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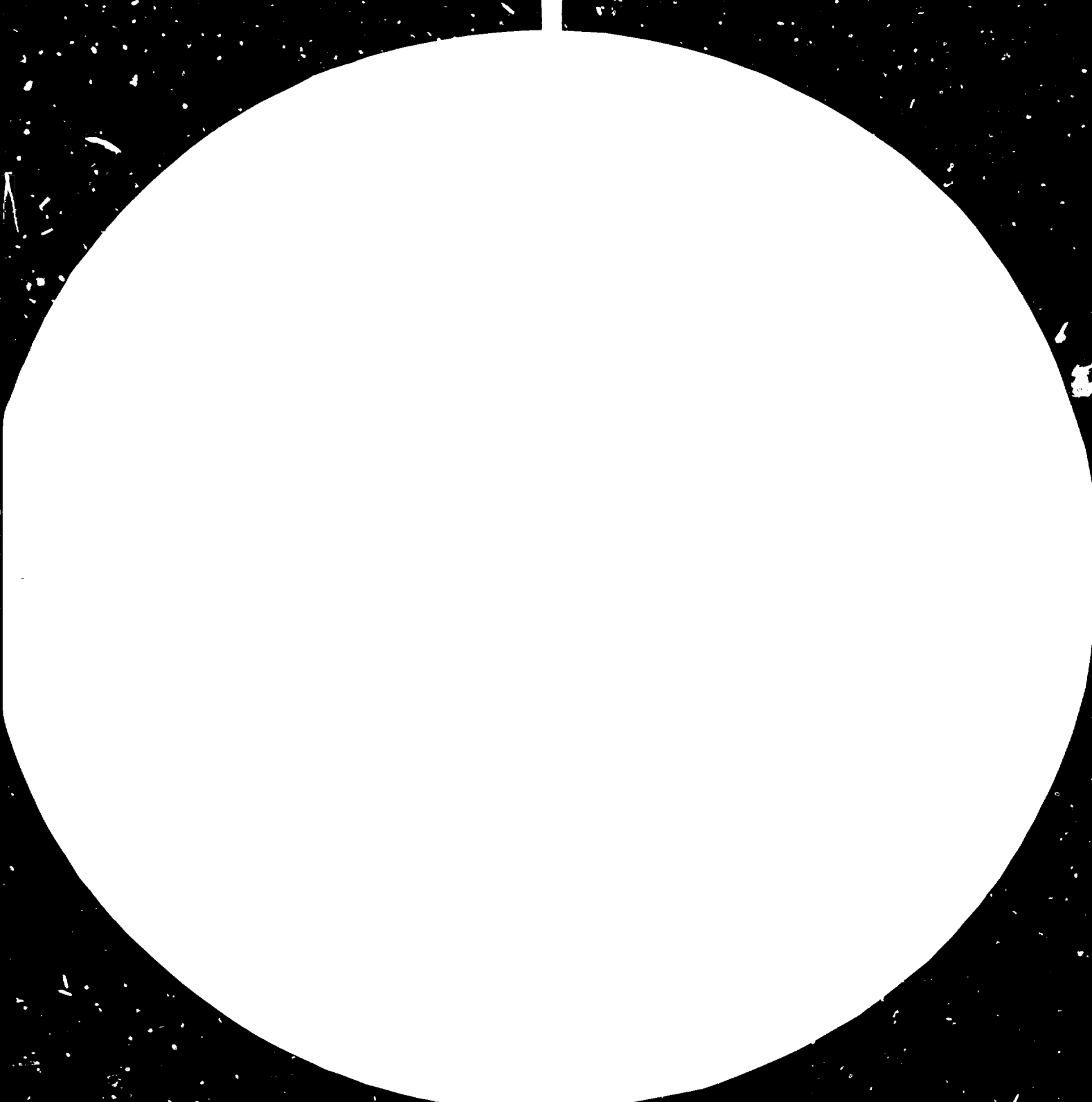


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GENETIC ENGINEERING:

THE TECHNOLOGY AND ITS IMPLICATIONS*

prepared by

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CONTENTS

INTRODUCTION

1. The use of micro-organisms has been known to mankind for the last four thousand years. Living organisms have been used for centuries to make yoghurt and cheese and to leaven dough for bread, but today we know much more about these simple beings than the mere procedures of these ago-old methods. With the aid of controlled laboratory experiments and powerful microscopes, we have come to understand the tiny microbes to be minute biochemical factories. We now view their long familiar actions as highly sophisticated chemical transformations.

2. The important role microbes play in a wide variety of natural processes intrigues scientists, and studies of natural processes have led to research into the use of micro-organisms in preserving human health, controlling pollution, building landscapes, and enriching the nutritional value of plant and animal food sources. In a world faced with a growing scarcity of natural resources, the startling efficiency of the chemical processes in microbes has been recognized as an important tool in preserving and enhancing the quality of human life.

3. When applied to the study of micro-organisms, the scientific advancements of the last thirty years in chemistry, biology, and genetics have now led mankind to the threshold of a new era of the industrial revolution, one that has the potential to touch nearly every phase of human existence. This revolution is based on the recognition that DNA, a type of large and complex organic molecule, directs the synthesis of proteins in all living organisms and therefore, controls the physical structure, growth, reproduction, and function of those living beings.

4. In recent years, scientists have discovered that DNA carries, coded within its chemical structure, the information for controlling protein synthesis. The code in which the information is stored has been discovered as have many of the subtle biochemical mechanisms by which livings cells transmit and maintain the integrity of that information. Furthermore, laboratory techniques now exist for making DNA molecules that are identical or analogous to those naturally occurring in living cells.

5. Most recently, scientists have discovered how to insert functional units of synthetic DNA, or genes, into special strains of micro-organisms. A foreign gene thus introduced enables the host cell to manufacture the protein molecules specified in the coded sequence of nuclectide building blocks in the gene. These new mutant strains of micro-organisms can be cultivated, or cloned, until a significant quantity of cells are available to produce specific, desirable protein molecules. Once a strain is established, the synthesis of the protein, whether for medical, agricultural, or industrial applications, is both efficient and economical.

6. Research on the techniques of splicing synthetic genes into single-celled micro-organisms comprises the scientific field of recombinant DNA or genetic engineering. This paper discusses the methods of genetic engineering, the future applications, and implications, both the benefits and the dangers, of this multidisciplinary field.

I. GENETIC ENGINEERING - THE METHOD

An Overview

7. Most fears and superficial criticisms of genetic engineering are based on ignorance of the chemical and biological processes involved in recombinant DNA research. Beneath the exaggeration and sensationalism is a superficial analysis and consequent omission that trivialize the difficulties encountered by actual research scientists. Scientists, awed by the complexity and mystery of biochemical processes in a single cell, scorn the extrapolations of their techniques from the microbial level to that of higher animals. Even the problems of genetic engineering at the simplest level have taxed the ingenuity of scientists around the world.

