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The Recombinant DNA Debate

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The questions surrounding the recombinant DNA research debate are not just questions of technique and safety. They involve the driving forces of scientific research, especially those premises and presuppositions concerning the expansion of knowledge versus our ability to use that knowledge wisely. Basically, we ask if policy--scientific, industrial, or political--should be an integral part of our future steps in recombinant DNA research and development.

It is obvious from past mistakes involving pollution, waste of fossil fuels, and overmechanization that we must try to avoid the crucial tendency that technology has of overrunning common sense and moral guidelines. This is especially true in light of the fact that we are looking at an area of research in which results are probably the most unpredictable of any area of biochemistry. There comes a point at which the scientist, seeing himself as providing good for mankind, becomes hostile at the thought of the regulator burdening down progress with red tape. The regulator, on the other hand, views the scientist as being too ambitious and uncontrolled, and sees himself as a protector of the "real world" from the eccentricities of the research scientist.

In the recombinant DNA question, this conflict becomes strikingly real. The techniques and ideas are no longer hypothetical or theory; they are available for widespread use.

A method of reducing risks while maintaining the potential benefits of recombinant DNA research must be found. On this point, there seems to be widespread agreement among scientists and lay-people. The real debate begins when the regulations for accomplishing this goal are proposed. Not only are the regulations themselves debated, but the validity of them is also a focal point of debate. Here, the challenge to validity comes from the rapidly changing knowledge concerning recombinant DNA techniques. Possibly, the answer is not in a set of fixed rules, but in rules that can be easily accommodated to current knowledge without endangering the public or the environment.

Therefore, the following is a review of facts concerning recombinant DNA research, starting with the basic premises of molecular genetics and then reviewing National Institutes of Health guidelines, testimony before the United States House of Representatives Subcommittee on Science and Technology, and transcripts of the National Academy of Sciences' Forum on Recombinant DNA research. Within this review, the risks, benefits, and existing regulations will be discussed, concluding with a general summary and commentary. It is hoped that this paper will serve as an informative summary of the author's three semester study of the recombinant DNA research policy debate.

To understand the nature of deoxyribonucleic acids, hereafter referred to as DNA, one must first gain an appreciation for the cells and some viruses in which DNA directs chemical reactions in the cycles which we recognize as life. In the case of viruses, the distinction between life and mere chemical reactions is still not clear, but the basic mechanisms of chemistry that are involved are the same as for the cells. The separation of organic and inorganic chemistry, as in living and non-living, has no real biological basis.

However, even through the first quarter of the twentieth century, there was a strong tendency towards a theory of "vitalism", that certain rules outside of the laws of chemistry differentiated between living and non-living entities.[1] As techniques became more sophisticated, the existence of certain macromolecules, especially proteins, was demonstrated. It was not until the elucidation of the basic structure of proteins as being alpha helical that immense strides were made towards understanding basic chemistry which determines the direction of life.[2] The direction of biochemical research before the elucidation of the molecular structure of proteins was toward studies on metabolism of the cell.[3] However, as metabolic pathways within the cell were worked out, it was found that a specific enzyme must mediate each step of individual pathways. This became especially obvious in the elucidation of the Embden-Meyerhof pathway, which is the stepwise degradation of glucose to pyruvic

acid. It was noted that enzymes caused reactions between molecules to occur at an increased rate, yet at a lower energy level than the reaction would normally require, i.e. at a lowered activation energy.[4] Yet the manner of the initial formation of enzymes was still unknown.

It is here that the role of DNA became of prime interest. Together with its associated proteins, DNA was discovered in 1890 by the German chemist, Miescher, in the nuclei obtained from pus. By the use of specific staining procedures, DNA was localized in the chromosomes in the nuclei of cells by Feulgen in 1924, and 20 years later, DNA was shown to be the crucial molecule of heredity by Avery, McCarty, and McLeod.[5] Chargaff, by use of paper chromotography, was able to analyze the nucleotide composition of DNA molecules from a number of organisms. He showed that the four nucleotides are not found in equal amounts and that their ratios to each other vary from species to species. This opened up the possibility of genetic specificity according to precise nucleotide arrangement. It later became evident that the amount of adenine was always equal to the amount of thymine, and the amount of guanine was always equal to the amount of cytosine. The real impact of these results was not really evident until the three dimensional structure of DNA was established.[6]

The development of X-ray diffraction analysis proved to be a tremendous asset in working out the structure of DNA. Using techniques developed in the X-ray analysis of proteins, high quality diffraction patterns were obtained by Wilkins and Franklin,

working in London at King's College in 1952. Then in 1953, following the theories of X-ray diffraction established by scientists working on protein structure, Watson and Crick were able to deduce the structure of DNA as being a complementary double helix. This was a momentous breakthrough, as scientists now had a real molecular object about which they could think objectively in terms of established chemical mechanisms such as hydrogen bonding. Immediately, the mechanism for replication could be theorized as one strand serving as a template for the building of another complementary strand. It was at this point that molecular genetics had its beginnings.[7]

The area of recombinant DNA research is deeply involved in the fundamentals of molecular biology, thus a rigorous treatment of the subject would seem most appropriate. However, it seems prudent to give a more concise and relevant background, thus we will look at what DNA is and then point out some of its activity in a living system.

DNA is the macromolecule that is the principal component of the chromosome, the structure within the cell that is the storage place for the "information" necessary to sustain the cell in the living state. The cell that we speak of may be a single-celled bacteria or one of a group of cells which form a higher organism. In either case, each cell carries the information it needs to sustain the organism. Not all of the information is used at once; instead, the chromosome also carries mechanisms for the timing of gene expression,

depending on stage of development or surrounding conditions.[8]

The DNA molecule itself varies in length from organism to organism and usually consists of two complementary polymeric chains twisted about each other in the form of a regular double helix. Each chain is a polynucleotide, each nucleotide being made up of a nitrogen base, a deoxyribose sugar and a phosphate group. There are four nitrogen bases: cytosine, guanine, adenine, and thymine. The two chains are joined together by weak bonds between complementary bases, that is, cytosine is always paired with guanine and adenine is always paired with thymine.[9] The number of different DNA molecules caused by arrangements of nucleotides is given by the expression 4^n, where n is the number of nucleotides in a given molecule.[10] Even in an extremely small virus, such as ϕ174, with only a single stranded DNA, there are 5,375 nucleotides, or 4^{5395} possible combinations. In a mammalian cell, there are approximately 1×10^7 nucleotides.[11]

Replication of DNA occurs by the unwinding of the strands and attachment of free deoxynucleoside-triphosphates to their now unpaired complementary nucleotides. It should be noted that some lower bacteria that do not have well defined nuclei, called prokaryotic cells, have single stranded DNA which replicates by a somewhat different mechanism, but complementary base pairing is still the essential step.[12]

In the case of the making of a protein, the weak bonds that make replication possible also make the first step of protein synthesis, transcription, possible. Again, complementary

bases attach to the DNA, but this time they are attached to ribose sugars instead of deoxyribose sugars. Another difference is that the nucleotide thymine is replaced by uracil. Thus, when the polynucleotide-ribose sugar chain is complete, it detaches and is known as RNA. It has the same information that DNA has, with the exception that each RNA nucleotide is the complement of the corresponding nucleotide on the original DNA strand. An important point here is that any mistake made in the copying of DNA will be continued through successive replications, but since RNA is not a self-replicating molecule, any mistake made in its transcription will not be propagated and will usually result in a nonfunctional protein being produced, if one is produced at all.[13]

The RNA strand contains successive bases which are "read" in a process called transcription by other RNA molecules with amino acids attached. The RNA units which result from transcription from DNA are known as messenger RNAs(m-RNA), while the RNA units that "read" the m-RNAs and link the proper proteins together are known as transfer RNAs, or t-RNAs. The t-RNA contains approximately 80 nucleotides in a chain that folds by means of weak bonds into a cloverleaf shape. Each loop of the cloverleaf (Fig.1) has a particular function; one loop binds to the ribosomal surface, another recognizes activating enzymes, and there is an area containing the anticodon, and an open end which holds the amino acid moiety which will become part of the protein to be made.

