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Asilomar Conference on Recombinant DNA Molecules*

Paul Berg, David Baltimore, Sydney Brenner, Richard O. Roblin III, Maxine F. Singer

1. Introduction and General Conclusions

This meeting was organized to review scientific progress in research on recombinant DNA molecules and to discuss appropriate ways to deal with the potential biohazards of this work. Impressive scientific achievements have already been made in this field, and these techniques have a remarkable potential for furthering our understanding of fundamental biochemical processes in pro- and eukaryotic cells. The use of recombinant DNA methodology promises to revolutionize the practice of molecular biology. Although there has as yet been no practical application of the new techniques, there is every reason to believe that they will have significant practical utility in the future.

Of particular concern to the participants at the meeting was the issue of whether the pause in certain aspects of research in this area, called for by the Committee on Recombinant DNA Molecules of the National Academy of Sciences in the letter published in July 1974 (*1*), should end, and, if so, how the scientific work could be undertaken with minimal risks to workers in laboratories, to the public at large, and to the animal and plant species sharing our ecosystems.

The new techniques, which permit combination of genetic information from very different organisms, place us in an area of biology with many unknowns. Even in the present, more limited conduct of research in this field, the evaluation of potential biohazards has proved to be extremely difficult. It is this ignorance that has compelled us to conclude that it would be wise to exercise considerable caution in performing this research. Nevertheless, the participants at the Conference agreed that most of the work on construction of recombinant DNA molecules should proceed, provided that appropriate safeguards, principally biological and physical barriers adequate to contain the newly created organisms, are employed. Moreover, the standards of protection should be

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greater at the beginning and modified as improvements in the methodology occur and assessments of the risks change. Furthermore, it was agreed that there are certain experiments in which the potential risks are of such a serious nature that they ought not to be done with presently available containment facilities. In the longer term serious problems may arise in the large-scale application of this methodology in industry, medicine, and agriculture. But it was also recognized that future research and experience may show that many of the potential biohazards are less serious and/or less probable than we now suspect.

II. Principles Guiding the Recommendations and Conclusions

Although our assessments of the risks involved with each of the various lines of research on recombinant DNA molecules may differ, few, if any, believe that this methodology is free from any risk. Reasonable principles for dealing with these potential risks are: (i) that containment be made an essential consideration in the experimental design and (ii) that the effectiveness of the containment should match, as closely as possible, the estimated risk. Consequently, whatever scale of risks is agreed upon, there should be a commensurate scale of containment. Estimating the risks will be difficult and intuitive at first, but this will improve as we acquire additional knowledge; at each stage we shall have to match the potential risk with an appropriate level of containment. Experiments requiring large-scale operations would seem to be riskier than equivalent experiments done on a small scale and therefore require more stringent containment procedures. The use of cloning vehicles or vectors (plasmids, phages) and bacterial hosts with a restricted capacity to multiply outside of the laboratory would reduce the potential biohazard of a particular experiment. Thus, the ways in which potential biohazards and different levels of containment are matched may vary from time to time, particularly as the containment technology is improved. The means for assessing and balancing risks with appropriate levels of containment will need to be reexamined from time to time. Hopefully, through formal and informal channels of information within and between nations of the world, the way in which potential biohazards and levels of containment are matched would be consistent.

Containment of potentially biohazardous agents can be achieved in several ways. The most significant contribution to limiting the spread of the recombinant DNA's is the use of biological barriers. These barriers are of two types: (i) fastidious bacterial hosts unable to survive in natural environments and (ii) nontransmissible and equally fastidious vectors (plasmids, bacteriophages, or other viruses) able to grow only in specified hosts. Physical containment, exemplified by the use of suitable hoods or, where applicable, limited access or negative pressure laboratories, provides an additional factor of safety. Particularly important is strict adherence to good microbiological practices which, to a large measure, can limit the escape of organisms from the experimental situation and thereby increase the safety of the operation. Consequently, education and training of all personnel involved in the experiments is essential to the effectiveness of all containment measures. In practice, these different means of containment will complement one another and documented substantial improvements in the ability to restrict the growth of bacterial hosts and vectors could permit modifications of the complementary physical containment requirements.