8. A solution to one such fundamental problem has, however, advanced genetic engineering to the point where the first practical benefits of the technology can be envisioned. When foreign DNA molecules enter a cell, special enzymes, called restriction enzymes, rapidly degrade the DNA before it can reach the nucleus and have any effect on the host organism. As a result, the stability of the foreign DNA is difficult to maintain even in simple bacterial cells. The way to solving this problem in single-celled organisms was opened by the discovery of small rings of DNA in the cell matter outside the nuclei of some bacteria.

9. In 1973, a technique was developed to use these circular DNA molecules, called plasmids, to transport synthetic DNA into bacterial cells and to protect it there from enzymatic degradation. Once the foreign DNA is joined to the plasmid and inserted into the host cell, the enzymes cannot recognize the synthetic segment of the augmented plasmid. As a result, when the cell reproduces, the added DNA is replicated along with the other constituents of the cell. Furthermore, when the cell carries on its normal metabolic functions, the synthetic gene in the plasmid directs the manufacture of the new protein coded for in the man-made sequence of nucleotides.

10. Three major advances in the field of recombinant DNA have made feasible the execution and verification of the approach to protein synthesis:

(a) The availability of well-defined sequences of DNA, whether by chemical or enyzmatic synthesis or by enzymatic or physical fragmentation of large segments of chromosomal DNA containing the target gene;

(b) The ability to analyze rapidly and precisely the sequence of nucleotides in long DNA molecules;

(c) The discovery that a number of restriction enzymes can be used to prepare the plasmid DNA to receive the synthetic DNA.

The Seven Phases Of Genetic Engineering

11. Currently, the general method of genetic engineering comprises seven procedural phases:

(a) Selection and isolation of the target gene by one of four methods:

(i) Fragmentation of chromosomal DNA by restriction enzymes;

(ii) Fragmentation of chromosomal DNA by hydrodynamic shear;

(iii) Enzymatic synthesis of DNA using an RNA template;

(iv) Chemical and enzymatic synthesis of well-defined sequences of DNA.

(b) Preparation of the plasmid DNA:

(i) Isolation of circular plasmid DNA;

(ii) Use of restriction enzymes to prepare the plasmids for receiving the foreign DNA.

(c) Combining the synthetic DNA and the plasmid to form a hybrid circular DNA;

(d) Insertion of the recombinant plasmids into bacterial cells;

(e) Isolation of clones with successfully incorporated recombinant plasmids;

(f) Verification of the nucleotide sequence of biologically active target genes;

(g) Use of the cloned cells to manufacture the biologically significant proteins.

12. Before the detailed discussion of this procedure, the two main advantages of this technique need to be pointed out. On the one hand, scarce biologically significant proteins, which are not easily or economically extracted from their natural sources, can be produced in large quantities. On the other hand, the technique gives scientists a powerful tool for purifying, selecting, and enriching well-defined sequences of DNA and for expanding knowledge of the biological and biochemical properties of genetic material.

II. THE PHASES OF GENETIC ENGINEERING - THE DETAILS

(see also Figure I)

Selection and Isolation of the Target Gene

13. The genetic engineer first selects a biologically significant protein, the production of which is the ultimate goal. A protein is a sequence of amino-acids, and the information about that sequence, the template or pattern, so to speak, is contained in a structural gene of the organism that produces the protein. The gene containing that template in the sequence of its nucleotide building blocks is called here the target gene. The more that is known about the sequences of the protein

and the target gene, the greater is the scientist's flexibility to choose among the various procedural options of genetic engineering.

14. The target gene can be isolated in either of two ways: it can be extracted from the genetic material of the organism that naturally produces the protein, or it can be synthesized in a laboratory. Isolation by extraction requires less knowledge of the target gene's structure than does the complete synthesis of the target gene. However, a nucleotide sequence for laboratory synthesis can be derived by using the genetic code to translate backward from the amino-acid sequence of the protein to a possible nucleotide sequence for the target gene. The redundancy of the genetic code, the fact that one amino-acid may have more than one code word, or codon, associated with it, allows for a vast variety of possible sequences in the target gene, all of which could result in the production of the same protein.

15. Once the target gene has been determined, the gentic engineer has four methods available to isolate it in preparation for joining it with the cloning vehicle.

Fragmentation of Chromosomes by Restriction Enzymes

16. DNA can be isolated in the form of chromosomes from tissue cultures or tissue samples containing the target gene. The chromosomes are then exposed to restriction enzymes that break the DNA into a mixture of polynucleotide fragments of various lengths. These fragments can be separated according to their sizes by the process of agarose gel electrophoresis, but because it is difficult to say with certainty which group of fragments contains the target gene, several groups are combined to increase the probability that the desired gene is contained in the mixture. Known sequences of RNA or DNA, called probes, can be used to select from that mixture those fragements containing the target gene.

17. Although these fragments have terminal ends suitable for making hybrid plasmids, there is a significant chance that the sites at which the restriction enzymes cleave the chromosomal DNA may fall within the boundaries of the target gene. In that case, the gene would not remain intact after the fragmentation by the enzymes.

Fragmentation of Chromosomes by Physical Force

18. Some of the disadvantages of isolating target genes with restriction enzymes can be overcome by fragmenting the chromosomes with physical force. Shear forces that break the DNA into polynucleotides of varying lengths can be produced, for example, by the use of ultrasound (sonification) or vigorous agitation (homogenization). The size of the fragments produced depends on the nature and extent of the shear forces.

19. DNA fragments produced by this method, however, do not have terminal ends suitable for making hybrid plasmids and must be chemically manipulated before the necessary joining with plasmid DNA is possible.

Enzymatic synthesis of DNA using an RNA template

20. This synthetic method requires the previous isolation of the RNA complement to the target gene and the use of several enzymes. The first enzyme, which derives from a virus that causes myeloblastosis in birds, is called a reverse transcriptase because it reverses the normal process of synthesis in which DNA is used as a template for making RNA. Reverse transcriptase uses the RNA to produce a complementary strand of DNA with a hairpin loop at one end, the so-called 3' ("three-prime") end.

21. This short segment of double-stranded DNA enables the synthesis of a complementary DNA strand to complete needed double-helix structure. The second enzyme (DNA polymerase I from the bacteria E. Coli) forms this complementary strand, and the RNA-DNA complex is then separated by washing it in an alkaline solution.

22. A third enzyme, SI nuclease, is used to cut the hairpin loop at one end of the double stranded DNA. The single-strand terminal ends needed to join the synthetic DNA to the plasmid DNA can then be added using either of two enzymes, terminal transferase or blunt-end ligatins.

23. The disadvantage of this method of back- ranslating from RNA to DNA is that the synthetic gene necessarily lacks the nucleotide sequences that form the initiation and termination signals of the gene.

Chemical and Enzymatic Synthesis of Target Genes

24. Once a nucleotide sequence analogous to that of the target gene has been designed, that well-defined sequence can be rapidly and accurately synthesized by a two step process. First, short segments of the desired sequence (units ten to twenty nucleotides long and called oligonucleotides) are made by chemical means. These oligonucleotides are then combined enzymatically to form the sequence exactly analogous to that of the target gene.

25. The product of this method includes the initiation and termination sequences of the gene, and includes the single-strand ends for joining with the plasmid DNA. Furthermore, the synthetic gene excludes the extraneous nucleotides characteristic of the fragmentation methods. Recently a new method, called the "modified phospotriester" method, has been developed, and it provides a highly efficient means of synthesizing well-defined DNA sequences analogous to those of target genes.

