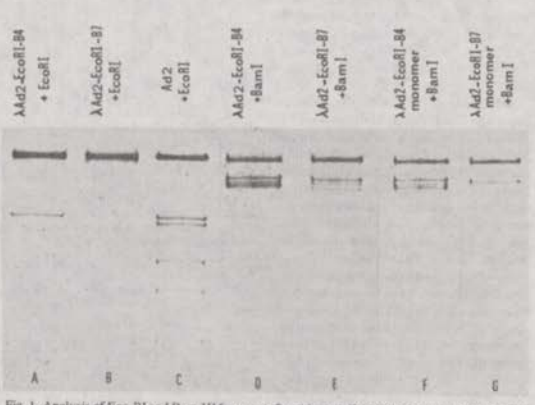


Fig. 1. Analysis of Eco RI and Bam HI fragments from the λ-Ad2-Eco RI-B4 and λ-Ad2-Eco RI-B7 hybrids. DNA from clones λ-Ad2-Eco RI-B4 and λ-Ad2-Eco RI-B7 was extracted and digested with endonucleases Eco RI and Bam HI (*1*). The DNA fragments were analyzed by electrophoresis in a slab gel containing a linear gradient of polyacrylamide from 2.5 to 7.5 percent (*1*). The Eco RI fragments of Ad2 DNA were used as size markers.

Bam HI endonuclease cleavage site close to one end of the Ad2-Eco RI-B fragment (3). There is also a Bam HI cleavage site close to the left end of the right arm of the λ2Pam vector (4). Consequently, after digestion of the hybrid DNA with Bam HI endonuclease two different cleavage patterns were observed after electrophoresis (1). When several clones were analyzed, an additional fragment was observed in some clones; this fragment comigrates with the Ad2-Eco RI-B fragment (in Fig. 1, compare D and E with C, and with A and B). This pattern suggests that some hybrids contain several Ad2-Eco RI-B fragments and that, at least, two adjacent fragments were inserted in the same direction.

The electrophoretic analysis does not allow us to determine the number of inserted fragments, or to establish whether the DNA analyzed comes from a homogenous or heterogenous phage population.

The hybrid phage λ-Ad2-Eco RI-B4 was grown on strain C600 r_k^+ m_k^+ and the phage preparation was analyzed by preparative isopycnic centrifugation in CsCl gradients. Three phage populations that varied in buoyant densities were observed (Fig. 2A). The buoyant density difference between the central and the two extreme phage bands are equal and correspond (5) to a difference in DNA content equal to one Ad2-Eco RI-B fragment. Similarly, the absolute amount of DNA in the hybrid phages was determined by comparing (5) their buoyant density with the density of phage λ2Pam (data not shown). Finally, both sets of data indicate that the three phage types correspond to the insertion of one, two, and three Ad2-Eco RI-B fragments, respectively. These classes of hybrid phages are therefore referred to as monomers, dimers, and trimers, respectively. A second clone of hybrid phage λ-Ad2-Eco RI-B7, which has a different Bam HI electrophoretic pattern (Fig. 1E), was also analyzed by CsCl centrifugation. The progeny were apparently composed of monomers, dimers, and trimers. The phage progeny of the third clone λ-Ad2-Eco RI-BA were composed only of monomers and trimers (Fig. 2B). The monomers of clones B1, B4, and B7 were purified by CsCl isopycnic centrifugation. Electrophoretic analysis of their Bam HI DNA fragments revealed that the fragment which comigrated with the Eco RI-B fragment had now disappeared (Fig. 1, F and G).

The genotypes of the B1, B4, and B7 clones could now be established by comparison of the electrophoretic pattern of the DNA's and the buoyant density analysis of the phages (Fig. 3). The λ vector has a 26 percent deletion. Therefore insertion of three Eco RI-B DNA fragments each of which contains 2.8 megadaltons of DNA yields a phage with a genome length slightly above that of the intact λ genome.

The observed multimers of the Ad2-Eco RI-B fragment may be due either to multiple insertions of the fragment when the hybrid DNA was constructed or to specific duplications during phage multiplication. In order to distinguish between these alternatives, monomers and trimers from clone B1 were extensively purified

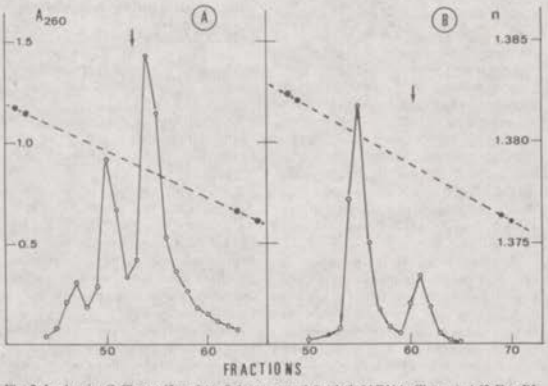


Fig. 2. Isodensity CsCl centrifugation of phages containing hybrid DNA. Clones λ-Ad2-Eco RI-B4 (A) and λ-Ad2-Eco RI-B1 (B) were grown in C 600 r_k^+ m_k^+ and the progeny were purified on CsCl gradients. Centrifugation was performed at 20,000 rev/min for 72 hours in an SW41 rotor.

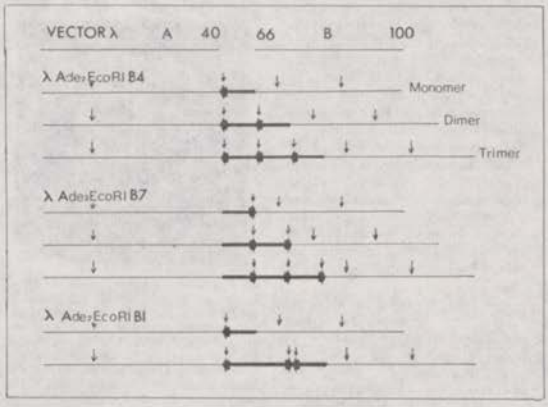


Fig. 3. Genotypes of the λ vector and the λ-Ad2-Eco RI-B hybrids. The λ vector consists of a left arm (A) (0 to 40 percent) and a right arm (B) (66 to 100 percent) which contains the sRI_L^-, sRI_R^-, and a Pam mutation. The Ad1-Eco RI-B DNA fragments of the dimer and the trimer of the clone B4 are all inserted in the same direction. The l-strand (1, 6) of the adenovirus fragment corresponds to the l-strand of λ. The Ad2-Eco RI-B DNA fragments of the dimer and the trimer of the clone B7 are all inserted in the same direction. The l-strand (1, 6) of the adenovirus fragment corresponds to the r-strand of λ. Two of the three fragments of the trimer of the clone B1 are inserted in opposite directions. The genotype of the monomer of the clone B1 is identical to the genotype of the clone B4.

by CsCl-isodensity centrifugation. After growth on C600 r_k^+ m_k^+ new progeny from the B1 monomer clone were again analyzed by centrifugation on CsCl gradients. Only monomers were observed, indicating that the monomer population was stable and that amplification did not occur. In contrast, the B1 trimer clone contained, after growth on C600 r_k^+ m_k^+, a mixture of monomers and trimers. Monomers were thus obtained from trimers by excision and recombination. We have, in addition, observed that the size of the plaques from monomer and trimer clones vary depending on the bacterial strain used for plating (Table 1). When plated on strain C600 r_k^- m_k^- Rec B^-C^-, monomers always give minute plaques, an observation that can be used for cloning monomer fragments. Since transfection with the hybrid DNA was initially carried out on this strain (*1*) it is not unexpected that multimers of the inserted fragment were isolated.

We may conclude from these observations that insertion of multimers of DNA fragments into the genome of λ leads to hybrids that can recombine within the foreign fragment. This recombination can be inter- or intramolecular (*5*). The rapidity of the conversion toward a population in which monomers are predominant suggests that intramolecular recombination is favored. Since the phage is Red^-, the bacterial Rec system is probably responsible for the recombination. Thus the bacterial recombination system works normally for DNA from a eukaryotic virus. This mechanism, if it exists, may yield adenovirus DNA circles. Our failure to observe circular DNA can be explained by the absence of a replication origin in the fragment which, in the present case, eliminates plasmid multiplications.

The experiments to construct the hybrid DNA were performed in a P3 laboratory in 1975 according to the Asilomar guidelines. Biohazards associated with the experiments described in this report have been examined previously by the French National control committee. The ultracentrifuge is located inside the P3 facility.

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References and Notes

1. P. Tiollais *et al.*, *Gene* **1**, 49 (1976).
2. A. Rambach and P. Tiollais, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 3927 (1974).
3. J. Sambrook, J. Williams, P. A. Sharp, T. Grodzicker, *J. Mol. Biol.* **97**, 369 (1975).
4. M. Perricaudet and P. Tiollais, *FEBS Lett.* **56**, 7 (1975).
5. A. J. D. Bellett, H. G. Bosse, R. L. Baldwin, in *The Bacteriophage Lambda*, A. D. Hershey, Ed. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1971), pp. 501–513.
6. P. A. Sharp, P. H. Gallimore, J. Flint, *Cold Spring Harbor Symp. Quant. Biol.* **39**, 457 (1974).

28 February 1977

Cloning of Cauliflower Mosaic Virus (CLMV) DNA in *Escherichia coli*

Abstract. *A plasmid containing cauliflower mosaic virus DNA can be faithfully cloned in* Escherichia coli, *but proved to be noninfective in test plants.*

The selection of novel plants having beauty or utility may be one of man's oldest activities (*1*). A major limitation to the formation of novel plant genomes is the fact that only related species can be crossed. While it is unreasonable to expect that the removal of the mating barrier will lead directly to the survival of plants containing arbitrarily constructed genomes, this barrier must be circumvented if these novel genetic systems are to be further explored.

One possible way to avoid the mating barrier is by the fusion of protoplasts and the subsequent growth of adult plants from the fused cells (*2*). Another is by the introduction of foreign genes directly. This route presents many attractive possibilities, but the frequency of genetic transformation in plants is low and the results up to now are highly variable (*3*). The introduction of genes into plant cells would be greatly facilitated if these DNA segments were included as part of a replicon that had the ability to replicate within the recipient cell. Such transduction experiments have been done by using a specifically deleted SV40 DNA into which bacterial or phage genes have been inserted (*4*). It is not unreasonable to expect that desirable genes can be introduced into plants by this route. Indeed, the only known beneficial virus to date infects tulips to produce variegated flowers of great beauty (*5*).