The start of protein synthesis (Fig.2) is the moving of

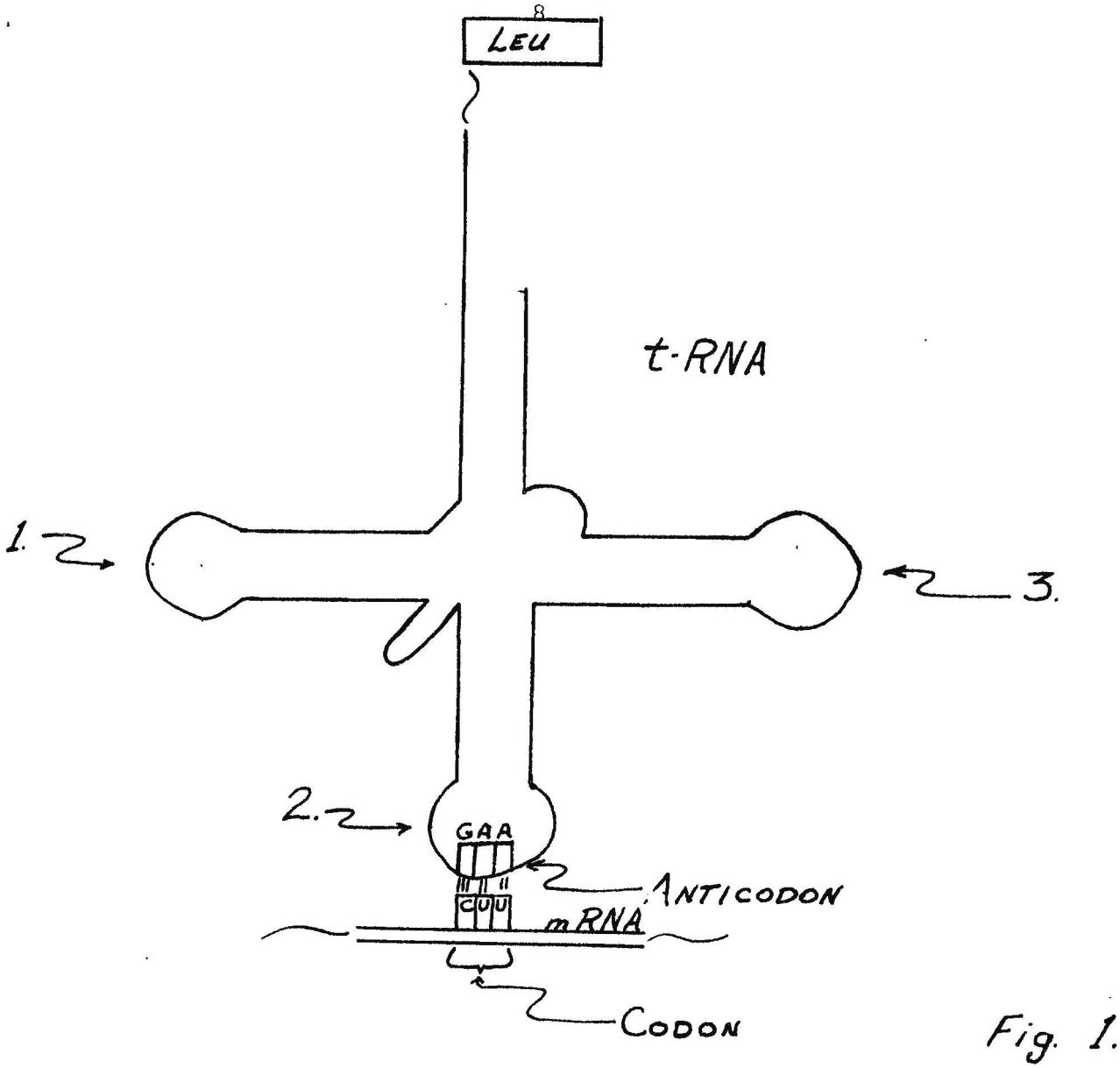

Fig. 1.

The above figure is a schematic diagram of a t- RNA molecule, attaching by means of its anticodon to the complementary bases of the codon on m- RNA. Both m- RNA and t- RNA are single stranded polynucleotides. Loops are formed by hydrogen bonding of complementary nucleotides within the t-RNA molecule.

1. Binding to the ribosomal surface may involve this loop.

2. Anticodon loop.

3. Possibly involved in binding to activating enzymes.

"Leu" represents the amino acid leucine, which will be positioned in the growing amino acid chain.

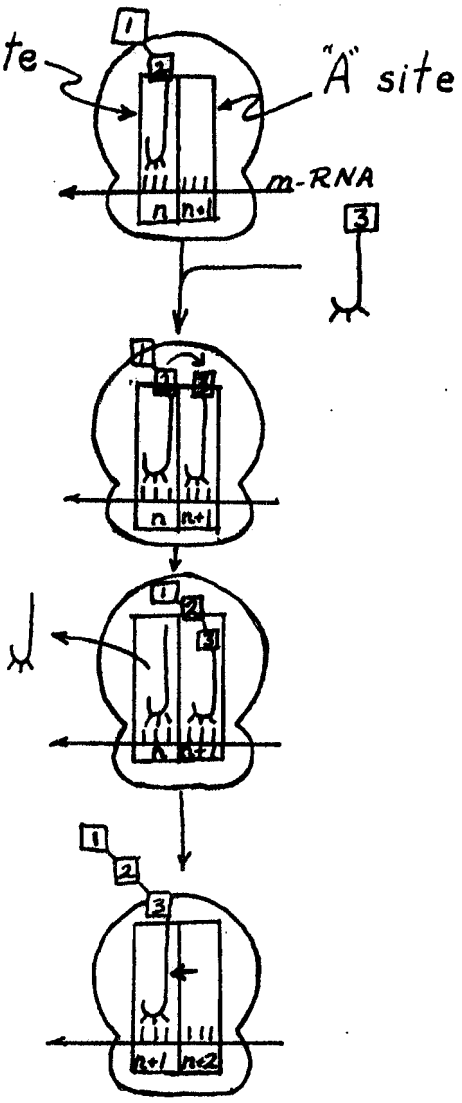

Fig. 2.

1. Growing polypeptide chain, attached by a t-RNA group to the protein binding site.

2. The next amino acid enters the amino acyl site attached to another t-RNA. A specific enzyme mediates the transfer of the first two amino acids to the third amino acid.

3. Ejection of t-RNA from the "P" site.

4. Growing polypeptide chain moves from the "A" site to the "P" site. Simultaneously, the m-RNA moves to place the next codon (n+2) at the "A" site.