Preparation of the Plasmid DNA

Isolating Plasmid DNA

26. The rings of plasmid DNA are found in the cell matter outside the nuclei of most types of cells. They carry genetic information for proteins that protect the host cell from attack by antibiotics. These proteins are called R factors (for "resistance"), and each one interferes with the action of a particular antibiotic. Plasmid DNA can be isolated from bacterial cells by a centrifugation technique that uses a special liquid medium to separate molecules according to their density.

Cloning Plasmid DNA

27. Once isolated, the plasmids can then be introduced into E. Coli bacteria which lack their own plasmids. To facilitate the incorporation of the plasmids, the bacteria are treated with calcium salts to increase the permeability of their cell walls. The proportion of plasmids successfully introduced into the bacterial cells is very small - one per million of E. Coli cells. Even though the event is rare, the few bacteria containing the plasmids can be easily selected

from the rest, because they live and multiply in the presence of the antibiotic to which the plasmid R-factor confers resistance. Meanwhile, the antibiotic kills the millions of cells that contain no plasmids The survivors then reproduce and multiply. The resulting identical cells are called clones, and each contains an exact replica of the plasmid DNA. These plasmids are the vehicles used for cloning the well-defined sequences of DNA that code for the biologically significant proteins.

Cleaving the Plasmid DNA

28. The discovery of endonuclease restriction enzymes has enabled scientists to produce fragments of DNA molecules with small, complementary, single-strand segments at each end. Each of these enzymes cleaves DNA molecules in a highly specific way. Research in the early 1970s showed that in most cases, the cleavage takes place at or near a site where the sequence of nucleotides is identical in both strands of the DNA helix. The application of these observations to nucleotide chemistry has greatly advanced genetic engineering. The concepts and techniques are so crucial to the synthesis of recombinant DNA that they are explained here in greater detail than other phases of the process.

29. The two ends of a single strand of DNA have different physical and chemical properties. For convenience in notation and discussion, the ends are called the 5' ("five prime") end and the 3' ("three prime") end. These names refer to the different carbon atoms in the ribose sugar rings at either end of a single strand of DNA. Two such strands of DNA can form the famous double helix structure only if the sequences of nucleotides in the two chains are complementary. In the double-stranded helix, the 5' end of one strand lines up with 3' end of the other, and the 3' end of the first lines up with 5' end of the second. The double helix structure is possible only when the 5' to 3' sequence of nucleotides of one strand is exactly complementary to the 3' to 5' sequence on the other.

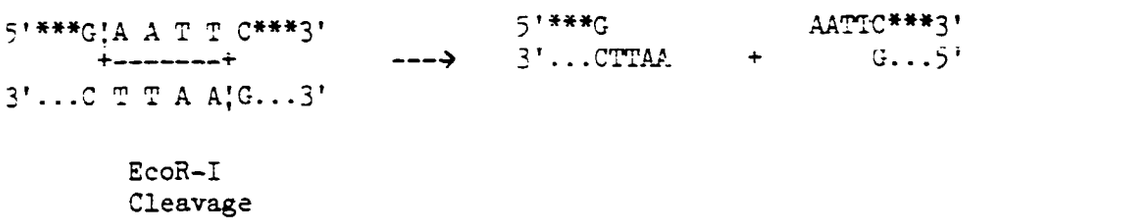
30. The complementarity of nucleotides is a function of the spatial relationships between the two pairs of DNA building blocks. Guanine (G) pairs with cytosine (C), and adenine (A) pairs with thymine (T).

So, for instance, the sequence of one chain, 5'-GCAT-3', complements the following sequence in the other chain: 3'-CGTA-5'. The conventions of notation dictate that references to single-strand nucleotide sequences begin with the 5' end, unless specifically marked otherwise.

31. This convention reflects the concept of polarity in DNA molecules. Each end of a linear DNA double helix has one strand with a 5' terminus and one with a 3' terminus, but only one of those four ends carries the transcription initiation code recognized by RNA-polymerase, the enzyme that synthesizes the messenger RNA. That initiation sequence is carried by one of the 3' ends. The location of that initiation signal establishes the polarity of the double helix and the framework for the conventions governing notation. The strand containing the initiation code is called the "lower" strand, and the other strand is called the "upper" strand. The lower strand, which contains the initiation sequence, serves as a template for the synthesis of messenger RNA, and thus the structure of the messenger RNA complements that of the lower strand. Because the lower strand also complements the upper strand, the nuclectide sequence of the messenger is identical to that of the upper strand, except that the messenger contains a uridine (U) nucleotide wherever the upper strand contains a thymine (T). As a result of this similarity, the upper strand is said to carry the DNA code.

32. At or near the site where an endonuclease cleaves a plasmid is a DNA sequence with a special complementary symmetry, called rotational symmetry. Although the sequences of the two strands are complementary, they are also identical when read from the 5' ends to 3' ends. For example, the EcoR-I enzyme cleaves DNA wherever it encounters a site of rotational symmetry based on the sequence 5'-GAATTC-3'. The complementary sequence on the other strand reads 3'-CTTAAG-5'. When both strands are read from the 5' end, the two sequences are identical: 5'-GAATTC-3'.

33. The EcoR-I cleaves this sequence in a way that leaves two single-strand segments at the two ends created by the cleavage. The following figure illustrates the EcoR-I cleavage of a linear double helix. The starred and dotted lines distinguish the two strands of the double helix and illustrate the concept of polarity.



EcoR-I
Cleavage

Because the single-strand fragments at the newly created ends are available for enzymatic binding with DNA strands bearing complementary single-strand segments, these ends are called cohesive or "sticky" ends.

34. When the fragments pictured above are mixed together with the enzyme DNA-ligase, the ligase joins the sticky ends, and three products result from the relinking:

5'***GAATTC***3'	5'***GAATTC...3'	5'...GAATTC***3'
3'...CTTAAG...5'	3'...CTTAAG***5'	3'***CTTAAG...5'
Original Sequence	Hybrid I	Hybrid II

The relinking does not return all the fragments to their original order or polarity. Some of the fragments combine with identical fragments to form hybrids, the sequences of which contain information different from that of the original.

35. This random recombination of DNA fragments with complementary sticky ends is an even greater problem for relinking the rings of plasmid DNA. When a restriction enzyme cleaves a ring containing one site of rotational symmetry, the result is one linear fragment with complementary sticky ends at each terminus, not two fragments with one sticky end each. When DNA-ligase relinks the fragments, the original ring is only one result among a wide variety of rings containing different numbers of original rings linked with random polarity, as illustrated in the following figure.