Fig. 1. Sal I cleavage pattern of CLMV DNA. About 1 μg of DNA was incubated with different levels of Sal I in 50-μl reaction volumes containing 60 m*M* tris, *p*H 7.6, 50 μg of BSA per milliliter, 5 m*M* $MgCl_2$, and 0.14*M* NaCl at 37°C for 3 hours. The reaction was terminated by the addition of 5 μl of bromphenol blue in 80 percent glycerol containing 0.1*M* EDTA. The sample was subjected to electrophoresis through 1 percent agarose gels in tubes, treated with fluorochrome, and photographed as described (*10*). The first four gels, from left to right, are CLMV DNA cleaved with different amounts of Sal I: gel 1, 1 unit; gel 2, 3 units; gel 3, 5 units; gel 4, 10 units; gel 5, uncleaved CLMV; gel 6, uncleaved CLMV—a fresh preparation.

The cauliflower mosaic virus (CLMV) is a prominent example, are the only plant viruses known to contain DNA (*6*). Therefore, in spite of its limited host range, the lack of a local lesion assay, and relatively weak infectivity, this viral DNA represents one of the few possible transducing vehicles for the introduction of foreign DNA segments into plant cells.

As a first step in developing this viral DNA as a transducing vehicle, we have formed a recombinant plasmid and cloned it in *Escherichia coli*. In a reversal of this experiment, we attempted to see whether the recombinant plasmid was replicated in plants. The results indicate that the first experiment was successful (although the cloned DNA could not be demonstrated to be infective to plants). The second experiment gave inconclusive results to be mentioned later. Since this work has now been halted for nonscientific reasons, we report on progress to date.

CLMV virus particles were isolated by the method of Shepard (*7*). Two milliliters of virus containing 0.2 to 0.3 μg of virus were mixed with 0.8 ml of proteinase K (600 μg/ml) (EM Laboratories) plus 6 percent sarkosyl in 0.25*M* EDTA, *p*H 8.0, which had been incubated at 37°C for 1 hour before use. After 16 hours at 37°C, the solution was extracted with one volume of redistilled phenol that was neu-

tralized with 1.0*M* tris, *p*H 9.0. The aqueous phase was extracted with an equal volume of $CHCl_3$, then dialyzed against 10 m*M* tris, 1 m*M* EDTA, *p*H 8.0. When examined in the electron microscope, mostly relaxed rings about 7500 base pairs in length were seen along with some linear and twisted structures. In 1.0 percent agarose gels a variety of bands with the major components corresponding to relaxed rings and linear molecules were observed. This distribution varies from preparation to preparation and changes with storage. However, when the preparations were treated with Sal or Bam endonucleases, a single band appeared having the mobility of a linear molecule about 7500 base pairs long (Fig. 1). In addition, there were some fainter bands in much less than molar yields, and these probably arose from variants purified with the predominant species. The mobility of the Sal and Bam products appear identical. The linearity of these molecules was confirmed by electron microscopy. We conclude that there is a single Sal and a single Bam site, but cannot rule out the unlikely possibility that both Sal and Bam have two sites equally spaced less than a few hundred base pairs apart.

In order to clone CLMV/Sal we used a composite plasmid, pGM706 (*8*), carrying both ampicillin and tetracycline resistance and a single Sal site. Insertion of DNA at this site inactivates the plasmid-specific tetracycline resistance. The CLMV/Sal was ligated to pGM706/Sal, and the reaction mixture was used to transform Ca^{2+}-treated GM4 (*E. coli* K12 r_B^- m_B^- *rec* A$^-$). The transformation mixtures were distributed to a large number of tubes from which multiple plates con-

Table 1. The length (in kilobase pairs) of various restriction segments of CLMV/Sal I and CL DNA based on the known lengths of λvir/RI and λvir/Hae (*10*). The bands in parentheses are considered minor because they are present in substantially less than molar yields.

Segments	CLMV/Sal I	CLS4	CLS8
Eco RI (1.4 percent agarose)			
1	5.0	5.0	5.0
1a (minor)	(4.9)		
2	2.4	2.4	2.4
2a (minor)	(1.8)		
3	0.5	0.5	0.5
	7.9	7.9	7.9
Bam (1.4 percent agarose)			
1	5.5	5.5	5.5
1a (minor)	(4.2)		
2	2.4	2.4	2.4
	7.9	7.9	7.9

Segments	CLMV/Sal I	CLS4	CLS8
Hind II + III (2.4 percent agarose)			
1	1.69	1.69	1.69
2	1.25	1.25	1.25
3	1.14	1.14	1.14
4	0.86	0.86	0.86
5	0.61*	0.60*	0.60*
6	0.59	0.59	0.59
7	0.51*	0.48*	0.48*
8	0.39	0.39	0.39
	7.04	7.00	7.00

(Some small fragments have run off the gel.)

Segments	CLMV/Sal I	CLS4	CLS8
Hae III (3.4 percent agarose)			
1	1.30	1.30	1.30
2	1.24*	1.23*	1.23*
3	1.13	1.13	1.13
4	0.99*	0.98*	0.98*
5	0.92	0.92	0.92
6	0.88	0.88	0.88
6a	(0.67)		
7	0.58	0.58	0.58
8	0.53	0.53	0.53
	7.57	7.55	7.55

(Perhaps some small segments cannot be seen.)

*The CLS4 and CLS8 bands have slightly different mobilities from asterisked CLMV/Sal I bands.

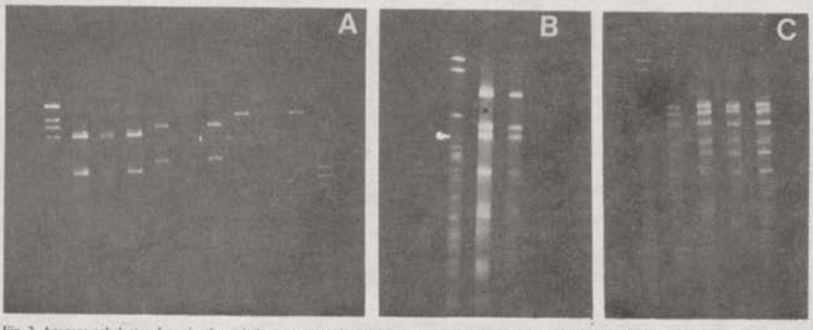


Fig. 2. Agarose gel electrophoresis of restriction segments obtained from cauliflower mosaic virus DNA and from two clones of this same DNA amplified in *E. coli* K. (A) Eco RI, prepared from *E. coli* RY13 by the procedure of Greene *et al.* (*11*), and Bam, prepared from *Bacillus amyloliquefaciens* by the procedure of Wilson and Young (*12*), was allowed to act on 500 ng of purified DNA's in 50-μl reaction volumes containing 50 m*M* NaCl, 10 m*M* $MgCl_2$, 50 m*M* tris, *p*H 7.6, at the level of 1 unit of enzyme per 1 μg of DNA for 3 hours at 37°C. At the end of this incubation, 5 μl of bromphenol blue in 80 percent glycerol and 0.1*M* EDTA was added and the sample was subjected to electrophoresis through 1.4 percent agarose gels in tubes, treated with fluorochrome, and photographed as described (*13*). The gels from left to right are: gel 1, λvir/Eco RI; gel 2, CLMV/Eco RI; gel 3, CLS4/Eco RI; gel 4, CLS8/Eco RI; gel 5, CLMV/Bam; gel 6, CLS4/Bam; gel 7, CLS8/Bam; gel 8, CLMV/Sal I; gel 9, CLS4; gel 10, CLS8; gel 11, λvir/Hae. The lettering scheme here refers to the origin of the DNA or the type of restriction endonuclease. For example, CLMV or λvir refers to DNA isolated from virus particles; CLS4 refers to the DNA segment obtained from the purified plasmid pCLS4, after treatment with Bam and recovery of the CL segment from agarose gels. The restriction endonucleases are abbreviated as RI (for Eco RI) Hind (for Hind II + III) and Hae (for Hae III). (B) Hind II + III, prepared from *Haemophilus influenzae* by the procedure of Danna *et al.* (*13*) was incubated under the conditions described in (A) in 50-μl mixtures containing 50 m*M* NaCl, 6 m*M* $MgCl_2$, 6 m*M* tris, *p*H 7.6, BSA (100 μg/ml), and DNA (50 μg/ml). Gel 1, λvir/Hae; gel 2, CLMV/Hind; gel 3, CLS4/Hind. CLS8 responds in an identical manner to CLS4. (C) Hae III, prepared from *Haemophilus aegypticus* (*14*), was incubated as described in (A) and (B) in mixtures containing 7 m*M* $MgCl_2$, 7 m*M* tris, *p*H 7.4, and 7 m*M* β-mercaptoethanol. Gel 1, λvir/Hae, gel 2, CLS4/Hae; gel 3, CLS8/Hae; gel 4, CLMV/Hae; gel 5, CLS4/Hae + CLMV/Hae.

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Table 2. Infectivity of DNA. The source of DNA is abbreviated as described in the legend to Fig. 2A. In the case of pCLS4, we mean covalently closed rings. The pCLS4/Sal I refers to this DNA treated with Sal I to produce a mixture of two linear molecules: the plasmid vehicle, p, and the cloned segment CLS4. In the case of lines 6 and 7 these linear segments were religated with T4 DNA ligase (gift of Dr. F. Asubel) to produce 30 percent and 60 percent rings, some of which may be nicked. These were assayed by scanning gels stained with ethidium bromide. Leaves of *Brassica* plants (*Brassica campestris*, "Tendergreen mustard" c.v. Burpee's seed) were washed with sterile 0.15*M* NaCl containing 0.015*M* sodium citrate, *p*H 7.0 (SSC), and then treated with DNA in SSC. Two young leaves were inoculated with carborundum on each plant, each with about half the amount of DNA shown. After 1 hour, the treated leaves were washed with sterile distilled water. The affected plants show an altered pattern of pigmentation and other symptoms that generally allow unambiguous scoring after 1 month (7).