This figure shows the steps of formation of a polypeptide chain. The "P" and "A" binding sites are shown within a schematic diagram of a ribosome. (Figures 1 and 2 taken from Molecular Biology of The Gene, 3d ed. by J.D. Watson.)

m-RNA to a ribosome which splits into two subunits. A complex is formed with the smaller subunit (30S), the t-RNA carrying the first amino acid, which is always formylated methionine, and the m-RNA molecule. This complex is joined by the larger ribosomal subunit (70S) which completes the full ribosome along which protein synthesis can occur. Specific proteins assist in all of these attachments. Each ribosome has two places for the t-RNA to attach to m-RNA. These are called the P (peptidyl) and A (amino acyl) sites. Each attachment locus on m-RNA is made up of three nucleotides called the codon which codes for one of twentv amino acids. The anticodon, three complementary nucleotides on a loop of the t-RNA, binds to the m-RNA codon. Thus the proper amino acid is coded for. When both the P and A binding sites are filled, peptidyl transferase, an enzyme, joins the amino acids together, and the first t-RNA moves out of the P binding site, leaving the two amino acids, formylated methionine and the second amino acid, attached to the t-RNA occupying the A site. Now the m-RNA and the ribosome move relative to each other, and the t-RNA carrying the two unit peptide chain is positioned in the P site. The now vacant A site accepts another t-RNA with its associated amino acid, which attaches to the growing peptide chain by a repetition of the above process. Thus, a protein is synthesized from the information coded originally on DNA. The time required to complete this process for a protein containing 300 to 400 amino acids is about 10 to 20 seconds.

Termination is accomplished by specific codons on the m-RNA that are not complementary to a t-RNA anticodon, but are read by specific protein release factors that stop elongation of the chain and release the t-RNA at the terminal end. Note that many ribosomes can attach to an m-RNA at once, thus several polypeptide chains can be synthesized simultaneously.[14]

The last area of relevant background is natural recombination. Recombinants have been observed even as early as Mendel's garden pea experiments. The recombinant part of his experiments dealt with the breeding of pea plants differing in more than one character, in this case, round versus wrinkled and yellow versus green. Mendel showed that round and yellow are dominant over wrinkled and green, that is if round and yellow peas which over successive generations bred true were crossed with wrinkled and green peas which also have bred true, the resulting peas would have a phenotype (physical structure) of round and yellow, but a genotype (genetic structure) of RrYy. (R is round, r is wrinkled, Y is yellow, and y is green.) The peas produced were called the F_1 generation. He then crossed the F_1 generation within itself and found that the resulting F_2 generation had the following phenotypes: the two original phenotypes (round, yellow; wrinkled, green) plus two new types, the recombinants, wrinkled yellow and round green. The interpretation is that any one gamete from the F_1 generation contains all the possible combinations of traits from each gene pair. Therefore, RrYy would yield four possible gametes: RY,

ry, rY, Ry, but never Rr, Yy, YY, or RR. All four of these gametes are produced in virtually equal numbers, and there is independent assortment of all the genes. The result is obtained by taking all possible combinations of RY, Ry, rY, ry, to produce the following ratio: 9 RY (round, yellow), 3Ry (round, green), 3rY (wrinkled, yellow), 1ry (wrinkled green). The middle two phenotypes would be recombinants, that is, they have expressed both a dominant and a recessive trait as a result of a new recombining of gametes.

This above phenomenon was explained using chromosomal theory in 1903 by Sutton, in his paper, The Chromosomes in Heredity. He said that the chromosomes are diploid and exist in identical pairs and during meiosis each gamete receives only one chromosome of each homologous pair. Thus, one pair could carry the gene for shape and another pair carry the gene for color. Thus a gamete from the F_2 generation could easily have any one of four possible color/shape combinations.

If, however, two genes for distinctly different traits are located on the same gene, they will not undergo independent assortment. Thus, the number of groups of linked genes should equal the number of chromosomal pairs. However, this is never 100% true. The Belgian cytologist, Janssens, first described the mechanism of crossing over. When meiosis begins, homologous chromosomes pair at a synapse, parallel to each other. Then the chromosomes of each duplicate, thus giving four parallel

strands, or a tetrad of chromatids. The chromatids coil, causing tension which breaks two chromatids at corresponding places and they cross over and recombine with the other chromatid. The closer genes are on a chromosome, the less likely a break will occur between them, thus by studying recombinants of crossing over, an effective method of mapping the genes was discovered.[15]

Random transfer of genetic material has also been observed. In the process called transformation, fragments of DNA are replicated and pass into the environment by excretion, or alternatively, a dying cell releases DNA fragments as its cell membrane ruptures. Another cell, which is in a state of competence, will allow these fragments to enter through the cell membrane. The recipient cell is termed to be in a state of competence when it is not producing DNA-ase, an enzyme which would break up the DNA chain when it reaches the cytoplasm, and when the cell membrane lacks its normal thick capsule which would not allow passage of macro-molecules such as DNA. Once the DNA fragments are within the recipient cell, there is only a five percent chance that the fragments will be similar enough to a segment of the host DNA that recombination will occur. If recombination does occur, the DNA fragment will become incorporated into the cellular DNA, with production of proteins coded for by the new DNA segment. If the newly produced proteins are toxic, a relatively harmless bacteria could become a source of toxic materials.

Another method of DNA exchange can come in the form of conjugation, where cells of the same species exchange genetic material. Conjugation is well documented in the bacteria Escherichia coli, which is a normal inhabitant of the lower intestinal tract of most primates, and has a circular DNA strand. The cell which initiates conjugation has a plasmid, in addition to its circular DNA. The plasmid is a piece of independent DNA that carries anywhere from 1 to 250 genes, and there can be as many as thirty plasmids per cell. The plasmid may replicate at any time, independent of the replication of the cell's "main" genetic material.

A plasmid is responsible for forming the sex pilus, a cellular extension which links two cells and enables the cell with the plasmid, called an F factor, to physically transport a copy of its circular DNA into the recipient cell. This is accomplished by the replication of the donor's DNA, and then a restriction endonuclease, an enzyme coded for by the F factor, cleaves the copy at a specific point. Now the DNA can pass through the sex pilus linearly, with the F factor attached to the end of the DNA that will enter the recipient cell last. Very seldom will the entire DNA copy and F factor pass through because the DNA is extremely fragile and usually breaks before the entire conjugation process is complete. Partial conjugation is sometimes referred to as sexduction.

One danger of recombination can be seen in the activity of

plasmids. It has been found that many plasmids carry genes which code for proteins which resist the actions of many antibiotics, such as penicillin or streptomyocin. The resistance to these drugs is passed on by conjugation. Staphlococcus bacteria are especially noted for this phenomenon. There are certain plasmids in E. coli which produce antibiotics known as colicins which kill off E. coli's competition in the primate intestine, and let E. coli proliferate, causing serious intestinal disorders.

A cell may obtain extracellular DNA via bacteriophages and viruses. A bacteriophage contains only DNA surrounded by a protein coat. It has a tail of protein fibers used for cellular attachment. A virus contains either DNA or RNA, but never both. It, too, has a protein coat, but usually does not contain the elaborate attachment fibers of the bacteriophage. In action, the bacteriophages are restricted to bacteria; viruses are found in cells making up larger organisms.

In the process known as transduction, a bacteriophage inserts its DNA into the host cell, leaving the protein coat outside the cell. Proteins coded for by the phage DNA rapidly break down host DNA and use cellular ribosomes for new protein coat production. New phages are produced that carry fragments of the host's DNA along with their own DNA. At this point, the cell lyses (splits open) and releases the phages into the environment. The phages find new hosts, and the DNA of the phage, still containing frag-

ments of the former host's DNA, may recombine with the new host's DNA. Thus, phages serve as a vector for the transfer of DNA from one cell to another. It is also possible, using the proper bacteriophage, that such transfer can occur between bacteria of different species.