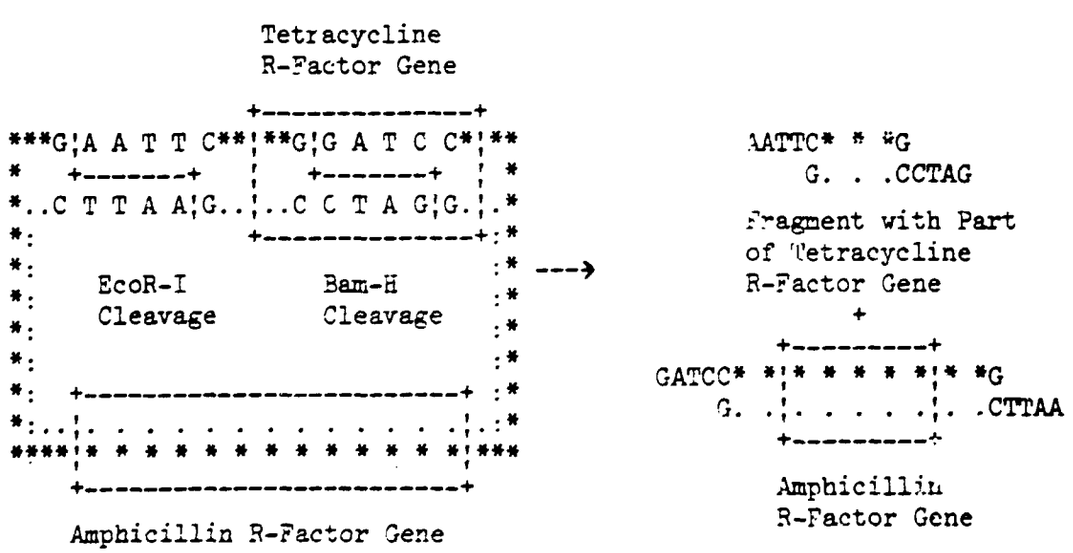
EcoR-I
Cleavage

Linear Fragment

One Possible
Hybrid Ring with
Mixed Polarity

36. This problem affects the use of plasmids to serve as vehicles for synthetic genes. To circumvent this problem, scientists have modified several naturally occurring plasmids to produce cloning vehicles with several important properties. One of the most widely used of these modified plasmids has been named PBR 322. It contains information for two R factors, one conferring resistance to tetracycline and one to amphicillin. Cells containing this plasmid can live and reproduce in the presence of those two antibiotics. In addition, PBR 322 contains six sites of rotational symmetry, each with a unique nucleotide sequence and each susceptible to cleavage by its own specific restriction enzyme.

37. To illustrate the use of a custom-tailored plasmid in the synthesis of recombinant DNA, only two of PBR 322's six sites or rotational symmetry will be discussed here. One of those cleavage sites, is located near the segment of the plasmid ring that carries the structural gene for the tetracycline R-factor. The nucleotide sequence of the site is GAATTC, and it is attacked by EcoR-I. The other site is contained within the structural gene. Its GGATCC seequence is the target of another restriction enzyme, Bam-H. When treated with both enzymes, PBR 322 forms two segments, one long and one short, each with a different single-strand terminus at each end.



There is an advantage conferred by this double cleavage: when the fragments are mixed with DNA-ligase, only one product can result - a ring with a sequence identical to that of the original plasmid. No hybrids can form. The significance of this specific type of recombining and of the presence of two sequences coding for different R-factors are explained in subsequent sections.

38. As preparation for joining with the synthetic DNA, the plasmid is treated with both EcoR-I and Bam-H enzymes to cleave it into two segments. The short segment of the tetracycline R-factor gene is separated and discarded, leaving the long segment for union with the synthetic DNA of the biologically significant gene.

Combining the Synthetic DNA and the Plasmid

39. To ensure the function of the synthetic gene, the synthetic DNA must be inserted into the plasmid ring in a way that aligns the polarity of the synthetic sequence with that of the plasmid. If the synthetic gene were inserted backwards, so to speak, the wrong chain would be read and the wrong protein would result. The asymmetric sticky ends produced by the action of the two enzymes on the plasmid prevent the misalignment of the synthetic gene.

40. The design of the synthetic gene includes the single-strand sticky ends that complement those of the long segment of the long plasmid fragment. When the properly constructed synthetic gene is mixed with the linear fragment of the plasmid in the presence of DNA-ligase, only one product results: a new form of plasmid that incorporates the synthetic gene in place of the excised segment of the tetracycline R-factor gene. The new form of plasmid carries the genetic information for the biologically significant protein, but it no longer carries the information for the tetracycline R-factor, and as a result, any cell containing this form of plasmid is vulnerable to attack by the antibiotic tetracycline. This result only provides a mechanism for isolating and purifying the target gene, but also gives scientists an important safeguard against the spread of organisms containing the synthetic gene.

Insertion of Recombinant Plasmids into Host Cells

41. As mentioned previously, the host bacterial cells are treated with calcium salts to increase the permeability of the cell membrane. When recombinant plasmids are mixed with these cells, a few of the plasmids cross host-cell membranes. The frequency of incorporation varies but is always quite small, often no greater than one successful incorporation per one million host cells.

42. The bacterial cells most frequently used as hosts for recombinant DNA are E. Coli K12 mutants. This strain of E. Coli is particularly suited for this type of research for three reasons. First, it lacks the restriction enzyme that ordinarily attacks foreign DNA and that would, if present, degrade any hybrid DNA entering the host. Second, it has no plasmid DNA of its own, but readily accepts foreign plasmids when the cell membrane is treated with calcium salts.

43. The third and most powerful control on the unregulated spread of experimental organisms is the set of metabolic deficiencies of the K12 strain. By selective breeding, scientists have produced successive generations of E. Coli that lack the ability to synthesize several essential nutrients. As a result, the K12 strain can grow only in a laboratory medium that supplies the nutrients that it cannot manufacture for itself. Even if a virulent gene were incorporated into an E. Coli K12 cell, that cell would pose no threat to any living being inside or outside the laboratory because it could not live outside its special laboratory medium. The nutrients K12 needs to survive are not found in natural environments but must be manufactured from more complex starting materials that the K12 strain cannot digest.

44. Research is currently underway to develop other micro-organisms as hosts for recombinant DNA. Bacterial hosts, such as E. Coli, are not ideal hosts because the replication of transformed bacteria requires a fermentation process that will prove cumbersome to adapt to the large scale necessary for the industrial production of the desired proteins. One of the most promising candidates is ordinary baker's yeast, which lacks its own plasmid and readily accepts foreign DNA. Once a method is discovered for stabilizing the plasmid after it enters the yeast cell, the safeguards of multiple metabolic deficiencies

can be developed by selected mutation until an ideal host strain of yeast results. Such an organism would have characteristics analogous to those of E. Coli K12, but would be easy to grow and readily adaptable industrial production.

Isolation of Clones with Recombinant Plasmids

45. Three methods have been developed to isolate cells containing the recombinant plasmids. Each method has its own application and its own justification.

The Genetic Method

46. The genetic method is most useful when cloning synthetic genes for the enzymes used in all applications of genetic engineering. This method requires that the host cell be deficient in the function of the target gene. The deficiency might result from a mutation that renders a natural gene inactive or from the absence of the target gene from the normal genetic material of the host. The recombinant plasmid contains a gene to remedy the deficiency, and the origin of that gene, whether extracted from another cell or synthesized in a laboratory, makes no difference.