DNA source	Amount (μg)	Plants infected/ inoculated
1. CLMV	10.0	6/9
	0.1	4/9
2. CLMV/Sal I	3.0	0/3
	1.0	0/6
3. pCLS4	10.0	0/6
	1.0	0/6
4. pCLS4/Sal I	10.0	0/3
	1.0	0/3
5. pML 21	20.0	0/2
	10.0	0/4
6. CLS4/Sal I/religated (30 percent rings)	10.0	0/6
	1.0	0/6
7. CLMV/Sal I/religated (60 percent rings)	10	0/4
	1	0/9

taining ampicillin (30 μg/ml) (Wyeth) were prepared. Many ampicillin resistant colonies were selected and tested on plates containing tetracycline (25 μg/ml) (Lederle). In this way, 13 *amp*^{R} *tet*^{R} clones which were derived from five separate tubes were selected, and therefore must contain at least five independent transformants. Plasmid DNA was prepared from all 13 strains by the Brij-lysis procedure (9); gel analysis before and after cleavage by Sal showed that each plasmid contained a long insert that proved to be the same length as CLMV/Sal. Further digestion by Bam and Eco RI gave identical patterns. Two strains, pCLS4 and pCLS8, were selected and plasmid DNA was purified by phenol extraction, ethanol precipitation, ethidium bromide–CsCl banding, and dialysis.

We attempted to clone CLMV/Bam by an equivalent procedure involving pGM 439 (8). However, the plasmid DNA's showed that none contained inserts characteristic of intact CLMV.

The plasmid DNA's pCLS4 and pCLS8 were each cleaved by Sal and subjected to electrophoresis in agarose tubes (1 cm in diameter); the zone containing the inserted viral segment, now called CL, was dissolved in saturated KI and the DNA was recovered on hydroxyapatite. These DNA's were cut by Eco RI, Bam, Hind, and Hae endonucleases. The results (Fig. 2 and Table 1) indicate that CLS4 and CLS8 have identical restriction segments and that these are nearly identical, with the same restriction segments formed from CLMV/Sal. Some possible differences are marked by the asterisks in Table 1.

Infectivity studies on "Tendergreen" mustard plants were performed as described (Table 2). In each case, CLMV DNA itself displayed infectivity. In contrast, CLMV DNA that was cleaved by Sal showed no infectivity even after religation. Likewise, the clonally amplified CLS4 showed no infectivity even after religation. Although the reason for this lack of infectivity is not known, some possibilities might be mentioned. (i) Perhaps there are closely spaced Sal sites, and the small segment between them is lost. (ii) Perhaps CLMV, like certain other plant viruses, has a bipartite genome, in which case two virus particles would be required to establish infection. Perhaps the minor bands mentioned above are derived from some helper virus. (iii) Perhaps the native CLMV DNA has some crucial secondary structure that is lost on cleavage or state of modification that is not present in the cloned material.

Passage experiments were initiated in leaf homogenates in an effort to determine whether the pCLS4 was replicated in the plant or acquired the ability to cause symptoms. Again no special symptoms could be observed, even after three and four passages.

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References

1. A. Leroi-Gourhan, *Science* **190**, 562 (1975); C. D. Darlington, *The Evolution of Man and Society* (Simon and Schuster, New York, 1969).
2. J. B. Power *et al.*, *Nature* **263**, 500 (1976); G. Melchers and G. Labib, *Mol. Gen. Genet.* **135**, 277 (1974); P. S. Carlson, H. H. Smith, R. Dearing, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 229 (1972).
3. L. Ledoux, *Genetic Manipulations with Plant Material* (Plenum, New York, 1975).
4. D. H. Hamer, D. Davoli, C. A. Thomas, Jr., G. C. Fareed, *J. Mol. Biol.*, in press; S. P. Goff and P. Berg, in preparation.
5. R. J. Dubos, in *Perspectives in Virology*, M. Pollard, Ed. (Wiley, New York, 1959), pp. 291–299.
6. R. J. Shepherd, *Adv. Virus Res.* **20**, 305 (1976).
7. P. P. Pirone, G. S. Pound, R. J. Shepherd, *Phytopathology* **51**, 540 (1961); R. J. Shepherd, R. J. Wakeman, R. R. Romanko, *Virology* **36**, 150 (1968); R. J. Shepherd, G. E. Bruenning, R. J. Wakeman, *ibid.* **4**, 339 (1970).
8. D. H. Hamer and C. A. Thomas, Jr., *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1537 (1976).
9. D. B. Clewell and D. R. Helinski, *ibid.* **62**, 1159 (1969).
10. D. H. Hamer and C. A. Thomas, Jr., *Chromosoma* **49**, 243 (1975).
11. P. J. Greene, M. C. Betlach, H. W. Boyer, H. M. Goodman, *Methods Mol. Biol.* **7**, 87 (1974).
12. G. A. Wilson and F. E. Young, *J. Mol. Biol.* **97**, 123 (1974).
13. K. J. Danna, G. H. Sack, D. Nathans, *ibid.* **78**, 363 (1973).
14. J. H. Middleton, M. H. Endgell, C. A. Hutchinson, *J. Virol.* **10**, 42 (1972).

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The Effects of *Escherichia coli* and Yeast DNA Insertions on the Growth of Lambda Bacteriophage

Abstract. *The effects of Eco RI endonuclease-cleaved* Escherichia coli *and yeast* (Saccharomyces cerevisiae) *DNA fragments on the propagation of the lambda bacteriophage vectors containing them were determined on a nonmutated and a PolA* E. coli *K12 host. Observable alterations in the growth of hybrids containing yeast DNA insertions were less frequent and less extreme than those seen in hybrids containing* E. coli *DNA. A lambda–*E. coli *hybrid was selected after extensive growth on the PolA (deficient in polymerase I) host which also grew very well on the PolA*$^+$ *host and may have resulted from some alteration in the hybrid. Hybrids selected on the PolA host gave no evidence for the expression of polymerase I activity. No lambda-yeast hybrid made from the λgt vector lacking lambda-specific recombination (*red$^-$*) had a yield of viable bacteriophage on infection greater than two-thirds that of "wild-type" lambda.*

Recent work has focused on the preparation in vitro of viable recombinant DNA molecules containing eukaryotic or prokaryotic DNA joined to a self-replicating prokaryotic vector DNA. An important concern in these experiments is the effect of the inserted DNA on the propagation of the vector in which it is inserted. A detrimental effect is of interest from the experimental standpoint because this may lead to difficulty in preparation, complete loss of the hybrid, or selection of a sequence alteration in the hybrid DNA which allows more efficient propagation. Any effect on growth of the hybrid is of importance since it might be indicative of expression of some function by the inserted DNA in the bacterial cell.

Related to this is the possible increase in the biohazard potential if the foreign DNA enables the hybrid to propagate more efficiently than the "wild-type" vector either under laboratory conditions or in the natural environment.

These phenomena have been investigated by using either yeast (*Saccharomyces cerevisiae*) DNA (*1*) or *Escherichia coli* DNA inserted into a suitable strain of bacteriophage λ, λgt-λB (*2*). These hybrid DNA's were constructed by first cleaving the DNA's with Eco RI endonuclease and covalently joining the fragments with *E. coli* DNA ligase. Bacteriophage from 10^4 to 10^5 independent plaques obtained after calcium transfection of these hybrid DNA's into *E. coli* cells were pooled to form stocks of λ hybrids which contain most sequences of the yeast or *E. coli* genomes in the form of cloned Eco RI DNA fragments. The formation of these hybrids and the genetic selection of a specific λ–*E. coli* hybrid is described in (*2*). Similar pools of λ-yeast hybrids were used for the genetic selection of a yeast Eco RI DNA fragment expressing a specific function in *E. coli* by Struhl *et al.* (*3*).

The constitution of the original λ-yeast hybrid pool may be examined by observation of its Eco RI restriction spectra (pattern of DNA bands produced on agarose gel electrophoresis of restriction endonuclease cleaved DNA). The DNA was prepared from the hybrid pool after passing the pool through only a few generations of growth in *E. coli*; the DNA was cleaved with Eco RI endonuclease, and the fragments electrophoresed on agarose gels. The resulting Eco RI restriction spectra is shown in Fig. 1c and is compared to whole yeast DNA in Fig. 1b. They are quite similar band for band, showing similar fragment sizes and abundances, except for the three strong bands originating from the original cloning vector, λgt-λB (two at the top and one in the middle of Fig. 1c). This suggests that the yeast fragments do not generally have an extreme effect on the growth of the λ hybrids in *E. coli*. Such is not the case when hybrids formed with *E. coli* DNA are examined. Previously (*2*), it was shown that the Eco RI restriction spectra of DNA's made from λ–*E. coli* hybrid pools consist of several prominent bands unlike that of *E. coli* DNA which has no very prominent bands. These results are reproduced in Fig. 2. The conclusion from this observation is that many *E. coli* DNA fragments have a strong differential effect on the propagation of λ hybrids, in which they are present. These conclusions were confirmed by measuring the burst sizes (mean yield of bacteriophage per infected cell) of several λ–*E. coli* and λ-yeast hybrids. Specific λ–*E. coli* hybrids were often found with burst sizes either considerably larger or smaller than the original λgt-λB bacteriophage. λ-Yeast hybrids did not have widely varying burst sizes. Thus, the expression of any functions affecting the propagation of λ hybrids is less frequently observed with inserted yeast DNA than with the homologous DNA.

It is desirable to define the maximum growth potential of yeast or *E. coli* hybrids in various hosts and to isolate such efficiently growing hybrids from the pools. This was accomplished by growing the hybrid pools on a particular bacterial host for many successive generations and preparing DNA from the resulting pools for restriction spectra analysis at different stages. This approach is termed a "grow-out" experiment. The λ hybrid pools were grown on plates, about 10^6 bacteriophages being used per plate. The resulting confluent lysis (*4*) represents three or four burst cycles per plating. The *E. coli* strain used in this grow-out experiment, W3110, is a representative of "wild-type" K12 *E. coli* (*5*). Another strain, *E. coli* C600 (*5*), was used as an example of a heavily mutagenized host and gave results identical to W3110. Grow-out experiments with the λ–*E. coli* hybrid pool on W3110 are illustrated in Fig. 3, b to d. Hybrids carrying one Eco RI DNA fragment are selected by eight platings. Grow-out experiments performed with pools of λ-yeast hybrids on W3110 gave results similar to those obtained with the λ–*E. coli* hybrids, with the exception that selection did not occur quite so rapidly. After eight consecutive platings the pool consisted primarily of hybrids containing one yeast Eco RI DNA fragment as seen by the prominent band in the restriction spectrum of Fig. 1d.