In some cases, the viral DNA will be inserted into the cellular DNA and remain dormant through several cell divisions. Thus, many copies of the viral DNA are made when the host DNA replicates just before cell division and the cells produced have a differing genetic makeup because of the viral DNA insertion. Proteins from this insertion may or may not be expressed, depending on the environment and state that the cell is in.[16]

Recombinant DNA technology has its origin in the principles expressed above. Basically, this type of experiment involves chemical synthesis or isolation of one or more genes from an organism followed by an insertion of this DNA into the DNA of a host organism. This insertion is done in such a way that the host will replicate the inserted gene along with the host's original DNA. The replication of the gene insertion by the host cell is referred to as cloning. The word cloning as used here refers to the replication of foreign DNA inside a host cell, and not the complete copying of a higher organism.

The most widely used organism for recombinant DNA experiments is Escherichia coli, because its genetic structure and biochemistry have been the most extensively studied of any organism. It is easy to obtain and grow in extremely pure cultures. The most widely

used strain is the K-12 strain of E. coli which exists naturally in the environment, but does not colonize the human intestinal tract.

Restriction endonucleases, previously mentioned in conjunction with the F factor and conjugation, have been found in many other forms and there exists specific endonucleases that cleave at specific sites on DNA. The most useful of these used in recombination experiments are those which produce DNA fragments with "sticky" ends. "Sticky" ends result from the cleavage of DNA at recognition sites known as palindromes. In the English language, a palindrome can be read as the same phrase both left to right and right to left, for example, "MADAM I'M ADAM." In DNA, such a sequence would be:

```
C  T  T  A  A  G
|||| ||  ||  ||  ||  ||||
G  A  A  T  T  C
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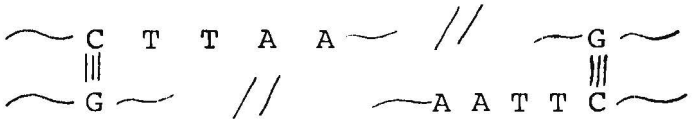
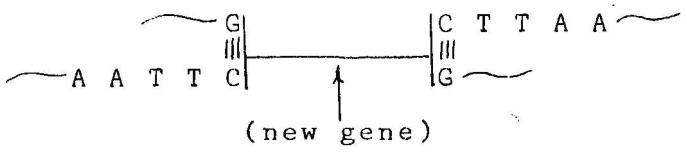
which, if in a plasmid, could be cleaved by the proper restriction endonuclease,

A.



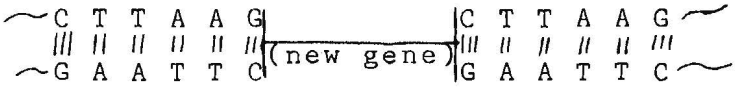
Now, if another DNA sequence were cleaved by the same endonuclease, with the exception that it has a series of other nucleotides forming a gene between the G and A, we would see:

B.



A. and B. are joined by a ligase enzyme to yield:

C.



The restriction ligase catalyses the formation of the phosphodiester bonds to yield an intact strand. Once the amino acid product of the recombinant is known, it can be sequenced, and the DNA sequence of the gene it came from can be predicted. Thus, certain genes can be synthesized in the laboratory, rather than isolated from a cell.

Once the plasmid has been recombined with the desired DNA segment, it can be inserted into a host cell, usually the bacteria Escherichia coli, and the cloning will occur. Normally, the host cell is not harmed by having as many as 100 plasmid copies inside it. To tell exactly which cells have the plasmid and which do not, the plasmid used contains a gene for antibiotic resistance. After the recombinant plasmids have been mixed with the desired host cells, an antibiotic is administered. Those cells that survive are the ones which have incorporated the plasmid.

In order to insure that the newly incorporated gene will

synthesize a desired protein, the gene must be recognized by the host system as being part of its DNA. This is done by splicing the gene into a section of the plasmid that is next to a DNA sequence that controls whether or not the gene will be transcribed.[17]

An excellent example of the utility of the recombinant DNA technique is in the production of the mammalian peptide hormone somatostatin. Somatostatin inhibits the secretion of a number of hormones including growth hormone, insulin, and glucagon. It has value in the treatment of pancreatitis and insulin dependent diabetes. Conventionally, it has been isolated in milligram quantities by extraction of the ground-up brain tissue of a half million sheep.

The DNA responsible for the production of somatostatin using recombinant DNA techniques was chemically synthesized and not isolated from mammalian DNA. The DNA fragments were linked using ligase enzymes. The gene was then inserted in a bacterial plasmid between a gene control sequence and a naturally occurring gene for a bacterial protein. Thus, the somatostatin was produced as an addition to the normally produced bacterial protein, and was not destroyed by the cell's natural defense systems. However, in this form, the somatostatin was not useable, so it was cleaved from the bacterial protein using chemical methods involving cyanogen bromide. One hundred grams of bacteria grown in approximately sixteen liters of culture resulted in milligram quantities of active somatostatin being produced.[18]

Another development showing the utility of recombinant DNA techniques involves the manufacture of insulin by Genetech Laboratories in California. The gene coding for the protein insulin has been isolated from pancreatic cells and inserted into the DNA of E. coli by a method similar to the one used to insert the somatostatin gene into a bacterial plasmid.[19]

In agriculture, recombinant DNA techniques are being used to enhance the efficiency of nitrogen fixation. All plants, including crop plants, need nitrogen in a useable form, such as ammonia, in order to sustain life. By recombination, the gene for nitrogen fixation may be isolated from bacteria which naturally exhibit this activity (which converts N_2 in the atmosphere to NH_4^+, ammonia). The isolated gene could be placed in the DNA of a crop plant, making the crop plant fix nitrogen for itself. Another possibility is that the gene could be placed in a bacteria or algae known to be part of the natural flora of the soil in which the crop grows. Then the source of ammonia for the plant would be the bacteria in the soil. Fertilization, which requires the making of fertilizer from petroleum products, could be vastly reduced.[20]

Other potential benefits, which will only be briefly mentioned here, include the discovery of the mechanism of gene regulation in mammalian systems, nucleotide sequencing in the complex DNA of higher animals and methods of differentiation of cells within the human embryo. Not only could inherited disorders be

pinpointed as to cause, but there is the possibility of gene repair to correct such defects by recombinant techniques. The manufacturing of pharmaceutical products could be made more efficient by reducing the use of animal tissue extracts. Proteins, such as the human clotting factor for hemophilacs, could be isolated in highly pure form. In cancer research, the protein interferon has been found to be a possible therapeutic substance with low toxicity. However, it is found in such low quantities that research concerning it has been slow at best. The cloning of interferon in bacteria is a possible means of obtaining workable quantities of the protein. Vaccines could be prepared in bacteria, using bacterial plasmids as the attachment point for viral genes. This would eliminate contamination by unidentifiable viruses in the vaccine culture, a phenomenon which plagues chicken embryo vaccine cultures that are presently used.[21]

The prospects and possibilities seem endless. Recombinant DNA techniques seem to be almost a panacea for even the greatest human scourges. But from the outset of this type of work, there were many scientists who warned against the misuse of gene control and manipulation. In the May, 1974, Proceedings of the National Academy of Sciences, Cohen and Chang from Stanford, Boyer, Hilling, and Goodman from the University of California at San Francisco, and Murrow of Johns Hopkins reported the successful combining of animal genes with a bacterial DNA strand. The recombinant DNA formed was called a "chimera," from the hideous monster of incongruous parts in Greek mythology.[22]

Shortly after this announcement, eleven scientists, making up the Committee on Recombinant DNA Molecules under the National Academy of Sciences, wrote a letter* requesting a ban on three types of recombinant DNA experiments. Type I is the addition of genes to bacteria that would confer antibiotic resistance or cause the production of a toxin not normal to that bacteria. Type II is linking DNA from tumor causing viruses to bacterial plasmids. Type III is the combining of animal cell DNA with bacterial DNA.