47. Host cells containing recombinant plasmids can be identified by the presence and function of the protein end product, such as an enzyme previously absent in the host cells: Restriction enzymes, in particular, can be produced in larger quantities by cloning of recombinant plasmids than by simple extraction from cellular matter.

Immunoassay

48. Immunoassay is also useful in identifying the proteins produced as a consequence of the successful incorporation of recombinant plasmids. Immune responses involve highly specific binding of antigens and antibodies. In some cases, the specific antibody against a desired protein is known - for instance, the antibody against human insolin has been isolated. Once isolated, a sample of the antibody can be made radioactive and exposed to colonies to be tested for the presence of the desired protein. In colonies containing protein from the successfully cloned genes, the radioactive antibodies bind to the protein and render the colony radioactive. The radioactivity can then be located by exposing X-ray

films to samples from the colonies and mapping the spots on the film to colonies on the culture medium. The colonies thus identified are isolated and cloned further to multiply the number of cells that can produce the desired protein.

The Shortcoming of the Genetic Method and Immunoassay

49. Both the genetic and immunoassay methods of isolation are effective only in cases where the target gene is biologically active, that is, in cases where the synthesis of the desired protein takes place. That synthesis, however, requires more than the successful incorporation of the target gene.

50. By itself, a structural gene, which carries the coded information about a desired protein, cannot function. It is only one of three parts of the larger functional unit, the operon. The other two parts of the operon must be present for the structural gene to convey its information to the messenger RNA, which in turn passes it on the desired protein.

51. The first element of the operon is the "promoter", a DNA sequence that tells the transcription enzyme, RNA polymerase, where to begin transcribing the DNA code. That enzyme preserves the information in the DNA sequence by building a complementary single strand of messenger RNA. That RNA molecule carries its genetic message to the site where the desired protein is syntesized, a nearby ribosome.

52. In order to read the code properly, the ribosome, too, must recognize where to start translating the RNA code into the amino-acid sequence of the desired protein. The ribosome recognizes the nucleotide sequence at one end of the messenger RNA, called the ribosomal binding site. The information for this RNA sequence is contained in the second element of the operon.

53. All three segments of the operon must function if the associated protein is to be synthesized. The structural gene in a successfully incorporated plasmid can be perfect in its sequence, but if either one or both of the other segments of the operon are missing or improperly sequenced, the chain of events leading to protein synthesis is broken. The gene is such cases is not biologically active, and without the presence of any desired protein in the host cells, neither the genetic or immunoassay methods can help isolate the

clones that contain the target gene. For cases where the lack of biological activity is the result of non-functioning promoters or ribosomal binding sequences, a third method has been developed to locate clones containing the successfully incorporated structural gene.

Labelled Probe Hybridization

54. Host cells containing successfully incorporated target genes can be located without depending on the synthesis of the desired protein. In fact, structural genes obtained by fragmentation with restriction enzymes or shear forces can be located if only a small portion of the nucleotide sequence is known. Using a portion of the known sequence as a pattern, a small chain of DNA or RNA, eight to fifteen nucleotides long, can be synthesized and used as "probes". The nucleotide sequence of a probe complements the corresponding segment of the target gene and is, therefore, capable of binding to plasmids containing that gene.

55. The probes are labelled with radioactive phosphorus and are exposed to samples of bacterial colonies grown from host cells incubated with plasmids carrying the target gene. First, nitrocellulose filter paper is pressed onto petri dishes containing colonies of host cells. Some of the members of each colony adhere to the filter, and are treated with an alkali solution to break open the cells and separate the two strands of all DNA in the sample. The filter containing this denatured DNA is then exposed to a solution of labelled probes, and wherever a probe finds a complementary sequence of DNA, it binds and forms a stable, radioactive hybrid. For a probe of twelve nucleotides, the chance of binding to a segment of DNA not in the target gene is less than one in sixteen million.

56. X-ray film is then exposed to the treated filter paper, and any spots produced on the film by radioactive hybrids, are mapped back to the location of the parent colonies. The living remnants of these colonies can be cloned to multiply the number of cells containing successfully incorporated plasmids. These cells then form the basis for further genetic manipulation to find a promoter and ribosomal binding sequence that work with the structural gene to produce the desired protein. The strength of labelled probe hybridization is that it can verify the successful incorporation of the target gene

without checking the biological activity of the gene.

Verification of Biologically Active Target Genes

57. Even if biological activity results from the successful incorporation of recombinant plasmids, proof of incorporation and cloning comes only when the plasmids are extracted from the host cells, and the nucleotide sequence of the gene is determined to be the same as that of the original DNA inserted in the plasmid. Plasmids are isolated from cells exhibiting the desired biological activity, and they are treated with the original combination of restriction enzymes used to make a place for the target gene. These enzymes cut the plasmids on either side of the target gene and separate it from the plasmid. The gene can then be isolated by gel-electrophoresis, and standard techniques applied to determine the nucleotide sequence of the gene. If the entire process has worked properly, that sequence matches that of the starting material.

Use of Successful Clones to Manufacture Proteins

58. Once a colony with the desired biological activity has been produced, it can be grown until sufficient quantities of protein-producing micro-organisms are available to manufacture the protein on an industrial scale.

59. One problem facing this type of industrial scale production is the focus of some current research. The biologically significant proteins at which recombinant research is directed are foreign to the bacterial host cells now used as clone vehicles. The proteins have no metabolic function in these hosts, and consequently, host cell enzymes degrade the desired protein shortly after it is produced. Two approaches to this problem are possible. The less desirable approach is to synthesize a hybrid protein too large for the host cell enzymes to degrade. That hybrid can then be extracted from the bacteria and the desired segment liberated by chemical treatment.

60. More promising than this inefficient method are the attempts being made to discover a transport mechanism to pass the protein through the cell membrane as soon as the protein is synthesized. The host cell could then continue to produce protein, and the difficulties of isolating the protein would be reduced.

III. POTENTIAL APPLICATIONS OF RECOMBINANT DNA

61. The techniques of genetic engineering seem to offer nearly unlimited opportunity to provide inexpensive solutions to some of humanity's most pressing problems: disease and the growing shortages of food and energy. At the present moment, any optimism suggested by these opportunities must be tempered with the fact that scientists are still years away from the first important and successfully demonstrated application of this fledgling technology.

62. Several general areas of application, however, are commonly recognized among researchers: industry, agriculture, and medicine.

Industrial Applications of Recombinant DNA

63. Recombinant DNA may be useful in the manufacture of organic compounds used in industry. Work is already in progress to isolate genetic material from organisms that convert decaying organic matter to methane and to transport it to certain anaerobic organisms that degrade cellulose, the common constituent of fibrous plants. This transformation might facilitate the direct and rapid conversion of cellulose waste products to methane, which can be used as environmentally clean fuel for industry, home-heating, and transportation.

64. Exchange of genes between yeast and organisms that degrade cellulose might lead to practical production of ethanol from plant wastes. Other alcohols and organic solvents might also be produced. The manufacture of various enzymes, oils, and starting materials for plastics might also result from other industrial applications of genetic engineering.