The most prevalent DNA fragment present in the λ–*E. coli* and λ-yeast hybrids following the grow-out on W3110 was found in either orientation relative to the vector. This was determined by electron microscopic observations of self-renatured DNA's. The fact that different hybrids were selected with the same fragment in both orientations suggests that it is some intrinsic property of the fragment itself which is responsible for the growth properties of the hybrids containing it.

The grow-out experiments with the λ hybrids in W3110 or C600 are considered

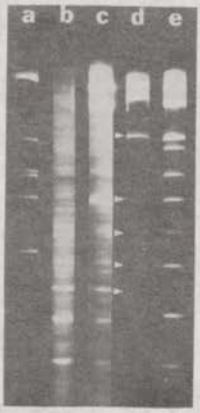


Fig. 1. Agarose gel (0.7 percent) electrophoresis of Eco RI endonuclease digests of DNA from (a) λcI857, 0.2 μg, (b) linear yeast DNA, 1 μg, (c) pool of λgt-yeast hybrids made with strain 231711-136, 4 μg, (d) pool grown out on W3110, eight platings, 0.4 μg, and (e) pool grown out on H560, two platings, 1 μg. The DNA preparation, digestions, and electrophoresis were done as in (*4*). The small arrows point to fragments determined to be yeast DNA, as discussed in the text.

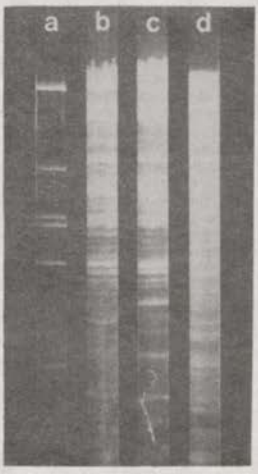


Fig. 2. Agarose gel (0.7 percent) electrophoresis of Eco RI endonuclease digests of DNA from (a) λcI857, 0.2 μg, (b) pool of 400 λgt–*E. coli* hybrid plaques, 2 μg, (c) pool of 8000 λgt–*E. coli* hybrid plaques, 2 μg, and (d) *E. coli*, 3 μg [from (*2*)].

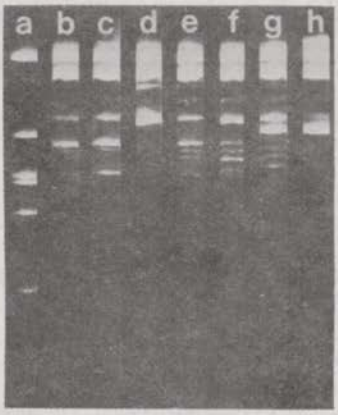


Fig. 3. Agarose gel (0.7 percent) electrophoresis of Eco RI endonuclease digests of DNA from (a) λcI857, 0.2 μg, λgt-*E. coli* hybrids grown out on W3110, three (b), six (c), and eight (d) platings, 0.5 μg, and λgt-*E. coli* hybrids grown out on H560, one (e), two (f), four (g), and seven (h) platings, 0.5 μg.

to be under conditions of a nonstringent environment. A strain which provides a more stringent environment is *E. coli* H560, a *thy*$^-$ and PolAl (amber mutation in the gene for DNA polymerase I) derivative of W3110. Lambda with a nonfunctional exonuclease gene (*red*$^-$), like the initial cloning vector λgt-λB, will not form plaques on PolAl strains (*2*, *6*). It was anticipated that growth of the λ–*E. coli* hybrid pool on H560 would select for those hybrids carrying the gene coding for DNA polymerase I. The pool grown on H560 for three to four generations was still heterogeneous, as shown by the Eco RI restriction spectrum of Fig. 3e. Continued growth on H560 resulted first in the enrichment for a hybrid containing one Eco RI DNA fragment and then, finally, enrichment for another hybrid containing a different Eco RI DNA fragment, as shown in Fig. 3, f to h. This fragment was present in only one orientation unlike the fragments in the hybrids selected on W3110. None of the selected hybrids carried a gene expressing DNA polymerase I activity as determined by enzyme assays of infected cell extracts (*7*) and by the lack of trans complementation with another coinfecting *red*$^-$ bacteriophage. Struhl (*8*) was able to isolate the genes analogous to *E. coli* PolA from *Klebsiella aerogenes* and *K. pneumoniae* using these same techniques.

Growth of the yeast-hybrid pool on H560 for two platings resulted in a pool that contained many different hybrids as shown in Fig. 1e. Individual isolates from these selected hybrids as well as those obtained by further growth on H560 all show moderate growth efficiency on W3110 and H560. Some of the hybrid DNA's contain the same Eco RI DNA fragments in different orientations. No evidence was found for the expression of a diffusible product resembling either DNA polymerase I or λ exonuclease. It was determined that the isolated yeast hybrids in fact contained yeast DNA by hybridizing ^{32}P-labeled complementary RNA (cRNA) from the hybrid DNA to yeast DNA from other sources. The hybridizations were conducted by first electrophoretically separating Eco RI yeast DNA fragments on agarose gels, then denaturing and eluting the DNA onto nitrocellulose strips followed by hybridization with the cRNA and autoradiography (*3*, *9*). In each case, hybridization was only seen at that position which corresponded to the size of the yeast DNA fragment present in the hybrid.

A quantitative evaluation of the growth potentials of the various selected hybrids can be obtained by determining their burst sizes. These are compared to the burst size of a hybrid containing a DNA fragment considered to have a neutral effect on the growth of λ. Such a hybrid is λgt-EcAra6, which contains just the A, B, and D genes of the arabinose operon of *E. coli* (*10*). In the absence of arabinose, this operon is not induced and the inserted DNA segment should be neutral with respect to growth of the phage. Its burst size is about 50 (half that of a "wild-type" *red*$^+$ λ). The λ–*E. coli* hybrids selected on W3110 have a slightly higher burst size than λgt-EcAra6 (60 as compared to 50). Unexpectedly, the λ–*E. coli* hybrid selected on H560 has a higher burst size on W3110 (80) than the hybrid selected on W3110. One explanation is that some alteration occurred during the selection process on H560 which allows the affected hybrid to grow better on both H560 and W3110 than any other unaltered hybrid present in the pool. Conversely, the hybrid selected on W3110 grows very poorly on H560.

The λ-yeast hybrids selected on W3110 gave a burst size of 60 like their *E. coli* counterparts. However, the λ-yeast hybrids selected on H560 grew only moderately well on H560, with burst sizes from 10 to 20, and on W3110, with burst sizes from 30 to 60 (25 hybrids examined).

The actual nature of the effects seen for particular yeast DNA inserts on the growth of the hybrid bacteriophage remains for speculation. Although the effects seem to be innate to the fragment, no function is expressed which can be ascertained other than a cis-acting effect on growth. An important consideration related to the expression of heterologous DNA insertions is the situation of the λ–*E. coli* hybrid selected on H560, which may be the result of a rare alteration selected by the pressure of 25 growth cycles in a restrictive environment. The possibility of DNA sequence changes which provide favorable growth rates must be considered whenever hybrid bacteriophage or plasmids are grown in an unfavorable environment. An example of this is selection for the expression of a particular function by eukaryotic DNA carried into a prokaryotic host.

No λ-yeast hybrid was present in these pools of *red*$^-$ bacteriophage which had a burst size larger than two-thirds of the burst size of "wild-type" λ. When compared to the growth of λgt-EcAra6 and λgt-λB, most inserted yeast DNA segments had a neutral or slightly detrimental effect on the growth of the hybrid λ bacteriophage. These hybrids would not, therefore, be expected to compete in nature with "wild-type" λ bacteriophage.

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References and Notes

1. J. R. Cameron, P. Philippsen, R. W. Davis, *Nucleic Acids Res.*, in press.
2. J. R. Cameron, S. M. Panasenko, I. R. Lehman, R. W. Davis, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3416 (1975).
3. K. Struhl, J. R. Cameron, R. W. Davis, *ibid.* **73**, 1471 (1976).
4. M. Thomas and R. W. Davis, *J. Mol. Biol.* **91**, 315 (1974).

5. B. J. Bachmann, *Bacteriol. Rev.* **36**, 525 (1972).
6. L. W. Enquist and A. Skalka, *J. Mol. Biol.* **75**, 185 (1973).
7. D. Uyemura and I. R. Lehman, *J. Biol. Chem.* **251**, 4078 (1976).
8. K. Struhl, in preparation.
9. E. M. Southern, *J. Mol. Biol.* **98**, 503 (1975).
10. M. Thomas and R. Davis, unpublished data.
11. We thank I. R. Lehman and D. Uyemura for valuable information related to polymerase I and B. D. Hall for some of the yeast DNA used in these experiments. L. Horn is appreciated for preparation of this paper. This work was supported in part by PHS grant GM 21891. J.R.C. is a postdoctoral fellow supported by National Cancer Institute grant CA 09151.

7 February 1977

Use of Isolators in Recombinant DNA Research

The use of negative pressure rooms has been recommended as a means of physical containment for recombinant DNA experiments. The major drawback of such rooms is that the experimenter, because he is in contact with the potentially hazardous material, may contaminate himself and serve as a dissemination vector. By using an isolator, the experimenter can avoid any direct contact with the hazardous materials, and he needs no special safety training. Isolators in positive or negative pressure have been used for many years for the manipulation of radioactive compounds and toxic chemicals and bacteria, and they have proved efficient in preventing outside contamination.

The aim of this report is to emphasize the advantages of using isolators in recombinant DNA research. To be really safe, an isolator must be equipped with a transfer system which allows the experimenter to take material in and out without ever coming into contact with it. The Elster isolator (*1*) (Fig. 1) which we started using recently possesses a particularly elaborate transfer system (*2*, *3*): material can be taken in or out in a tightly closed container, and this container can be transferred to another isolator or directly autoclaved. The isolator is in negative pressure, with air filtrated through Hepa filters, and its internal temperatures can be regulated from 20° to 40°C so that it can be used as an incubator for plates and cultures.