Stringent physical containment and rigorous laboratory procedures can reduce but not eliminate the possibility of spreading potentially hazardous agents. Therefore, investigators relying upon "disarmed" hosts and vectors for additional safety must rigorously test the effectiveness of these agents before accepting their validity as biological barriers.

III. Recommendations for Matching Types of Containment with Types of Experiments

No classification of experiments as to risk and no set of containment procedures can anticipate all situations. Given our present uncertainties about the hazards, the parameters proposed here are broadly conceived and meant to provide provisional guidelines for investigators and agencies concerned with research on combinant DNA's. However, each inve gator bears a responsibility for determ ing whether, in his particular case, spec circumstances warrant a higher level containment than is suggested here.

A. Types of Containment

1) *Minimal risk.* This type of conta ment is intended for experiments in wh the biohazards may be accurately asses and are expected to be minimal. Such c tainment can be achieved by following operating procedures recommended clinical microbiological laboratories. sential features of such facilities are drinking, eating, or smoking in the labo tory, wearing laboratory coats in the w area, the use of cotton-plugged pipettes preferably mechanical pipetting devic and prompt disinfection of contamina materials.

2) *Low risk.* This level of containmen appropriate for experiments which gen ate novel biotypes but where the availa information indicates that the recombin DNA cannot alter appreciably the ecolo cal behavior of the recipient species, crease significantly its pathogenicity, prevent effective treatment of any result infections. The key features of this c tainment (in addition to the minimal p cedures mentioned above) are a proh tion of mouth pipetting, access limited laboratory personnel, and the use of b logical safety cabinets for procedu likely to produce aerosols (for exam blending and sonication). Though exist vectors may be used in conjunction w low-risk procedures, safer vectors hosts should be adopted as they beco available.

3) *Moderate risk.* Such containment cilities are intended for experiments which there is a probability of generat an agent with a significant potential pathogenicity or ecological disruption. principal features of this level of conta ment, in addition to those of the two p ceding classes, are that transfer operati should be carried out in biological sa cabinets (for example, laminar f hoods), gloves should be worn during handling of infectious materials, vacu lines must be protected by filters, and n ative pressure should be maintained in limited access laboratories. Moreove periments posing a moderate risk m done only with vectors and hosts tha an appreciably impaired capacity to ply outside of the laboratory.

4) *High risk.* This level of contain is intended for experiments in whic

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potential for ecological disruption or pathogenicity of the modified organism could severe and thereby pose a serious biohazard to laboratory personnel or the public. The main features of this type of facility, which was designed to contain highly infectious microbiological agents, are its isolation from other areas by air locks, a negative pressure environment, a requirement for clothing changes and showers for entering personnel, and laboratories fitted with treatment systems to inactivate or remove biological agents that may be contaminants in exhaust air and liquid and solid wastes. All persons occupying these areas should wear protective laboratory clothing and shower at each exit from the containment facility. The handling of agents should be confined to biological safety cabinets in which the exhaust air is incinerated or passed through Hepa filters. High-risk containment includes, in addition to the physical and procedural features described above, the use of rigorously tested vectors and hosts whose growth can be confined to the laboratory.

B. Types of Experiments

Accurate estimates of the risks associated with different types of experiments are difficult to obtain because of our ignorance of the probability that the anticipated dangers will manifest themselves. Nevertheless, experiments involving the construction and propagation of recombinant DNA molecules using DNA's from (i) prokaryotes, bacteriophages, and other plasmids; (ii) animal viruses; and (iii) eukaryotes have been characterized as minimal, low, moderate, and high risks to guide investigators in their choice of the appropriate containment. These designations should be viewed as interim assignments which will need to be revised upward or downward in the light of future experience.

The recombinant DNA molecules themselves, as distinct from cells carrying them, may be infectious to bacteria or higher organisms. DNA preparations from these experiments, particularly in large quantities, should be chemically inactivated before disposal.