The letter stemmed from a meeting of the Gordon Research Conference on Nucleic Acids in 1973, in which Paul Berg of Stanford University formed a committee which began the investigation of the problem under the auspices of the National Academy of Sciences.[23] Some members of the committee were actively involved in recombinant DNA experiments, including Cohen and Boyer from Stanford, and Daniel Nathans, whose work on restriction enzymes in 1969 started the field of recombinant DNA research and won for him a share of the 1978 Nobel Prize in Medicine.[24]

Immediately, there was a furor in the scientific community. A new precedent had been set, that is, scientists asking other scientists to curtail research, and to decide among themselves, in public, how and/or if recombinant DNA research was to continue.[25] To compound problems, the potential benefits and risks were purely speculative, as no previous work had been done on genetic material crossing the species barrier.

*APPENDIX I

Speculative risks include animal tumor viruses which might contaminate community air and water supplies or the escape into air and water of normally harmless bacteria containing gene additions coding for toxic proteins.[26] Since E. coli is a natural inhabitant of the human intestinal tract, fears exist that recombinant E. coli could escape a research laboratory via human vectors, and cause massive epidemics of intestinal disorders, or infections of the blood stream. It has also been found that one segment of DNA may encode for several different proteins, depending on the location of the segment in question in relation to the segment being transcribed. In other words, an inserted segment might code for one protein by itself, plus be at the beginning of the code for another protein, and at the end of the code for yet another. This overlapping cannot always be predicted, and thus the proteins from a single recombinant insertion may be quite varied and unpredictable.

The above stated negative aspects of the research, spurred on by the self-imposed moratorium, caused a tempest of press interest. Scientists who were used to a secluded atmosphere of research and decision making were suddenly thrust in front of the camera and microphone. The main issue to the public was safety and headlines like "Bid to Ban Test Tube Super Germ" left the public with an impression of a few mad-cap, eccentric scientists fooling around with dangerous substances for lack of anything better to work on.

The first attempt at self-regulation took place in an atmos-

phere of eagerness to clear up the safety issue and put the facts before the public. One hundred forty scientists from seventeen countries gathered at Asilomar, California, in February of 1975, to discuss effects of the now eight-month old moratorium on research with recombinant DNA and to come up with recommendations that might permit the research to continue.

The first three days of the conference were mostly condolence sessions. Scientists were telling each other that their research was too good and important for safety regulations to intrude. It was observed by one person as like "having the chairman of General Motors write the specification for safety belts."[28] However, the evening before the final session, three lawyers gave presentations on the legal aspects of the research and the legal responsibilities of the researchers. The final speaker of the three, Professor Harold Green of the George Washington University Law School, hit home with his topic, "Conventional aspects of the law and how they may sneak up on you--in the form, say, of a multi-million dollar lawsuit."[29]

The results of the final session showed the impact of the previous evening's speakers. A two-point safety program was outlined, one part dealing with physical laboratory containment of recombinant molecules and organisms, based on risk. The second point was a novel concept of biological containment, where a strain of E. coli was to be developed that would not colonize in the natural environment.[30]

The next major step in regulation was taken by the National Institutes of Health. Basically, the NIH detailed the physical and biological containment recommendations of the Asilomar Conference. These guidelines, explained briefly below, were published in June of 1976.[31]

Physical containment will be discussed first, followed by biological containment. The lowest level of physical containment, P1, involves the standard microbiological procedures of sterile technique and autoclaving. Open bench tops may be used, but all wastes must be stored and decontaminated daily before release into the environment. Experiments using P1 can at most involve transfer of genetic material resulting from biological gene exchanges that can occur in nature. The P2 level involves experiments that use bacteria that do not naturally exchange genes. It uses the same procedures as P1, plus restricted entrance to the laboratory,[32] and an open front cabinet with inward air flow over the work surface, so that bacteria laden aerosols do not contaminate the worker. The air exhausted to the environment by these cabinets is first filtered to remove 99.997 percent of the organisms present in the air. At the P3 level, all of the above would be used, plus protective gloves, wrap around disposable gowns, and vacuum systems protected by filters and disinfectant traps. The P3 room itself is isolated from all other laboratories; air pressure is slightly below outside pressure so that organisms can pass in but not out when the doors are opened, and double door

systems are used. Ultraviolet light shields are used in all hoods in the laboratory. Experiments done under these conditions include use of embryonic, vertebrate and primate tissue, and DNA transfer from these tissues and their tumors to "crippled" host systems. "Crippled" hosts will be discussed under biological containment. P4 procedures are maximum containment, using class III cabinets used within the laboratory of all conduits. The class III cabinets used within the laboratory are gas tight and all materials passing in or out of them must be autoclaved. This requires attaching an autoclave to the cabinet. A second sterilization must be done before any materials leave the laboratory proper. Personnel entering and leaving the laboratory must shower and wear completely separate clothing when in the laboratory. Under the original regulations, P4 facilities would accommodate any recombinant DNA research, except DNA from cancer causing viruses, pathogens, drug resistant organisms, or genes for toxins. These experiments would be completely banned.

Experience with these procedures of containment has been gained in the biological warfare laboratories of Fort Detrick, Maryland. The infection rates for workers in similarly equipped laboratories ranged from 7 infections per 100 person-years worked for P1 to 0.4 infections per 100 person-years worked for P3. P4 data is not available as no lab meeting such stringent requirements has even been built. Construction of a P4 facility is in progress at Fort Detrick.[33]

Biological containment, using so called "crippled" hosts contains recombinant DNA molecules in vectors that will not endanger the environment. Usually, these "crippled" hosts are ones that will only survive in the laboratory, or will self destruct by not possessing the genes to synthesize their own cell wall or replicate DNA outside of a narrow environment.[34]

E. coli K-12 was the first strain used in biological containment because of its inability to survive more than 48 hours within the human intestinal tract. It was originally isolated from a human patient 50 years ago and since then has been cultured under laboratory conditions with regularity. However, approximately 1 in every one hundred million cells will pass through the intestinal tract unharmed. In March, 1976, the NIH approved a safer host strain of E. coli K-12 developed by Roy Curtiss of the University of Alabama, and dubbed it E. coli K-12 Chi 1776. Chi 1776 requires certain laboratory nutrients, is sensitive to bile salts, and is destroyed by sunlight.[35]