Isolators of this type but in positive pressure have been used for surgical operations (*3*), the protection of immunodeficient patients (*2*, *4*), and for the



Fig. 1. Photograph of an Elster isolator measuring 2.5 m long, 0.7 m broad, and 1 m high. It is made of a flexible (though resistant) transparent plastic sheet held by a tubular frame, set up on a table with filters and machinery attached underneath. There are five regularly spaced gloves along the front, which permit manipulation in all parts of the internal volume. The isolator is maintained under negative pressure (which can be varied from 0 to −7 mm of water). The incoming and extracted air is passed through Hepa filters. Because of the limited air flow, these filters will last for many years. There are two circular openings, one on each side. The biggest one, on the left, is for taking in or out large sized equipment. The other, which is the transfer system door, shown here with a container plugged in, is used to take materials in or out during experiments. When the container with its lid is applied onto the transfer door and rotated, it triggers the isolator's door which can now be opened from inside. The lid of the container is stuck to the door in such a way that the external faces of both the lid and the door are protected from contamination. The tightly closed container can be transferred to another isolator or autoclaved (with metal containers not shown). The inlet for formol or peracetic acid is at the bottom left. The internal volume is closed and remains safe even if air extraction stops. Since plates may have to be incubated or cultures grown at temperatures ranging from 30° to 40°C, the entire isolator is thermally regulated, and the internal temperature can be varied at will. Plates are incubated on the bench, and cultures are grown on a shaking platform. A magnetic stirrer is available for cultures of 1 to 2 liters, and a small electric pump can be used to bubble air through the culture. Samples could be subjected to ultracentrifugation by sealing the opening of the ultracentrifuge in the same or another isolator while the centrifuge itself remains on the outside.

breeding of axenic animals (5). For this reason, a large body of data on the efficiency of the decontamination and air filtration systems is available. The data show excellent efficiency, at least as far as bacterial contamination is concerned (6).

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References and Notes

1. Elster Company, 9 avenue Fremiet, 75016 Paris.
2. B. Saint-Martin, R. Duchuzeau, J. C. Ghnassia, C. Griscelli, B. Lauvergeon, *Rev. Fr. Gynécol. Obstet.* **70**, 585 (1975).
3. J. Lannelongue, P. Burdin, J. Pelicot, J. Castaing, *Rev. Chirurg. Orthopéd.* **62**, 389 (1976).
4. J. C. Ghnassia and C. Griscelli, *Nouv. Presse Méd.* **3**, 1429 (1974).
5. P. Raibaud, R. Duchuzeau, M. C. Muller, *Ann. Microbiol. (Paris)* **126 B**, 357 (1975).
6. J. Pelicot, *Aérobiocontamination en Milieu Hospitalier* (Thèse de Médecine, Tours, 1974).

8 February 1977

Degradation of DNA by Nucleases in Intestinal Tract of Rats

Abstract. *Strains of* Escherichia coli *K12 have been constructed as safer hosts for use in recombinant DNA research. These strains are unable to survive passage through the intestinal tracts of rats because of a constellation of mutations conferring bile sensitivity and requirements for diaminopimelic acid and thymine. Since death caused by diaminopimelic acid deprivation could release recombinant DNA before DNA is degraded because of thymine starvation, it is important to determine the "survival potential" of the released DNA's. Bacterial and plasmid DNA's extracted from bacterial cells are rapidly degraded when added to low dilutions of rat intestinal contents. This observation, coupled with the stringent requirements necessary for in vitro transformation or transfection, make in vivo transmission of naked recombinant DNA in the rat intestinal tract highly improbable.*

The design, construction, and testing of strains of *Escherichia coli* K12 that are more useful and safer for recombinant DNA molecule research is one of the goals of our laboratory (*1*). The first of such strains, χ1776, is unable to survive passage through the intestinal tracts of rats at frequencies of less than 10^{-11} bacteria per milliliter because of a constellation of mutations that cause sensitivity to bile and requirements for diaminopimelic acid (DAP) and thymine (*1*). Although DNA degradation accompanies thymine-less death in in vitro experiments and is an all-or-none response for any given cell (*1*), it is likely that lysis associated with DAP-less death may liberate DNA prior to its degradation. Since undegraded recombinant DNA might be taken up by robust microorganisms encountered in natural environments by transformation or as a consequence of concomitant phage infection (*2*), it is important to determine the "survival potential" of such DNA in nonlaboratory controlled environments. We determined the rate of degradation of *E. coli* chromosomal and plasmid DNA in extracts of the intestine contents of rats. We were unable to locate any literature reports indicating the fate of "naked" DNA's in the intestines of warm-blooded animals and believed that a likely route of escape of chimeric microorganisms from the laboratory is ingestion by laboratory workers.

The small and large intestines were aseptically removed from asphyxiated rats (Charles River) (*3*), and after longitudinal incisions were made the contents were removed by washing with 5.0 ml of buffered saline with gelatin (BSG) (*4*) containing 10 m*M* $MgSO_4$. Each extract had a volume of approximately 6.0 ml and thus was a 1 : 6 dilution of the original intestine contents. The data in Fig. 1 show that there is an initial rapid degradation of the DNA in the 1 : 6, 1 : 12, and 1 : 60 dilutions of the small intestine extracts while no detectable degradation is observable in the large intestine extracts. After 4 hours, the level of DNA degradation in the diluted large intestine contents is comparable to the initial level of degradation in the small intestine contents, while complete degradation occurs in the 1 : 6, 1 : 12, and 1 : 60 dilutions of the small intestine contents. There was no DNA degradation during the experiment in the boiled extracts and controls containing BSG and 10 m*M* $MgSO_4$. In order to ascertain if the immediate reduction in acid-insoluble radioactivity in the diluted small intestine contents was due to quenching, the total soluble and insoluble radioactivity was monitored throughout the experiment and found to be equal to that in the input DNA. Results similar to those in Fig. 1 were obtained in another experiment in which the intestine contents were diluted in BSG without added $MgSO_4$. Thus a suitable ionic environment exists within the intestine for nuclease activity and our results are not enhanced by the addition of Mg^{2+} to the buffer used for suspending and diluting intestine contents. Comparable but somewhat more extensive DNA degradation occurs in the intestine contents from gnotobiotic rats (Fig. 2). These results rule out bacterial nucleases as being the sole cause for DNA degradation in the intestine contents from conventional rats. It therefore appears that the intestine contents of rats contain nucleases that rapidly degrade free bacterial DNA and are probably secreted into the lumen of the intestine by cells in the intestinal wall (*5*) or from the pancreas (*6*).

Data on the rate of bacterial DNA deg-

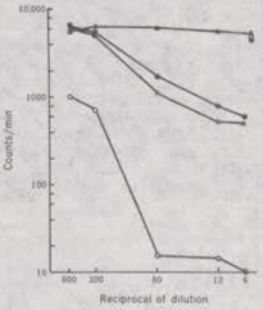


Fig. 1. Degradation of bacterial DNA by intestine contents from conventional rats. DNA labeled with tritiated thymidine was isolated from *E. coli* χ1776 (*1*). Lysozyme-generated spheroplasts were made in buffer containing 50 m*M* tris, 50 m*M* EDTA, 50 m*M* NaCl, and 10 percent sucrose (pH 8.0) and were lysed by adding 0.2 ml of spheroplasts to 1.0 ml of the intestine contents that were diluted to the extent indicated in BSG plus 10 m*M* $MgSO_4$. This method was believed to most closely mimic the in vivo entry of an enfeebled host into the lumen of the intestine. All incubations were at 37°C. Samples of 25 μl were taken immediately after mixing (0 hours) and at 4 hours and spotted on Whatman No. 3 filters. The filters were immersed sequentially in 10 and 5 percent TCA and 95 percent ethanol by standard methods and dried. The acid-insoluble counts were determined in a Beckman scintillation counter with 2,5-bis-2(5-*tert*-butylbenzoxazolyl)thiophene (BBOT) in toluene as a scintillation fluid. Data are shown for small intestine contents at 0 hours (●) and after 4 hours (○); large intestine contents at 0 hours (▲) and after 4 hours (△); small intestine contents boiled for 10 minutes before adding DNA at both 0 and 4 hours (■); and large intestine contents boiled for 10 minutes before adding DNA at both 0 and 4 hours (□). The control was DNA diluted into BSG plus 10 m*M* $MgSO_4$; its radioactivity remained at approximately 6200 count/min at 0 and 4 hours.

radation by small intestine contents from conventional rats are illustrated in Fig. 3. The 1 : 240 dilution of the small intestine contents was the lowest dilution that did not give DNA degradation in the time it takes to add the DNA and then sample the intestine contents. The rate of degradation in the 1 : 240 dilution is in good agreement with the degradation in the 1 : 300 dilution after 4 hours (Fig. 1).

The degradation of pMB9 DNA in 1 : 6 and 1 : 12 dilutions of small intestine contents from a conventional rat was qualitatively similar to that obtained with chromosomal DNA (Fig. 1); however, complete degradation of the plasmid DNA was achieved more rapidly (data not shown).

The data in Fig. 4 show the rate of degradation of plasmid pMB9 by small intestine contents from conventional rats. In order to properly evaluate this data it is necessary to attempt to relate the disappearance of trichloroacetic acid (TCA) insoluble counts to the number of surviving intact pMB9 molecules. The instantaneous rate of degradation of plasmid DNA is initially 24 min^{-1} and decreases to 0.48 min^{-1}. The decrease in rate probably reflects an inhibition of enzyme activity due to the generation of small polynucleotide fragments. The rate of inactivation of the plasmid, in terms of biological function, is much higher since only the action of endonuclease cuts is required to render the molecule linear and thereby lower its efficiency of transformation.