Agricultural Applications of Recombinant DNA

65. Two approaches have been pursued by people applying genetic engineering to agriculture. On the one hand, productivity of crop plants can be increased by changing the genetic characteristics of the plants; on the other, recombinant DNA technology can be used to develop an alternative source of fertilizer for agricultural crops.

Recombinant Hybridization of Divergent Species

66. Traditional techniques of selective breeding and hybridization have produced crop plants with many desirable characteristics, but the plant breeder can only work with chance mutations and species closely enough related to permit conventional cross-breeding.

67. Recombinant DNA technology, coupled with known techniques for growing entire plants from tissue cultures, will widen the range of genetic material from which desirable genes may be extracted to enhance the hereditary characteristics of crop plants. New varieties of crop plants will result from hybridizations of widely divergent species.

68. It may be possible to increase crop yields by making plants more hardy to extend their growing season and their geographical range, by reducing their growth cycle to permit more harvests per growing season, by increasing the density of plants per acre, by strengthening resistence to insects, disease, heat, frost, drought, and flooding, and by increasing the size of the produce and the ratio of edible matter to waste. It may also be possible to increase the nutritional value and taste of crops and to improve the transportability of produce without sacrificing that nutritional value or taste.

Nitrogen Fertilizer

69. Two conventional methods of providing nitrogen fertilizer to plants are to add synthetic petroleum-based compounds to the soil, or to rotate crops, that is, to alternate planting of crops that use nitrogen with those that enrich the nitrogen content of the soil. The process of nitrogen fixation is actually carried out micro-organisms that live in the roots of certain plants called legumes. The need for these conventional methods of fertilization would be eliminated if the genes that control nitrogen fixation in those micro-organisms could be extracted and incorporated in other micro-organisms that live in soil and are not dependent on a symbiotic relationship with any particular crop plant, or if those genes could be incorporated directly into the genetic material of the crop plants.

Obstacles to Agricultural Applications of Recombinant DNA

70. At present, little is known about the means of achieving the functional expression of foreign genes in plant cells; consequently, no reasonable assessment of the limits of recombinant hybridization can be made. No one knows, for instance, if DNA sequences derived from animal genes will function in the reproductive system of higher plants. To solve or circumvent these problems, techniques need to be developed for introducing foreign DNA into various types of soil bacteria and plant cells.

71. Two other problems confront the attempts to apply genetic engineering to nitrogen fertilization. The process of nitrogen fixation consumes a great amount of energy, and, as a result, cells that fix nitrogen must also produce large quantities of ATP, the main source of energy in all cells. Hence, the genetic information for producing adequate levels of ATP must be incorporated in the host cells along with the genes that control the nitrogen fixation. A further complication arises because the enzymes involved in fixing the nitrogen do not function in the presence of oxygen, a common and necessary constituent of all plant cells. Either anaerobic plants cells will have to be developed, or varieties of anaerobic bacteria will have to be relied upon.

Medical Applications of Recombinant DNA

72. Genetic engineering offers the opportunity for large-scale and economical manufacture of many drugs and vaccines, which because of their modest cost can be widely distributed among the peoples of the world and can thus significantly improve the general health of those peoples. In addition, it may be possible to treat certain genetic diseases among humans.

Drugs and Vaccines

73. Significant research is currently trying to develop living systems that use recombinant DNA to manufacture biologically significant proteins such as human insulin, interferon, and blood clotting factor. Using conventional methods of extraction, these substances of their analogs are available only in small amounts from tissues of animals or from blood donated by humans. The techniques of recombinant DNA applied

on a large scale will enable the economical and efficient production of large quantities of drugs needed throughout the world.

74. Vaccines against a variety of diseases may also be produced with genetic engineering. In fact, recombinant DNA may provide a new approach to numerous viral diseases that affect humans, such as hepatitis, infectious mononucleosis, measles, polio, influenza, and the the common cold. In place of the conventional vaccines or serums, specific antiviral coat proteins could be made for each of these diseases. Such vaccines would be both more effective and less expensive than the vaccines and serums currently in use.

Treatment of Genetic Diseases

75. In recent years, important progress has been made toward understanding the molecular mechanism underlying inherited genetic disease. Genetic engineering offers two approaches to relieving the suffering and death caused by these diseases. Genetic diseases are caused by mutations in human chromosomes that disrupt the normal metabolic functions of specific tissues. Often cells in the tissue either fail to produce a necessary protein or produce a defective one that lacks the normal activity. The new technology can be used to manufacture the needed proteins in recombinant clones, which proteins can then be administered to the victims of the disease. A more complex and difficult alternative may be to insert functioning genes into the genetic material of the afflicted person and restore to the tissues their original metabolic functions.

General Problems of Applying Genetic Engineering

76. Although a variety of genes have been cloned in bacteria, there are still some basic unresolved problems blocking many of the applications discussed here. These problems deal primarily with making the genes function efficiently. Not enough is currently known about the various recognition signals and mechanisms used to regulate the transcription of the genetic code and govern the translation of genetic information into functioning proteins. These problems are the central focus of much current research, and scientists are optimistic that many of the outstanding problems will be solved within the next five years.

IV. PRESENT STATUS OF GENETIC ENGINEERING IN DEVELOPED COUNTRIES

77. Since the first report of gene splicing experiments in 1973, rapid progress has been made in North America. Efficient methods of gene synthesis and sequencing have been developed, and plasmids and restriction enzymes have been discovered. As a result of this impressive work, almost any gene that can be isolated or synthesized can be cloned in a precise manner.

78. As a result of these developments in genetic engineering, a new industry has been established by molecular biologists. The recent and rapid expansion of this industry is expected to continue, especially in the light of the recent legal decisions governing patenting of recombinant micro-organisms. These corporate biologists are expected to move beyond the production of common chemicals and to address the challenges offered by applications of their techniques to the pharmaceutical, food-processing, energy-related, and agricultural industries. Before genetic engineering is implemented in these various industries, scientists will have to solve the problems of making the genes function efficiently in the host micro-organisms.

79. At present, there is strong competition in the minds of the many molecular biologists between the desire for financial gain on the one hand and the altruistic concern for human welfare and the drive to acquire new scientific knowledge on the other. It is hoped that this conflict will not result in any lessening of the commitment to developing applications of genetic engineering to the problems of the modern world.

80. Progress in genetic engineering is likely to continue at ever-increasing rates of discovery. Research will expand significanly and derive financial support from industry, governments, and the academic institutions. The competition in all these areas is sufficiently strong to ensure the development of the knowledge needed to solve many of the current problems in the field. The greatest need may well be to establish the principles that will govern the legal and political decisions required by the possible application and control of this new technology.

V. POTENTIAL FOR GENETIC ENGINEERING IN DEVELOPING COUNTRIES

81. Historically, developing countries have a long tradition of using microbes in the daily lives of their peoples. Recent scientific literature, however, indicates that people in developing countries know little if anything about genetic engineering and the importance of recent advancements in research. Most developing countries have academic and industrial institutions capable of adapting to the requirements of genetic engineering, but the people who help determine institutional policies on scientific research and who control the allocation of the countries' financial and material resources need to be educated about the benefits and risks of developing recombinant DNA technology in their countries.