Another method of biological containment includes the use of the Charon Lambda bacteriophage, where the recombinant molecule is not in a bacteria, but is present in a type of virus that only attacks bacteria. The bacteriophage only stays in the bacteria long enough to replicate, then lyses the cell. Propagation by these means outside the laboratory is extremely difficult. It has been found that fewer than 1 in 10^{10} phages survive stomach conditions (pH 3 for 2.75 hours), fewer than 2 in 10^{7} survive 30 minutes in detergent conditions (1 percent sodium dodecyl sulfate) and

fewer than 3 in 10^6 survive in raw sewage. Those that do survive however, do not encounter bacteria in natural surroundings that will support their growth. There are two strains of bacteria used for their growth in the laboratory, E. coli Chi 1953 and Chi 2098. Each has a survival rate 1000 times less than E. coli K-12. Neither colonizes the human or rat intestinal tract.[36]

The year after the publication of the NIH guidelines, there were two important sets of hearings on the recombinant DNA research issue. The National Academy of Sciences Forum reviewed the advances in developing safe strains of E. coli and heard scientists who presented their cases concerning pharmaceutical and industrial applications, food production, genetic engineering, and problems of regulation and control. This forum differed from the Asilomar Conference in that the atmosphere was both philosophical and safety oriented indicating that more scientists were willing to take a long, hard look at recombinant DNA research before going into it headlong. Excerpts of this forum have been previously cited.

The other significant hearings were held before the Subcommittee on Science, Research and Technology of the Committee on Science and Technology, United States House of Representatives. These hearings were similar in content to the NAS Forum and were also characterized by a philosophical slant on the future uses of the technique and public involvement in decisions concerning recombinant DNA research.

Philosophically, a major question has been how far should we go with the recombinant DNA techniques. Critics contend that the possibilities of bio-hazards have not been resolved, but merely covered up. They claim that even one case of bacterial escape from a containment facility will prevent a re-containment of it or its prodigy. Dr. Ruth Hubbard of Harvard asks how safety guidelines can be established when the risks are unpredictable and unquantifiable. Dr. Willard Gaylin warns that by nature, disasters are not anticipated, otherwise, they are prevented.

Inherent to the technique is the potentially dangerous violation of three billion years of evolution. According to Dr. Robert Sinsheimer, we cannot prove that such "quantum jumps" in genetics occur naturally in the evolutionary process. Some feel that this technique will take the path of nuclear technology, demonstrating itself in a "Hiroshima style" with a disaster caused by an escape of a lethal recombinant DNA chimera, followed by more peaceful, but nonetheless controversial uses such as genetic engineering on human cloning. According to those who oppose genetic engineering, the proliferation of recombinant DNA techniques would encourage a symbiotic relationship between geneticists and social visionaries who envision a "super race" much as was done during Hitler's Third Reich.[37]

Those who favor recombinant DNA research usually have accepted the NIH guidelines and believe that genetic engineering in humans is far fetched and not applicable to the direction of the research at the present time. It is the safety factor rather than the

philosophical factor which concerns citizens.

Dr. Sheldon Krimsky served on the Cambridge (Massachusetts) Experimentation Review Board, which advised local officials whether recombinant DNA procedures should be allowed in the city. The citizen review board unanimously voted to allow the research up to the P3 level. However, the committee also required research to have separate regulators and promotors, additional review and monitoring of the facilities, done by a group independent of the sponsoring institution.[38]

In other cities, the same concern was arising, especially in those towns with large universities or pharmaceutical firms. Ann Arbor, Michigan, has both. Albert Wheeler, the mayor in 1977, stated that he is concerned that the NIH guidelines have not been enforced in industry. In testimony before the House Subcommittee on Science Research and Technology, Mr. Wheeler said that he would allow research up to but not including the P4 level, and city officials would have to be notified when recombinant DNA experiments were being done at any level. He also proposed the formation of a citizens review board on the subject.

The apparent lack of acceptance of NIH guidelines in industry is unfortunate, but the increasing influence of citizen review boards may force compliance.[39] Groups such as Genetics Group of Science for the People have pressured industry to comply with NIH guidelines and have included the general public in the decision-making process on allowing recombinant DNA experiments to be done in their communities.[40]

In a statement by the Pharmaceutical Manufacturers Association, acceptance of NIH guidelines by that group and most pharmaceutical manufacturers seemed widespread. In early 1977, NIH proposed a national registry for all recombinant DNA work, and the PMA seemed likely to accept this measure also.

Most environmentalists ask for strict controls and along with them, prominent anti-DNA research scientists have asked that all recombinant DNA work be carried out in P4 facilities. This, in 1977, would have amounted to a complete ban on recombinant DNA work, as no P4 laboratories were in existence.[41] Presently, only the NIH and Fort Detrick, Maryland, have the proper P4 facilities.

Actual DNA legislation was proposed in early 1977 and for the most part, placed tremendous restrictions on recombinant DNA research. However, in late summer of 1977, many Congressmen backed away from their "worst case scenario" philosophy, at the urging of scientists and industry alike. One of the most tenable sets of principales was proposed by the American Society for Microbiology which basically asked for a national regulatory commission, preemption of local or state laws by federal regulations, and fines for failure to comply with federal legislation.

The A.S.M. principles caused Senator Edward Kennedy to withdraw his bill that would virtually halt recombinant DNA work and caused relaxed revisions in both House and Senate bills dealing with recombinant DNA research regulation.[42]

A major question still stands unresolved and that is of

enforcement. In the research done on the insulin gene and its transfer to bacteria, a vector was used that was not NIH approved as a biological containment vector. This prompted the statement that among those in graduate research, some follow the guidelines to a degree, others not at all, and it seems "almost chic not to know the NIH rules." Even if the vector were safe, the question of unfair advantage arises. Are those who abide by the rules being taken advantage of by those who do not? The short time it took to produce a successful insulin producing bacteria indicates that an unfair advantage may be possible.[43]

The last section will describe events in recombinant regulation during 1978. In the July 28, 1978, Federal Register, the NIH published revisions of the original NIH guidelines for work on recombinant DNA. The major revisions include:

1. Exemption from the restrictions of the guidelines certain experiments which are now considered to be safe. (Most of these experiments fell under the old P1 containment requirements.)

2. Placing primary responsibility for assuring guideline compliance on the institution where research is done.

3. Dropping the requirement for NIH notification when a P1 experiment was changed to a P3 experiment.

4. Providing voluntary registration of recombinant DNA experiments.[44]

During hearings before the Senate Subcommittee on Science Technology and Space, a recommendation was made that the current NIH guidelines should not be enforced by the NIH but by another unnamed agency. They also suggested that all laws regulating the

research be national laws with a deemphasis on local and state regulations, except in special cases. In a minority report Senator Harrison Schmitt said the recommendations would result in "unwarranted and excessive regulations."[45]