1) *Prokaryotes, bacteriophages, and bacterial plasmids.* Where the construction of recombinant DNA molecules and their propagation involve prokaryotic agents that are known to exchange genetic information naturally, the experiments can be performed in minimal-risk containment facilities. Where such experiments pose a potential hazard, more stringent containment may be warranted.

Experiments involving the creation and propagation of recombinant DNA molecules from DNA's of species that ordinarily do not exchange genetic information generate novel biotypes. Because such experiments may pose biohazards greater than those associated with the original organisms, they should be performed, at least, in low-risk containment facilities. If the experiments involve either pathogenic organisms or genetic determinants that may increase the pathogenicity of the recipient species, or if the transferred DNA can confer upon the recipient organisms new metabolic activities not native to these species and thereby modify its relationship with the environment, then moderate- or high-risk containment should be used.

Experiments extending the range of resistance of established human pathogens to therapeutically useful antibiotics or disinfectants should be undertaken only under moderate- or high-risk containment, depending upon the virulence of the organism involved.

2) *Animal viruses.* Experiments involving linkage of viral genomes or genome segments to prokaryotic vectors and their propagation in prokaryotic cells should be performed only with vector-host systems having demonstrably restricted growth capabilities outside the laboratory and with moderate-risk containment facilities. Rigorously purified and characterized segments of nononcogenic viral genomes or of the demonstrably nontransforming regions of oncogenic viral DNA's can be attached to presently existing vectors and propagated in moderate-risk containment facilities; as safer vector-host systems become available such experiments may be performed in low-risk facilities.

Experiments designed to introduce or propagate DNA from nonviral or other low-risk agents in animal cells should use only low-risk animal DNA's as vectors (for example, viral or mitochondrial), and manipulations should be confined to moderate-risk containment facilities.

3) *Eukaryotes.* The risks associated with joining random fragments of eukaryote DNA to prokaryotic DNA vectors and the propagation of these recombinant DNA's in prokaryotic hosts are the most difficult to assess.

A priori, the DNA from warm-blooded vertebrates is more likely to contain cryptic viral genomes potentially pathogenic for man than is the DNA from other eukaryotes. Consequently, attempts to clone segments of DNA from such animals and particularly primate genomes should be performed only with vector-host systems having demonstrably restricted growth capabilities outside the laboratory and in a moderate-risk containment facility. Until cloned segments of warm blood vertebrate DNA are completely characterized, they should continue to be maintained in the most restricted vector-host system in moderate-risk containment laboratories; when such cloned segments are characterized, the may be propagated as suggested above for purified segments of virus genomes.

Unless the organism makes a product known to be dangerous (for example, a toxin or virus), recombinant DNA's from cold-blooded vertebrates and all other lower eukaryotes can be constructed and propagated with the safest vector-host system available in low-risk containment facilities.

Purified DNA from any source that performs known functions and can be judged to be nontoxic may be cloned with currently available vectors in low-risk containment facilities. (Toxic here includes potentially oncogenic products or substances that might perturb normal metabolism if produced in an animal or plant by a resident microorganism.)

4) *Experiments to be deferred.* There are feasible experiments which present such serious dangers that their performance should not be undertaken at this time with the currently available vector-host systems and the presently available containment capability. These include the cloning of recombinant DNA's derived from highly pathogenic organisms (that is, Class III, IV, and V etiologic agents as classified by the U.S. Department of Health, Education and Welfare), DNA containing toxin genes, and large-scale experiments (more than 10 liters of culture) using recombinant DNA's that are able to make products potentially harmful to man, animals, or plants.

IV. Implementation

In many countries steps are already being taken by national bodies to formulate codes of practice for the conduct of experiments with known or potential biohazard (2). Until these are established, we urge individual scientists to use the proposals in this document as a guide. In addition, there are some recommendations which could be immediately and directly implemented by the scientific community.