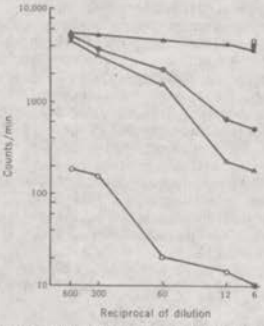


Fig. 2. Degradation of bacterial DNA by intestine contents from gnotobiotic rats. Methods were as described in the legend to Fig. 1. The intestine contents were found to be sterile. Data are for small intestine contents at 0 hours (●) and after 4 hours (○); large intestine contents at 0 hours (▲) and after 4 hours (△); small intestine contents boiled for 10 minutes before adding DNA at both 0 and 4 hours (■); and large intestine contents boiled for 10 minutes before adding DNA at both 0 and 4 hours (□). Radioactivity of the control, DNA diluted into BSG plus 10 m*M* $MgSO_4$, remained, at approximately 4800 count/min at 0 and 4 hours.

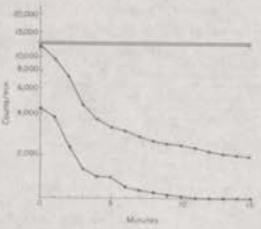


Fig. 3. Rate of degradation of bacterial DNA by small intestine contents from conventional rats. Methods were as described in the legend to Fig. 1. Data are for a 1 : 60 dilution of small intestine contents (■); a 1 : 60 dilution of boiled small intestine contents (□); a 1 : 240 dilution of small intestine contents (●); and a 1 : 240 dilution of boiled small intestine contents (○). Radioactivity of the control, DNA diluted into BSG plus 10 m*M* $MgSO_4$, remained at approximately 12,400 count/min at 0 and 15 minutes.

From our results, it is evident that a (gratuitous) safety feature exists in the rat intestinal tract that aids in rapidly degrading potentially hazardous DNA. We are concerned with the "survival potential" of the chimeric DNA in the small and large intestines because that is where the robust bacteria exist ($\sim 10^9$ bacteria per milliliter in the former and $\sim 10^{12}$ bacteria per milliliter in the latter) that could possibly be transformed by the chimeric DNA. Although we have not determined the actual site in the alimentary canal where χ1776 dies, we surmise that considerable killing would occur in the stomach due to gastric acid and in the duodenum at or after the juncture with the bile duct. If death of χ1776 in the small intestine is associated with cell lysis and release of DNA, then such DNA would immediately be exposed to pancreatic secretions, a most likely source of the intestinal nucleases, since the pancreatic and bile ducts are either joined together or adjacent in entering the duodenum (7). If some χ1776 cells survive passage through the small intestine and then lyse because of DAP-less death with release of DNA in the large intestine, this DNA would not be degraded as rapidly as in the small intestine (Figs. 1 and 2). However, the conditions that seem to be necessary for in vitro transformation of enteric bacteria with phage and plasmid DNA (8) (that is, cold $CaCl_2$ treatment of bacteria followed by a heat shock) are not likely to be found in nature and certainly not in the intestines of warm-blooded animals; this further reduces the probability that recombinant DNA released from phage or bacterial cells would be perpetuated in robust microorganisms in the intestine. Alternatively, DNA might be taken up concomitantly with phage infection (2). Although there is no readily available quantitative data on the titer of free phage in the intestines and no information on the ability of this mechanism to facilitate uptake of chimeric plasmid DNA, the rates of DNA degradation observed would seem to make such a mechanism of in vivo transmission improbable. Based on our data and the above considerations, we are of the opinion that death of disabled hosts such as χ1776 in the intestine will not lead to transmission of recombinant DNA to other enteric bacteria. The only qualification to this conclusion involves the question of whether the intestine contents of recombinant DNA molecule researchers, who are most likely to inadvertently ingest organisms containing recombinant DNA, are comparable to those of rats. However, should the pancreas be the major source of nucleases, it might be suggested that all recombinant DNA molecule researchers eat heartily before undertaking their experiments.

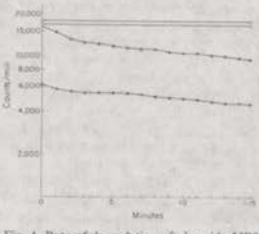


Fig. 4. Rate of degradation of plasmid pMB9 by small intestine contents from conventional rats. Covalently closed circles of the plasmid pMB9 were isolated in a cesium chloride-ethidium bromide gradient (7). The plasmid DNA was added to the intestine contents that were diluted to the extent indicated in BSG plus 10 m*M* $MgSO_4$. Methods were as described in the legend to Fig. 1. Data are for a 1 : 60 dilution of small intestine contents (■); 1 : 60 dilution of boiled small intestine contents (□); a 1 : 240 dilution of small intestine contents (●); and a 1 : 240 dilution of boiled small intestine contents (○). The control, plasmid DNA diluted into BSG plus 10 m*M* $MgSO_4$, remained at approximately 16,900 count/min at 0 and 15 minutes.

since feeding in normal humans stimulates both pancreatic and bile secretions into the duodenum (7). This last suggestion, although somewhat contrary to the advice given in the National Institutes of Health recombinant DNA research guidelines, may prove to be a safer practice when conducting recombinant DNA research with disabled hosts such as χ1776.

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References and Notes

1. R. Curtiss III *et al.*, in *Proceedings of the Tenth Miles International Symposium* (Raven, New York, in press); R. Curtiss III, D. A. Pereira, J. E. Clark, J. C. Hsu, R. Goldschmidt, S. I. Hull, R. Moody, L. Maturin, M. Inoue, in preparation.
2. A. D. Kaiser and D. S. Hogness, *J. Mol. Biol.* **2**, 392 (1960).
3. Conventional rats were COBS derived from Sprague-Dawley whereas the germfree rats were Fischer CDF (344) GN; both stocks were originally from Charles River Breeding Laboratories, Wilmington, Mass.
4. R. Curtiss III, *J. Bacteriol.* **89**, 28 (1965).
5. S. Azhar, H. Mukhtar, G. P. Dutta, V. K. Mohan Rao, *Indian J. Med. Res.* **62**, 573 (1974).
6. S. B. Zimmerman and G. Sardeen, *Anal. Biochem.* **14**, 269 (1966); P. A. Price, T. Y. Liu, W. H. Stein, S. Moore, *J. Biol. Chem.* **244**, 917 (1969).
7. W. F. Ganong, *Review of Medical Physiology* (Lange, Los Altos, Calif., 1967), pp. 404–405.
8. M. Mandel and A. Higa, *J. Mol. Biol.* **53**, 159 (1970); S. N. Cohen, A. C. Y. Chang, L. Hsu, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 2110 (1972); E. M. Lederberg and S. N. Cohen, *J. Bacteriol.* **119**, 1072 (1974).
9. Lysozyme spheroplasts were lysed with 0.1 percent Sarkosyl, heated 10 minutes at 68°C and mixed with cesium chloride-ethidium bromide (density 1.576) and centrifuged to equilibrium. Isopropanol was used to remove ethidium bromide; the dialysis buffer contained 20 m*M* tris, 2 m*M* EDTA, and 10 m*M* NaCl, pH 8.4.
10. The rats were provided by J. McGhee. Supported by NSF grant GB-37546 and NIH grants AI-11456 and DE-02670.

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Probability of Establishing Chimeric Plasmids in Natural Populations of Bacteria

Abstract. *Formulas for estimating the probability that chimeric plasmids carried by disarmed hosts will become established in natural populations of bacteria are presented and their use illustrated with a series of realistic numerical examples. The implications of these a priori probability estimates for the problem of containment for recombinant DNA research is discussed.*

At the very best, physical containment will retard the rate at which the bacteria, phage, and plasmids used in recombinant DNA research enter the environment. Some, and potentially great numbers, of these cloning vectors will escape. However, escape from containment does not necessarily imply that these renegade organisms and plasmids or their chimeric DNA will become established in natural populations of bacteria, or that carriers of this synthetic genetic material will achieve and maintain sufficient densities to represent a threat to existing communities. This contamination of natural populations of bacteria with chimeric DNA ultimately depends on the population biology of the cloning vectors. In recognition of the need for biological containment, "disarmed" strains of bacteria with low fitness have been developed, and phage and plasmids with limited infectious capabilities have been identified for use in recombinant DNA research (*1*). Although there is no question that employing the recommended cloning vectors offers a much lower likelihood of contaminating natural populations with chimeric DNA than that associated with the use of normal laboratory strains, just what this contamination probability is remains uncertain. We believe that there are two difficulties with the estimates of it that have been offered (*1*, *2*): (i) failure of the estimation procedure to consider the population dynamics of the contamination process and (ii) inability to obtain accurate estimates of some of the parameters necessary to compute the contamination probability.

In this report we present a stochastic model of the establishment of a plasmid carried by a disarmed host into a natural population of bacteria. We do consider the population dynamics of the contamination process and, we believe, reasonably accurate estimates of all the parameters of this model could be obtained. Although we restrict this formal consideration to conjugative factors, the theory is appropriate to certain situations where nonconjugative plasmids can be mobilized (*3*). We present formulas for the probability of establishment of a chimeric plasmid and illustrate their use with a series of realistic numerical examples.

In this treatment we define establishment as the indefinite persistence of the chimeric plasmid in a natural population of bacteria. That is, bacteria carrying that plasmid will continue to exist and increase in numbers after all of the original, disarmed, hosts are eliminated. What densities "wild" bacteria carrying that plasmid will maintain and whether these factors will persist if the plasmid reduces the fitness of its host cell have been considered elsewhere (*4*).

Consider the release of m_0 disarmed, plasmid-bearing bacteria into a natural population containing r (bacteria per milliliter) potential hosts of that plasmid. The original hosts are incapable of growth in the natural habitat and, in the course of time, die or effectively die by losing the plasmid (segregation) or the capacity to transmit it. Let the probability of any particular disarmed plasmid-carrying bacterium dying or becoming infertile during the short period δt be $d\delta t$. We assume that all the wild bacteria have the same rates of cell division and cell mortality and that these rates are not influenced by carrying the chimeric plasmid. Let the probabilities of a particular wild bacterium dividing or dying during the period δt be $b\delta t$ and $e\delta t$, respectively. We further assume that transmission of the plasmid by conjugation occurs at random at a frequency that is proportional to the concentration of potential recipients. We let the probability that a single disarmed host will transmit the plasmid during time δt be $g\delta t = \gamma_\theta r\delta t$, where γ_θ (milliliters per cell per hour) is the conjugational transfer rate constant (*4*). The analogous transmission probability for a single wild bacterium carrying that factor is $h\delta t = \gamma_\theta r\delta t$.