As an additional signal that regulations and bans were relaxing within the scientific community, Genetech, Inc. agreed to work with Eli Lily and Company to manufacture insulin by insertion of artificial insulin genes into plasmids of E. coli. Large scale production is predicted in 2 to 5 years.[46] However, bans and restrictions have not been so much the issue this year as has the role of the NIH. Prompted by the Senate Hearings during the late summer, a public hearing was held in Washington to discuss the proposed revisions in NIH guidelines. Very little medium ground was covered; the guidelines were either too stringent or too relaxed. Ambiguities were pointed out often. The NIH planned to publish final revisions, based on the hearings in November, 1978.[47]

Finally, Joseph Califano announced the revisions in the NIH guidelines. Basically, they were unchanged from the guideline changes mentioned previously, but the reporting procedures for violations, illness, and accidents were clarified. The NIH retained control over recombinant DNA guidelines, but greater public representation in hearings and decision on policy was required. Therefore, the recombinant DNA question has not been completely resolved, but an interim set of guidelines that have shown themselves adaptable to new facts and findings may help us to control a new and powerful research tool.[48]

In writing this paper, the author was amazed at the number of plausible arguments both for and against continued use of recombinant DNA techniques. It seems that each potential risk is counterbalanced by an equally convincing benefit. It would be ridiculous to assign numerical values to each risk and benefit and settle the question forevermore by a mere bookkeeping system. Each individual experiment must be judged primarily on its specific risks and specific benefits, and secondarily, on its relationship to other recombinant DNA experiments and vice-versa.

However, we must have broad guidelines to assist not only our cost-benefit analysis of individual experiments, but also to insure that once an experiment is approved for use, the experiment will be done safely. A safe experiment is one that combines biological and physical containment procedures such that organisms containing artificially recombined DNA will not be able to reproduce in the environment. In this writer's opinion, the current NIH guidelines, if enforced, would accomplish this goal. It must be noted that these guidelines must be open to change, as basic research reveals more about the nature of recombinant DNA techniques. The purpose of the NIH guidelines should not be to dictate a set of one time rules, but to be flexible guidelines with respect to the current findings of basic research in the area of recombinant DNA.

Another major factor to consider is enforcement of the guidelines. A workable set of guidelines without enforcement are of little value. Enforcement must come from the national as well as

local levels. Communities must take more responsibility towards deciding what types of experiments should be allowed to take place in their institutions, both academic and industrial. However, the communities must also have the assurance that a federal agency will back them up in their decision. Here again, we see that the federal government must show flexibility in guidelines and in recognizing the rights of communities to make intelligent decisions of scientific importance.

This writer certainly does not call for a ban on recombinant DNA research. Rather, there should be regulations, such as the current NIH guidelines that can change as current knowledge dictates. There should also be enforcement from both the community and federal levels in order to assure compliance with research guidelines. The future of recombinant DNA research lies not in doomsday scenarios of plague caused by recombinant bacteria. Nor does it lie in surrealistic extrapolations of potential uses, such as human cloning and super-races. The future of recombinant DNA research lies in the scientists' ability to conduct accurate research within the bounds set mutually by science and society, each being willing to change their position as the facts permit.

APPENDIX 1.

Potential Biohazards of Recombinant DNA Molecules

Recent advances in techniques for the isolation and rejoining of segments of DNA now permit construction of biologically active recombinant DNA molecules in vitro. For example, DNA restriction endonucleases, which generate DNA fragments containing cohesive ends especially suitable for rejoining, have been used to create new types of biologically functional bacterial plasmids carrying antibiotic resistance markers (*1*) and to link *Xenopus laevis* ribosomal DNA to DNA from a bacterial plasmid. This latter recombinant plasmid has been shown to replicate stably in *Escherichia coli* where it synthesizes RNA that is complementary to *X. laevis* ribsomal DNA (*2*). Similarly, segments of *Drosophila* chromosomal DNA have been incorporated into both plasmid and bacteriophage DNA's to yield hybrid molecules that can infect and replicate in *E. coli* (*3*).

Several groups of scientists are now planning to use this technology to create recombinant DNA's from a variety of other viral, animal, and bacterial sources. Although such experiments are likely to facilitate the solution of important theoretical and practical biological problems, they would also result in the creation of novel types of infectious DNA elements whose biological properties cannot be completely predicted in advance.

There is serious concern that some of these artificial recombinant DNA molecules could prove biologically hazardous. One potential hazard in current experiments derives from the need to use a bacterium like *E. coli* to clone the recombinant DNA molecules and to amplify their number. Strains of *E. coli* commonly reside in the human intestinal tract, and they are capable of exchanging genetic information with other types of bacteria, some of which are pathogenic to man. Thus, new DNA elements introduced into *E. coli* might possibly become widely disseminated among human, bacterial, plant, or animal populations with unpredictable effects.

Concern for these emerging capabilities was raised by scientists attending the 1973 Gordon Research Conference on Nucleic Acids (*4*), who requested that the National Academy of Sciences give consideration to these matters. The undersigned members of a committee, acting on behalf of and with the endorsement of the Assembly of Life Sciences of the National Research Council on this matter, propose the following recommendations.

First, and most important, that until the potential hazards of such recombinant DNA molecules have been better evaluated or until adequate methods are developed for preventing their spread, scientists throughout the world join with the members of this committee in voluntarily deferring the following types of experiments.

► *Type 1*: Construction of new, autonomously replicating bacterial plasmids that might result in the introduction of genetic determinants for antibiotic resistance or bacterial toxin formation into bacterial strains that do not at present carry such determinants; or construction of new bacterial plasmids containing combinations of resistance to clinically useful antibiotics unless plasmids containing such combinations of antibiotic resistance determinants already exist in nature.

► *Type 2*: Linkage of all or segments of the DNA's from oncogenic or other animal viruses to autonomously replicating DNA elements such as bacterial plasmids or other viral DNA's. Such recombinant DNA molecules might be more easily disseminated to bacterial populations in humans and other species, and thus possibly increase the incidence of cancer or other diseases.

Second, plans to link fragments of animal DNA's to bacterial plasmid DNA or bacteriophage DNA should be carefully weighed in light of the fact that many types of animal cell DNA's contain sequences common to RNA tumor viruses. Since joining of any foreign DNA to a DNA replication system creates new recombinant DNA molecules whose biological properties cannot be predicted with certainty, such experiments should not be undertaken lightly.

Third, the director of the National Institutes of Health is requested to give immediate consideration to establishing an advisory committee charged with (i) overseeing an experimental program to evaluate the potential biological and ecological hazards of the above types of recombinant DNA molecules; (ii) developing procedures which will minimize the spread of such molecules within human and other populations; and (iii) devising guidelines to be followed by investigators working with potentially hazardous recombinant DNA molecules.