A. Development of Safer Vectors and Hosts

An important and encouraging accomplishment of the meeting was the realiza-

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tion that special bacteria and vectors, which have a restricted capacity to multiply outside the laboratory, can be constructed genetically, and that the use of these organisms could enhance the safety of recombinant DNA experiments by many orders of magnitude. Experiments along these lines are presently in progress and, in the near future, variants of λ bacteriophage; nontransmissible plasmids, and special strains of *Escherichia coli* will become available. All of these vectors could reduce the potential biohazards by very large factors and improve the methodology as well. Other vector-host systems, particularly modified strains of *Bacillus subtilis* and their relevant bacteriophages and plasmids, may also be useful for particular purposes. Quite possibly safe and suitable vectors may be found for eukaryotic hosts such as yeast and readily cultured plant and animal cells. There is likely to be a continuous development in this area, and the participants at the meeting agreed that improved vector-host systems which reduce the biohazards of recombinant DNA research will be made freely available to all interested investigators

B. Laboratory Procedures

It is the clear responsibility of the principal investigator to inform the staff of the laboratory of the potential hazards of such experiments, before they are initiated. Free and open discussion is necessary so that each individual participating in the experiment fully understands the nature of the experiment and any risk that might be involved. All workers must be properly trained in the containment procedures that are designed to control the hazard, including emergency actions in the event of a hazard. It is also recommended that appropriate health surveillance of all personnel, including serological monitoring, be conducted periodically.

C. Education and Reassessment

Research in this area will develop very quickly, and the methods will be applied to many different biological problems. At any given time it is impossible to foresee the entire range of all potential experiments and make judgments on them. Therefore, it is essential to undertake a continuing reassessment of the problems in the light of new scientific knowledge. This could be achieved by a series of annual workshops and meetings, some of which should be at the international level. There should also be courses to train individuals in the relevant methods, since it is likely that the work will be taken up by laboratories which may not have had extensive experience in this area. High priority should also be given to research that could improve and evaluate the containment effectiveness of new and existing vector-host systems.

V. New Knowledge

This document represents our first assessment of the potential biohazards in the light of current knowledge. However, little is known about the survival of laboratory strains of bacteria and bacteriophages in different ecological niches in the outside world. Even less is known about whether recombinant DNA molecules will enhance or depress the survival of their vectors and hosts in nature. These questions are fundamental to the testing of any new organism that may be constructed. Research in this area needs to be undertaken and should be given high priority. In general, however, molecular biologists who may construct DNA recombinant molecules do not undertake these experiments and it will be necessary to facilitate collaborative research between them and groups skilled in the study of bacterial infection or ecological microbiology. Work should also be undertaken which would enable us to monitor the escape or dissemination of cloning vehicles and their hosts.

Nothing is known about the potential infectivity in higher organisms of phages or bacteria containing segments of eukaryotic DNA, and very little is known about the infectivity of the DNA molecules themselves. Genetic transformation of bacteria does occur in animals, suggesting that recombinant DNA molecules can retain their biological potency in this environment. There are many questions in this area, the answers to which are essential for our assessment of the biohazards of experiments with recombinant DNA molecules. It will be necessary to ensure that this work will be planned and carried out; and it will be particularly important to have this information before large-scale applications of the use of recombinant DNA molecules are attempted.

References and Notes

1. P. Berg *et al.*, *Science* 185, 303 (1974).
2. Advisory Board for the Research Councils, "Report of the Working Party on the Experiments: Manipulation of the Genetic Composition of Micro-Organisms, Presented to Parliament by the Secretary of State for Education and Science by Command of Her Majesty, January 1975" (Her Majesty's Stationery Office, London, 1975). National Institutes of Health Recombinant DNA Molecule Program Advisory Committee, Bethesda, Maryland.
3. The work of the committee was assisted by the National Academy of Sciences–National Research Council Staff: Artemis P. Simopoulos, Executive Secretary, Division of Medical Sciences, Assembly of Life Sciences; Elena O. Nightingale, Resident Fellow, Division of Medical Sciences, Assembly of Life Sciences. Supported by the National Institutes of Health (contract NO1-OD-52123) and the National Science Foundation (grant GEMS7-05293). Requests for reprints should be addressed to: Division of Medical Sciences, Assembly of Life Sciences, National Academy of Sciences, 2101 Constitution Avenue, NW, Washington, D.C. 20418.

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