With the above definitions and assumptions, the establishment of the plasmid can be regarded as a population process (*5*) to be analyzed by means of generating functions. Using this approach, and after some relatively straightforward, albeit cumbersome, calculations, we concluded that the probability that the plasmid will be lost after the escape of a *single*, disarmed plasmid-bearing cell is

$$P_{\text{loss}} = \frac{d(b + h)}{d(b + h) + g(b + h - e)} \quad (1)$$

and hence the probability that the plasmid will become established is

$$P_{\text{est}} = 1 - P_{\text{loss}} = \frac{g(b + h - e)}{d(b + h) + g(b + h - e)} \quad (2)$$

The calculations that formally prove these results can be obtained from (*6*). In this report we restrict the justification of this probability estimator to an intuitive argument.

With biologically realistic values of the

parameters, the establishment probability is small and Eq. 2 can be very closely approximated by

$$P_{est} = \frac{g}{d} \cdot \frac{b + h - e}{b + h} \quad (3)$$

In this form, the formula more closely approximates one's biological intuition. The first factor, g/d, is the probability that a copy of the plasmid will be transmitted from the original, disarmed host to a wild cell before the original host dies or becomes infertile. The second factor, $(b + h - e)/(b + h)$, is the conditional probability that the wild bearer of this single copy will leave copies of the plasmid in all subsequent generations, given that the copy was transmitted to some wild bacterium. The quotient $1/d$ is the average persistence time of the disarmed host, so g/d is the expected number of copies of that plasmid that will be transmitted by the disarmed host before it dies or becomes infertile. However, it is most unlikely that it will transmit more than one copy during this period. Consequently, this average number of transmitted copies is very nearly the probability that it will transmit one copy. The second factor, $(b + h - e)/(b + h)$, is analogous to the probability that a family name will survive indefinitely in Galton's classical problem (*7*). In this situation the plasmid is the analog of the family name. When a change in the number of plasmid copies occurs, it occurs either because one copy has doubled (by cell division or conjugational transmission) or because the plasmid is lost (due to its host's death or infertility), the probability of doubling being $B = (b + h)/(b + h + e)$ and the probability of loss being $D = e/(b + h + e)$. The probability that the plasmid will be established, given that a wild bacterium has acquired it, is then

$$\frac{B - D}{B} = \frac{b + h - e}{b + h}$$

If, instead of a single disarmed, plasmid-bearing bacterium, a number m_0 escaped, establishment of the plasmid would be a consequence of m_0 stochastically independent trials. The probability of establishment is then

$$P_{est;m_0} = 1 - (P_{loss})^{m_0} = 1 - (1 - P_{est})^{m_0}$$

which, for the anticipated small values of P_{est}, is

$$P_{est;m_0} = 1 - \exp(-m_0 P_{est}) \quad (4)$$

For very low values of P_{est}

$$P_{est;m_0} \approx m_0 P_{est}$$

Table 1. Probability that chimeric plasmids will be established in natural populations of bacteria following the release of different numbers of disarmed carriers. The parameters are g, the product of the concentration of potential recipients, r, and the transfer rate constant of the plasmid in a disarmed host, γ_0; h, the product of r and the transfer rate constant of the plasmid in a wild host, γ_h; d, the death rate of disarmed cells; b, the probability a wild bacterium dividing; and e, the probability of a wild bacterium dying. The choice of these parameter values is justified in the text. The probability of establishment is computed for single cells ($m_0 = 1$) from Eq. 2 and extended for the release of multiple cells ($m_0 > 1$) with Eq. 4.

Parameter values			Probability of establishment			
g,h	d	b,e	$m_0 = 1$	$m_0 = 10^2$	$m_0 = 10^3$	$m_0 = 10^4$
5×10^{-5}	0.58	0.20	1.7×10^{-5}	8.2×10^{-3}	1.0*	1.0*
		0.02	5.8×10^{-6}	1.0*	1.0*	1.0*
	2.30	0.20	4.3×10^{-5}	3.5×10^{-3}	1.0*	1.0*
		0.02	1.5×10^{-5}	8.6×10^{-3}	1.0*	1.0*
5×10^{-4}	0.58	0.20	2.1×10^{-4}	2.1×10^{-2}	7.0×10^{-1}	1.0*
		0.02	2.1×10^{-5}	2.1×10^{-3}	8.7×10^{-1}	1.0*
	2.30	0.20	5.4×10^{-5}	5.4×10^{-3}	5.3×10^{-2}	1.0*
		0.02	5.3×10^{-6}	5.3×10^{-4}	4.1×10^{-1}	1.0*
5×10^{-5}	0.58	0.20	2.2×10^{-5}	2.2×10^{-3}	2.1×10^{-2}	8.8×10^{-1}
		0.02	2.2×10^{-7}	2.2×10^{-5}	2.1×10^{-3}	1.0*
	2.30	0.20	5.4×10^{-9}	5.4×10^{-7}	9.4×10^{-5}	4.2×10^{-1}
		0.02	5.4×10^{-6}	5.4×10^{-4}	5.4×10^{-2}	9.9×10^{-1}
5×10^{-7}	0.58	0.20	2.2×10^{-10}	2.2×10^{-8}	2.2×10^{-6}	2.2×10^{-4}
		0.02	2.2×10^{-11}	2.2×10^{-9}	2.2×10^{-7}	2.2×10^{-5}
	2.30	0.20	5.4×10^{-10}	5.4×10^{-11}	5.4×10^{-9}	5.4×10^{-7}
		0.02	5.4×10^{-10}	5.4×10^{-10}	5.4×10^{-7}	5.4×10^{-4}

*The probability of this event exceeds .995.

The numerical values used to generate the examples presented in Table 1 were chosen to illustrate the relative contributions of the different parameters to the establishment probability. However, these sample parameter values are within a realistic range for natural populations of enteric bacteria and some of their conjugative plasmids. In these examples, the conjugational transfer rate constants of the plasmid in the disarmed host and in the wild host are equal, $\gamma_0 = \gamma_h = \gamma$. The two values of γ used, 5×10^{-10} and 5×10^{-13} ml per cell per hour, are, respectively, the value we estimated for an F-lac$^+$pro$^+$ plasmid in an *Escherichia coli* K12 host growing exponentially in glucose-limited minimal medium at 37°C, and the value for these bacteria at equilibrium in chemostats at a dilution rate of 0.2 hour^{-1} with that medium and at that temperature. We used two values for the concentration of potential recipients $r = 10^6$ and $r = 10^8$ cell/ml. The first is the density considered by Curtiss and co-workers (*1*, *2*) in their estimates of the contamination probability. However, we believe that the higher value, $r = 10^8$, is realistic for some natural populations of enteric bacteria. The two mortality probabilities for disarmed bacteria that we used, $d = 0.58$ and $d = 2.30$ hour^{-1}, are those estimated by Curtiss and his colleagues (*8*) for EK2 (strain χ1776) in minimal medium and in broth without the specific EK2 supplements, respectively. In these examples we consider the natural population to be at a steady state so that the probability of a wild bacterium dividing is equal to the probability of a wild cell dying, $b = e$. The two values of these parameters used, 0.20 and 0.02 hour^{-1}, specify steady-state generation times of 5 and 50 hours, respectively.

In interpreting the estimates presented in Table 1, it is necessary to consider that the probabilities of establishment given are those for single escape events. The inadvertent release of bacteria carrying chimeric plasmids is, however, an event that can be anticipated to occur many times and from many different laboratories doing recombinant DNA research. Nevertheless, even for single escape events, the establishment probabilities for the conjugative plasmids considered in the numerical examples above are extremely high. These results unquestionably support the existing ban on using conjugative plasmids as cloning vectors. These theoretical considerations also illustrate the utility of efforts at biological containment. In the range of parameter values we anticipate for most recombinant DNA research, the probability of establishment of the chimeric plasmid is directly proportional to the fertility of the host-plasmid combination, as measured by the transfer rate constant, γ_d, and inversely proportional to the death rate of the host cell, d. An order of magnitude decrease in this fertility or an order of magnitude increase in the death rate of the disarmed host is reflected by an order of magnitude decline in

the establishment probability. These results also illustrate the value of combining biological containment with strict physical containment. In the anticipated parameter range, the establishment probability is directly proportional to the number of plasmid-carrying bacteria released into the environment.

Since the rate of transfer of nonconjugative plasmids is proportional to the density of bacteria carrying mobilizing conjugative plasmids (*1*, *2*), the model used here is not appropriate as a general analog of this situation. However, this model and the probability estimators derived from its analysis do apply to two special cases of nonconjugative plasmids. (i) In steady-state natural populations it is reasonable to assume that the frequency of bacteria carrying potentially mobilizing plasmids would be relatively constant and, as a result, the average transfer rate parameters, γ_0 and γ_n, would also be relatively constant. Although it is reasonable to assume that, in general, these "mobilization-transfer" rate parameters would be lower than the analogous parameters for conjugative plasmids, there is evidence that for some nonconjugative plasmids transfer by mobilization can occur at substantial rates (*2*). (ii) It is conceivable that chimeric DNA carried by a nonconjugative plasmid will become incorporated into a conjugative factor. After such an event, the probabilities that chimeric DNA will persist could well be as high as or even higher than those presented in the numerical examples. At this juncture it is not at all clear just how great the probability is that DNA from a nonconjugative plasmid will be permanently incorporated into a conjugative factor. It is, however, our contention that estimates of this probability are essential to a full evaluation of the dangers of recombinant DNA research.

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References and Notes

1. R. Curtiss, III, *Annu. Rev. Microbiol.* **30**, 507 (1976).
2. ——— *et al.*, in *Proceedings of the Third International Symposium on Antibiotic Resistance* (Castle of Smolenice, Czechoslovakia, in press).
3. R. P. Novick, R. C. Clowes, S. N. Cohen, R. Curtiss, III, N. Datta, S. Falkow, *Bacteriol. Rev.* **40**, 169 (1976).
4. F. M. Stewart and B. R. Levin, unpublished submitted manuscript.
5. M. S. Bartlett, *Stochastic Population Models in Ecology and Epidemiology* (Methuen, London, 1960).
6. F. M. Stewart, Department of Mathematics, Brown University, Providence, Rhode Island.
7. A. J. Lotka, *Theorie Analytique des Associations Biologique* (Actualités Scientifique et Industrielle No. 780, Hermann, Paris, 1939), vol. 2, pp. 123–136; D. Ludwig, *Stochastic Population Theories*, notes taken by M. Levandowsky (Springer-Verlag, Berlin, 1974).
8. R. Curtiss, III, personal communication.