82. This needed awareness can be built through symposia and exchange programmes developed through the co-operative efforts of the United Nations, the universities and appropriate government agencies of both developed and developing countries, and the individual representatives of groups researching the principles and applications of genetic engineering. These presentations will introduce the technology and its implications and help each country analyze its own national needs, establish its own research priorities, and develop its own strategy for implementing the new technology.

83. Many developing countries will need both financial and technical foreign aid to establish up-to-date laboratories to support their research efforts. But the primary problem is to train people to conduct that research. To effect an orderly and systematic transfer of technology from the developed to the developing countries, and international research and training centre should be established under the auspices of the United Nations. This facility would have three purposes:

(a) To help each developing country establish its own core of trained professionals responsible for educating their own community and up-grading and managing their own research facilities;

(b) To assist developing countries solve particular problems of applying genetic engineering;

(c) To conduct basic research into the principles and applications of recombinant DNA and to share the results with all interested countries.

Such an Institute of Biotechnology should employ at least thirty professionals with significant experience in the field, and in addition post-doctoral fellows and technicians. A staff of this composition would provide a strong core of research and teaching talent to pursue the goals of the institute.

VI. THE RISKS OF GENETIC ENGINEERING

84. Th risks of genetic engineering divide into two catagories, those pertaining to the technology itself and those pertaining to its end products.

The Implications of Non-proliferation of the Technology

85. Without international co-operation of the sort needed to establish an Institute of Biotechnology or similar institution, it is possible that the entire technology and its benefits could be withheld from the developing countries of the world. This isolation of genetic engineering might result from a collective oversight or incompetence of international leaders upon whom the responsibility for action rests. This lack of co-operation might also result from an international climate of distrust in which the developed countries would withhold new knowledge in the field from each other and from the developing countries.

86. An analogous situation already exists to control the spread of nuclear technology and is codified in the Nuclear Non-proliferation Treaty, an explicit agreement among the nuclear powers to prevent other countries from developing nuclear weapons. The difference between nuclear and recombinant DNA technologies is that an international non-proliferation agreement would withhold the benefits as well as the risks. On the one hand, the gap between the standards of living in the developed and developing countries could be magnified tremendously. On the other hand, the potential for international coercion and manipulation in such a situation demands careful consideration by the people who influence and control

international relations.

Exploitation of the Technology by Commercial Interests

87. In the absence of adequate international co-operation to safeguard the economic interests of the developing countries, privately-funded commercial interests could exploit the uneven distribution of the technology to sell their products on the world market and manage their profits to suit their own whims. The precedent for such economic manipulation already exists in the actions of countries controlling important energy resources.

The Development and Use of Harmful Organisms

88. The potential harm to people and to the natural environment could come from the accidental or purposeful development and use of virulent organisms.

The Accidental Production of Virulent Organisms

89. Recent advancements in the field of recombinant DNA have generated public discussion among scientists and concerned citizens about the risks of producing virulent organisms that might escape from the laboratory, propagate themselves, and create unforeseen hazards to human health and to the natural environment.

90. Some of the safeguards of recombinant DNA technology to prevent the accidental spread of experimental micro-organisms have already been mentioned, for example, the use of special mutant organisms that cannot live outside their special laboratory environments, and the manipulation of plasmid R-factor sites to produce clones vulnerable to common antibiotics.

91. These safeguards are the result of guidelines established in each of the developed countries to define the conditions under which these experiments should be carried out to protect laboratory workers and the general public from unforeseen accidents. Although most members of the scientific community agree that current experiments present no hazards, no one can claim that all types of recombinant DNA experiments are safe.

92. To insure a constant vigilance governing the conduct of recombinant DNA experiments, each country implementing genetic engineering should provide itself with a series of committees to perform the following functions:

(a) To set national goals and priorities for research;

(b) To co-ordinate the exchange of relevant information with other countries;

(c) To evaluate all research projects using recombinant DNA technology;

(d) To establish guidelines for laboratory facilities, working conditions, and safety measures to ensure the proper handling of the starting materials and products used in genetic engineering experiments;

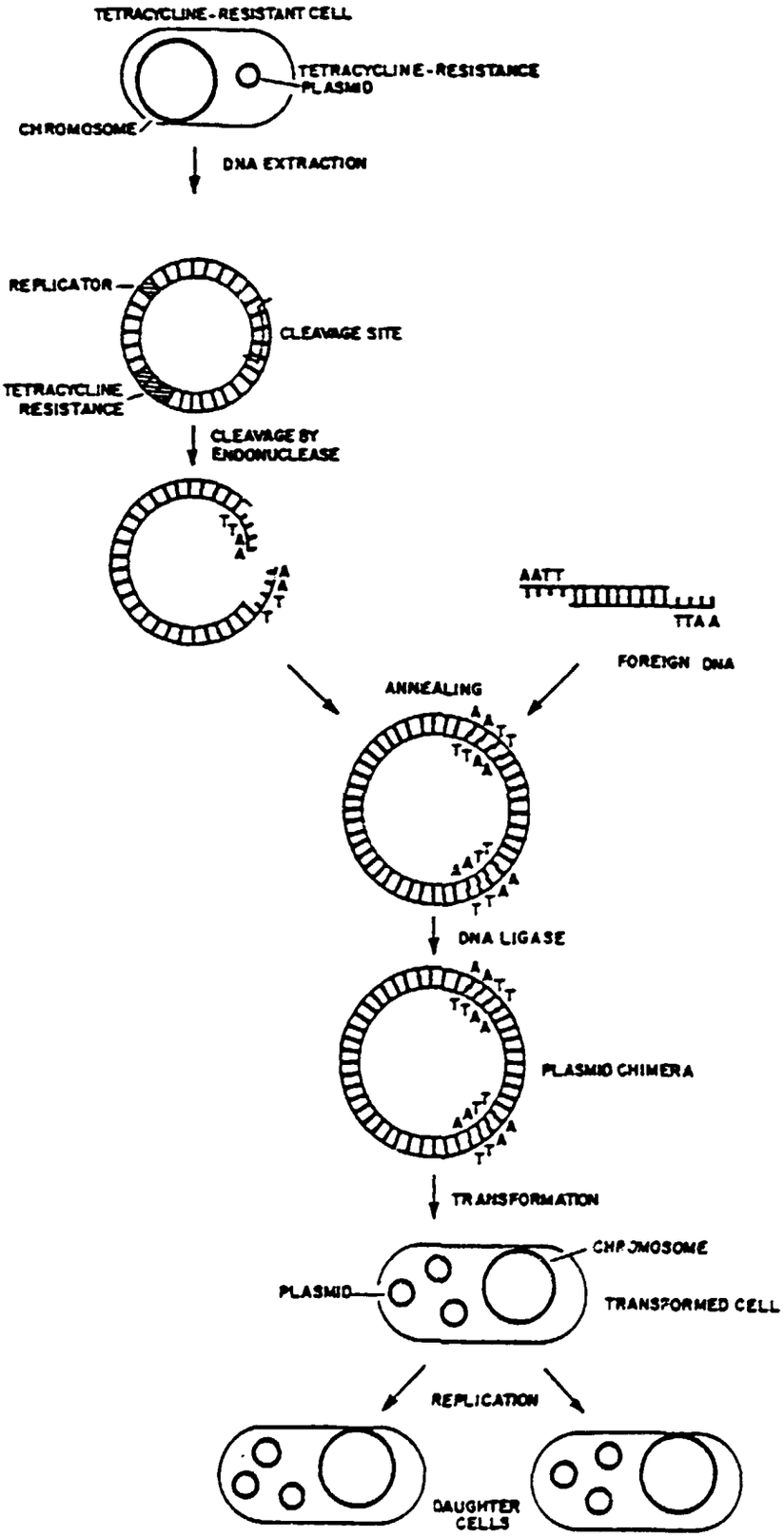
(e) To formulate legislative policies governing the patenting and commercial use of the organisms produced by these experiments.