Fourth, an international meeting of involved scientists from all over the world should be convened early in the coming year to review scientific progress in this area and to further discuss appropriate ways to deal with the potential biohazards of recombinant DNA molecules.

The above recommendations are made with the realization (i) that our concern is based on judgments of potential rather than demonstrated risk since there are few available experimental data on the hazards of such DNA molecules and (ii) that adherence to our major recommendations will entail postponement or possibly abandonment of certain types of scientifically worthwhile experiments. Moreover, we are aware of many theoretical and practical difficulties involved in evaluating the human hazards of such recombinant DNA molecules. Nonetheless, our concern for the possible unfortunate consequences of indiscriminate application of these techniques motivates us to urge all scientists working in this area to join us in agreeing not to initiate experiments of types 1 and 2 above until attempts have been made to evaluate the hazards and some resolution of the outstanding questions has been achieved.

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References and Notes

1. S. N. Cohen, A. C. Y. Chang, H. Boyer, R. B. Helling, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 3240 (1973); A. C. Y. Chang and S. N. Cohen, *ibid.*, **71**, 1030 (1974).
2. J. F. Morrow, S. N. Cohen, A. C. Y. Chang, H. Boyer, H. M. Goodman, R. B. Helling, *ibid.*, in press.
3. D. S. Hogness, unpublished results; R. W. Davis, unpublished results; H. W. Boyer, unpublished results.
4. M. Singer and D. Soll, *Science* **181**, 1114 (1973).

END NOTES

[1]J. D. Watson, Molecular Biology of the Gene (2d ed; New York: W. A. Benjamin, 1970), p.72.

[2]Ibid., p.64.

[3]Ibid., p.38.

[4]Ibid., p.51.

[5]Swenson, Mertz, and Young, Cytogenetics (New Jersey: Prentiss Hall, Inc., 1967), p.8.

[6]Watson, p.32.

[7]Watson, pp. 64-67.

[8]U.S. Congress, House, Committee on Science and Technology, Subcommittee on science, Research and Technology, Science Policy Implications of Recombinant DNA Molecule Research, Hearing, 95th Congress, 1st Sess., March 29, 1977, (Washington: Government Printing Office, 1977), p.22

[9]Watson, 3d, ed., p.208.

[10]Watson, p.299.

[11]Clifford Grobstein,"The Recombinant DNA Debate" Scientific American (July, 1977) p.24.

[12]Watson, p.299.

[13]Watson, pp. 330-341.

[14]Watson, pp. 355-395.

[15]Watson, pp. 16-21.

[16]Pelczar, Reid, and Chan, Microbiology (4th ed; New York: McGraw, Hill, 1977), pp. 226-239.

[17]Vaughn Vandegrift, "Recombinant DNA: Scientific and Social Perspectives," Journal of Chemical Education (February, 1979) pp 78-79.

[18]Itakura, et al, Science (December 9, 1977), p. 1056.

[19]"1978 Nobel Prizes Reflect the Controversial Nature of Science," Chemistry (January, 1979) p.28.

[20]Raymond C. Valentine, "Genetic Engineering in Agriculture, with Emphasis on Biological Nitrogen Fixation," Science Policy Implications of Research with Recombinant DNA: An Academy Forum (Washington, D.C.: National Academy of Sciences, 1977) pp. 224-231.

[21]David Nathans, "Potential Benefits of the Research, "Research With Recombinant DNA: An Academy Forum (Washington, D.C.: National Academy of Sciences, 1977) pp. 49-54.

[22]"Animal Genes Transplanted to Bacteria," Science News (June 1, 1974) p. 348.

[23]"Geneticists and Gene Transplants: A Historic Call For a Ban on Research," Science News (July 27, 1974) p. 106.

[24]"1978 Nobel Prizes Reflect the Controversial Nature of Science", Chemistry (January, 1979) p.28.

[25]Maxine Singer, "The Involvement of Scientists," Research With Recombinant DNA: An Academy Forum (Washington, D.C.: National Academy of Sciences, 1977) p.24.

[26]Laura Brunton Riesenberg, "Recombinant DNA- The Containment Debate," Chemistry (December, 1977) p.14.

[27]U.S. Congress, House, Committee on Sciences and Technology, Subcommittee on Science, Research and Technology, Science Policy Implications of Recombinant DNA Molecule Research , Hearing, 95th Congress, 1st Sess., March 30, 1977 (Washington: Government Printing Office, 1977) pp. 139-141.

[28]Ted Howard and Jeremy Rifkin, Who Should Play God? (New York: Delacorte Press, 1977).

[29]Ibid.

[30]Ibid., p.38.

[31]Singer, p.25.

[32]Riesenberg, p.16.

[33]U.S. Congress, House, Committee on Science and Technology, Subcommittee on Science Research and Technology, Science Policy Implications of Recombinant DNA Molecule Research, Hearing, 95th Congress, 1st Sess., March 29, 1977 (Washington: Government Printing Office, 1977) pp. 35-38.

[34] Riesenberg, p.16.

[35] Vandegrift, p.81.

[36] Blattner, et al, "Charon Phages: Safer Derivatives of Bacteriophage Lambda for DNA Cloning", Science (April 8, 1977) pp 161-169.

[37] Howard and Rifkin, pp. 41-44.

[38] U.S. Congress, House, Committee on Science and Technology, Subcommittee on Science, Research and Technology, Science Policy Implications of Recombinant DNA Molecule Research, Hearing, 95th Dongress, 1st Sess., May 3, 1977 (Washington: Government Printing Office, 1977) pp. 490-491.

[39] Ibid., pp. 503-509.

[40] Ibid., p. 520.

[41] E. M. Leeper, "Scientists Ask Congress to Control DNA Research," BioScience (February, 1977) pp. 141-143.

[42] Harlyn O. Halvorson, "Recombinant DNA Legislation--What Next?," Science (October 28, 1977) p. 357.

[43] Nicholas Wade, "Recombinan DNA: NIH Rules Broken in Gene Project," Science (September 30, 1977), pp. 1342-1345.

[44] "DNA Guideline Changes," Science News (August 5, 1978), p. 87.

[45] "Senate Report on DNA," Science News (September 9, 1978) p. 182.

[46] "Human Insulin: Seizing the Golden Plasmid," Science News (September 16, 1978) p. 195.

[47] "DNA Guidelines Near," Science News (September 23, 1978), p. 213.

[48] "Recombinant DNA Guidelines Relaxed," Science News (December 30, 1978) p. 437.

SOURCES CONSULTED

Books

Howard, Ted, and Rifkin, Jeremy. Who Should Play God? New York: Delacorte Press, 1977.

National Academy of Sciences. Research With Recombinant DNA: An Academy Forum. Washington, D.C.: National Academy of Sciences, 1977.

Pelczar, Reid, and Chan. Microbiology. 4th ed. New York: McGraw, Hill, 1977.

Swenson, Mertz, and Young. Cytogenetics. New Jersey: Prentiss Hall, Inc., 1967.

Watson, J.D. Molecular Biology of The Gene. 2d ed. New York: W.A. Benjamin, 1970.

Watson, J.D. Molecular Biology of The Gene. 3d ed. New York: W.A. Benjamin, 1976.

Periodicals

Abelson, John. "Recombinant DNA: Examples of Present-Day Research." Science, April 8, 1977, pp.159-160.

"Animal Genes Transplanted to Bacteria." Science News, June 1, 1974, p. 348.

Berg, Paul, et al. "Potential Biohazards of Recombinant DNA Molecules." Science, July 26, 1974, p. 303.

Blattner, et al. "Charon Phages: Safer Derivatives of Bacteriophage Lambda for DNA Cloning." Science, April 8, 1977, pp. 161-169.

"DNA Guideline Changes." Science News, August 5, 1978, p. 87.

"DNA Guidelines Near." Science News, September 23, 1978, p. 213.

"Geneticists and Gene Transplants: A Historic Call For a Ban On Research." Science News, July 27, 1974, p. 106.

Grobstein, Clifford. "The Recombinant DNA Debate." Scientific American, July 1977,p. 24.

Halvorson, Harlyn O. "Recombinant DNA Legislation- What Next?" Science, October 28,1977.

"Human Insulin: Seizing the Golden Plasmid." Science News, September 16, 1978, p. 195.

Itakura, et al. Science, December 9, 1977, p. 1056.

Leeper, E.M. "Scientists Ask Congress to Control DNA Research." BioScience, February 1977, pp. 141-143.

"1978 Nobel Prizes Reflect the Controversial Nature of Science." Chemistry, January 1979, p. 28.

"Recombinant DNA Guidelines Relaxed." Science News, December 30, 1978, p. 437.

Riesenberg, Laura Brunton. "Recombinant DNA - The Containment Debate." Chemistry, December 1977, p. 14.

"Senate Report On DNA." Science News, September 9, 1978, p. 182.

Vandegrift,Vaughn. "Recombinant DNA: Scientific and Social Perspectives." Journal of Chemical Education, February 1979, pp. 78-79.

Wade, Nicholas. "Recombinant DNA: NIH Rules Broken in Gene Project." Science, September 30, 1977, pp. 1342-1345.

Government Documents

U.S. Congress. House. Committee on Science and Technology, Subcommittee on Science, Research and Technology. Science Policy Implications of Recombinant DNA Molecule Research. Hearing, 95th Congress, 1st Session. Washington: Government Printing Office,1977.