9. We wish to thank R. M. Curtiss, III, for making his unpublished data available to us and for the very useful comments he made during our preparation of this report. Supported by NIH grant GM 19648 and NIH research career development award K04 GM 00112 to B.R.L.

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Interbacterial Transfer of *Escherichia coli*–*Drosophila melanogaster* Recombinant Plasmids

Abstract. *Recombinants were constructed between various* Escherichia coli *plasmids and fragments of* Drosophila melanogaster *DNA. These recombinant plasmids are nonconjugative, but can be mobilized from one cell to another by conjugative sex factors. Of 47 recombinants studied, 46 were mobilized at approximately the same or slightly lower frequencies than the parental plasmids, whereas one was mobilized 1000 times less efficiently.*

Most of the plasmids used as molecular cloning vehicles are nonconjugative; that is, they are not transferred from one cell to another under normal mating conditions. However, when a cell harboring a nonconjugative plasmid also carries a conjugative sex factor, the nonconjugative plasmid can be passively transferred to an appropriate recipient. This process, referred to as mobilization, is one possible route by which recombinant plasmids might be disseminated in animal and human populations (*1*).

Does foreign DNA inserted into a plasmid affect its mobilization? To approach this question, I determined mobilization frequencies for a series of recombinants between various *Escherichia coli* plasmids and fragments of *Drosophila melanogaster* DNA. Of 47 recombinants studied, 46 were mobilized with approximately the same or slightly lower efficiencies than the parental plasmids, whereas one was mobilized very poorly. The low mobilizability of the latter recombinant was shown to be due to some specific effect of the inserted *D. melanogaster* DNA.

The first determinations were made on recombinants between pSC101 (*2*), a tetracycline resistance factor that replicates under stringent control, and Eco RI fragments of *D. melanogaster* DNA. Preliminary experiments showed that pSC101 could be mobilized readily by the *Salmonella* sex factors Col Ib and I. Table 1 presents the results of triparental matings in which the donor harbored pSC101 or a pSC101/Eco RI–*D. melanogaster*/Eco RI recombinant, and the intermediate carried Col Ib or I. Of the five recombinants studied, four were mobilized at about the same frequency as the parental plasmid. However, one of the recombinants, pDm2, was mobilized 500 to 1000 times less efficiently by both sex factors.

Why is pDm2 mobilized so poorly?

Table 1. Mobilization of pSC101 and pSC101/Eco RI–*Drosophila melanogaster*/Eco RI recombinants by Col Ib and I. Triparental matings were performed by the method of Anderson and Lewis (*6*), using *E. coli* C spc^R as the final recipient. No transconjugants were observed in control matings in which one of the parents was omitted, or in which the donor carried no plasmid. Strain GM4 is *E. coli* HB101 r_K^- m_K^- pro$^-$ str^R gal$^-$ lac$^-$ ara$^-$ arg$^-$ *rec*A (*7*). The construction and characterization of the recombinant plasmids has been described (*8*).

Donor	Length of *D. melanogaster* DNA fragment (kilobase pairs)	Intermediate	Frequency of mobilization (transconjugants per donor)
GM4(pSC101)	0	*S. typhimurium* (Col Ib)	3.0×10^{-1}
		S. panama (I)	1.4×10^{-1}
GM4(pDm1)	4.4, 3.3	*S. typhimurium* (Col Ib)	1.2×10^{-1}
		S. panama (I)	0.8×10^{-1}
GM4(pDm2)	3.1	*S. typhimurium* (Col Ib)	2.8×10^{-3}
		S. panama (I)	3.1×10^{-3}
GM4(pDM3)	2.9	*S. typhimurium* (Col Ib)	1.8×10^{-1}
		S. panama (I)	1.2×10^{-1}
GM4(pDm4)	0.3	*S. typhimurium* (Col Ib)	2.7×10^{-1}
		S. panama (I)	1.2×10^{-1}
GM4(pDm5)	1.5	*S. typhimurium* (Col Ib)	2.8×10^{-1}
		S. panama (I)	0.9×10^{-1}

Table 2. Studies on the low mobilizability of pDm2. The final recipient was *E. coli* C spc^R, except in experiment 3, in which it was *E. coli* C str^R. Strain GM3 is *E. coli* C600 r_k^- m_k^+ thr$^-$ leu$^-$ lac$^-$ Bl$^-$ (9). The construction and characterization of the recombinant plasmids has been described (*4*, *8*).

Donor	Intermediate	Frequency of mobilization (transconjugants per donor)
	Experiment 1	
GM4 (pSC101)	*S. typhimurium* (Col Ib)	0.8×10^{-4}
GM4 (pDm2)	*S. typhimurium* (Col Ib)	1.2×10^{-5}
GM3 (pSC101)	*S. typhimurium* (Col Ib)	3.2×10^{-4}
GM3 (pDm2)	*S. typhimurium* (Col Ib)	4.8×10^{-5}
	Experiment 2	
GM4 (pSC101)	*S. typhimurium* (Col Ib)	5.2×10^{-4}
GM4 (pDm2)	*S. typhimurium* (Col Ib)	7.4×10^{-5}
GM4 (pDm2/Eco RI → pSC101)	*S. typhimurium* (Col Ib)	6.8×10^{-4}
	Experiment 3	
C spc^R (pSC101, Col Ib)		8.1×10^{-4}
C spc^R (pDm2, Col Ib)		7.4×10^{-4}
	Experiment 4	
GM4 (pSC101)	*S. typhimurium* (Col Ib)	4.2×10^{-5}
	S. panama (I)	1.6×10^{-5}
GM4 (pML21)	*S. typhimurium* (Col Ib)	1.4×10^{-4}
	S. panama (I)	0.7×10^{-4}
GM4 (pML21/Eco RI–Dm2)	*S. typhimurium* (Col Ib)	2.1×10^{-5}
	S. panama (I)	0.6×10^{-5}
GM4 (pGM437 = pSC101/Sal I–pML21/Sal I)	*S. typhimurium* (Col Ib)	0.8×10^{-5}
	S. panama (I)	1.6×10^{-5}
GM4 (pGM439 = pSC101/Sal I–pML21/Sal I)	*S. typhimurium* (Col Ib)	1.2×10^{-5}
	S. panama (I)	0.9×10^{-5}
GM4 (pGM16 = pSC101/Eco RI–pML21/Eco RI)	*S. typhimurium* (Col Ib)	1.5×10^{-4}
	S. panama (I)	0.8×10^{-5}

Table 2, experiment 1, shows that this was not due to a host mutation, nor to a specific interaction with the host strain, since the same result was obtained after transformation into a new donor. Table 2, experiment 2, shows that the low mobilizability of pDm2 was not due to a mutation in the bacterial fragment, since a pSC101 plasmid generated by Eco RI cleavage of pDm2 was mobilized at the same frequency as the original pSC101. As shown in Table 2, experiment 3, the low mobilizability of pDm2 persisted in two parent crosses in which the donor harbored both the recombinant and Col Ib. Furthermore, strains harboring pDm2 were as good recipients for Col Ib as were strains harboring pSC101 (results not shown). This rules out the possibility that pDm2 renders its host resistant to infection by the sex factor. Thus, the low mobilizability of pDm2 must be attributed to some specific effect of the inserted *D. melanogaster* DNA.

Is this effect exerted only on pSC101, or is it general? To answer this, the Dm2 fragment was translocated to pML21 (*3*), a mini Col EI–kanamycin resistance factor. Table 2, experiment 4, shows that this recombinant was mobilized at the same frequency as pML21 by both Col Ib and I. This suggests that pSC101 and pML21 are mobilized by different mechanisms. Further evidence for this comes from the surprising finding that hybrids between pSC101 and pML21 are mobilized up to 100 times more efficiently than either parental plasmid (Table 2, experiment 4).

Frequencies of Col Ib–mediated mobilization were also determined for 20 recombinants carrying Sal I fragments and for 22 recombinants carrying Bam I fragments of *D. melanogaster* DNA. The Sal I fragments were cloned on pML21 (*3*), whereas the Bam I fragments were cloned on pGM16 (*4*); both of these vehicles replicate under relaxed control. All of the recombinants were mobilized 20 to 100 percent as well as the parental plasmids (results not shown).

These limited data suggest that, in general, the insertion of foreign DNA into a plasmid has little effect on its mobilizability. In the one case where a significant change was noted, it was to decrease the mobilization frequency. No cases of increased mobilizability have been noted.

The transfer experiments described in this report were conducted under conditions of low risk as advised by the "Summary statement of the Asilomar conference on recombinant DNA molecules" (*5*). More stringent containment procedures are required by the National Institutes of Health guidelines (*1*).

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References and Notes

1. "Guidelines for research involving recombinant DNA molecules," *Fed. Regist.* **41** (No. 131), 27917 (1976).
2. S. N. Cohen and A. C. Y. Chang, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 1293 (1973); ———, H. W. Boyer, R. B. Helling, *ibid.*, p. 3240.
3. V. Hershfield, H. W. Boyer, C. Yanofsky, M. A. Lovett, D. R. Helinski, *ibid.* **71**, 3455 (1974).
4. D. H. Hamer and C. A. Thomas, Jr., *ibid.* **73**, 1437 (1976).
5. P. Berg, D. Baltimore, S. Brenner, R. O. Roblin, M. F. Singer, *ibid.* **72**, 1981 (1975).
6. E. S. Anderson and M. J. Lewis, *Nature (London)* **208**, 843 (1965).
7. H. W. Boyer and R. Roylland-Dussoix, *J. Mol. Biol.* **41**, 459 (1969).
8. D. H. Hamer, thesis, Harvard Medical School (1976).
9. Strain GM3 was provided by J. Morrow.
10. I thank C. A. Thomas, Jr., for support, facilities, and suggestions.

8 February 1977

Senator KENNEDY. The subcommittee will adjourn, and we will keep the record open until Monday noon.

[Whereupon, at 4:07 p.m., the subcommittee was adjourned.]

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