The Purposeful Production and Use of Virulent Organisms

93. No one can guarantee that irresponsible people with adequate training will not use recombinant DNA technology to develop instruments of destruction. Virulent organisms of frightening potency might be developed and used as weapons for use in war, political terrorism, or repressive manipulation of whole populations.

94. This danger has always been inherent in the development of any technology, and the response to this threat must answer the question, "Do the potential benefits outweigh the potential risks?" As suggested in the foregoing discussion, the potential benefits for humanity are enormous. At the same time the potential risks are equally unprecedented. Policy makers will have to assess the risk that some person or group of people will develop an organism that jeopardizes the very existence of a target population or, indeed, or the entire human race. The responsibility for making such an assessment is awesome. A resolution to this dilemma may come, however, with the assertion that genetic engineering itself also provides the techniques for creating organisms of defense to counteract and neutralize any threat of the misuse and abuse of recombinant DNA technology.

Figure 1: The Phases of Genetic Engineering



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Glossary

Active site

The region of a protein molecule at which direct interaction with a substrate or regulatory molecule takes place.

Amplification

1. Treatment (for example, chloramphenicol) designed to increase the proportion of plasmid DNA relative to that of bacterial DNA;
2. Replication of a gene library in bulk.

Antiparallel

Describes molecules that are parallel but point in opposite directions (the strands of DNA are antiparallel).

Antisense

Strand of DNA that has the same sequence as mRNA

Autoradiography

A technique for the detection of radioactively labelled molecules by overlaying the specimen with photographic film. When the film is developed an image is produced which corresponds to the location of the radio-activity.

Cistron

A DNA fragment or portion that specifies or codes for particular polypeptide.

Codon

A group of three nucleotides that codes for an amino acid.

Cohesive termini

(Cohesive end) DNA molecules with single-stranded ends that show complementarity, making it possible, for example, to join end to end with introduced fragments.

Colony hybridization

Is a procedure for identification of plasmid clones with a DNA or RNA molecule radiolabelled to a high specific radioactivity. The procedure is employed to detect the presence of a complementary sequence.

Episome

Is a plasmid which can integrate (reversely) with the chromosome of its bacterial host. In the integrated state the plasmid behaves as part of the chromosome. Integration appears to require a region of homology between the plasmid and the chromosome. The homologous regions can pair, and a single cross-over between the two circular molecules results in integration.

Exon

Portion of DNA that codes for the final mRNA.

Gap

A double-stranded DNA is said to be gapped when one strand is missing over a short region.

Gene library

Random collection of cloned fragments in a vector that ideally includes all the genetic information of that species; for example, chicken, human; sometimes called shotgun collection.

Gene splicing

Manipulations, the object of which is to attach one DNA molecule to another.

Genome

All the genes of an organism or individual.

Heteroduplex

A DNA molecule, the two strands of which come from different individuals so that there may be some base pairs or blocks of base pairs that do not match.

Initiation codon

(AUG; sometimes GUG); codes for the first amino acid in protein sequences, which is formylmethionine; fMET is often removed post-translationally.

Integration and excision

Integration is a recombination in which a genetic element is inserted. Excision is the reverse of integration.

Intervening sequence

A portion of a gene that is transcribed but does not appear in the final mRNA transcript.

Jumping genes

Genes associated with transposable elements.

Ligase

DNA ligase, ligase catalyzes the formation of a phosphodiester bond at the site of a single-strand break in duplex DNA (RNA can also act as a substrate to some extent).

Linker

A small fragment of synthetic DNA that has a restriction site useful for gene splicing.

mRNA

Is messenger RNA.

Nearest neighbour analysis

Biochemical technique for estimating the frequencies that pairs off the bases that are next to one another.

Nick

A single-strand scission of the DNA (can be made with deoxyribonuclease and ethidium bromide).

Operator

A region of DNA that interacts with repressor protein to control the expression of an adjacent gene or group of genes.

Operon

A gene unit consisting of one or more genes that specify a polypeptide and an "operator" that regulates the transcription of the structural gene (the regulator and the coding genes are adjacent on the DNA molecule).

Palindrome

A self-complementary nucleic acid sequence; that is a sequence identical to its complementary stand (both read in the same 5' to 3' direction); perfect palindromes (for example, GAATTC) frequently occur as sites of recognition for restriction enzymes; less perfect palindromes (for example, TACCTCTGGGTGATA) frequently occur in binding sites for other protein, such as repressors; interrupted palindromes (for example, an inverted repeat such as GGTTXXXAACC) afford the possibility in single-stranded nucleic acids for the loop stem (hairpin) structure as in tRNA.

Phosphodiesterase I

Removes, by hydrolysis, 5' nucleotides from the 3' hydroxy termini of oligonucleotides with 3' ends; also called 5' exonuclease.

Plasmid

A extrachromosomal genetic structure which can replicate independently within a bacterial cell and which is normally dispensible to the cell although its presence may be advantageous to the cell in certain circumstances. Those plasmids which have been investigated appear to be circular double-stranded DNA molecules of molecular weight approximately 10^6-10^8.

Polymerase

An enzyme that catalyzes the assembly of nucleotides into RNA and of deoxynucleotides into DNA.

Probe

(Hybridization) DNA or RNA molecule radiolabelled to a high specific radioactivity, used to detect the presence of a complementary sequence by molecular hybridization.

Pribnow box

TATAATG; consensus sequence near the RNA start point of prokaryotic promoters.

Promotor

A DNA polymerase binds, and then initiates transcription.

Reading

One-way linear process by which nucleotide sequences are decoded, for example, by protein synthesizing systmes.

Regulatory gene

A gene whose product is involved in the regulation of an other gene, such as a repressor gene.

Restriction endonuclease

Or site-specific endodeoxyribonuclease; cleavage is sequence-specific; both strands are cleaved; usually have been isolated from bacteria; there are many, for example, Eco RI; Bam^{H}1; Hind III.

Southern blot technique

Is a method of transferring DNA fragments that have been separated by gel electrophoresis (agarose) to a nitrocellulose filter such that the relative positions of the DNA fragments are maintained; the DNA is usually visualized by hybridization with a ^{32}p-labelled DNA or RNA probe.

Spheroplast

A bacterial cell whose wall is partially, or nearly completely, removed, so that the cell assumes a spherical shape.

Split gene

One that is not continuous but has been interrupted.

Structural gene

A gene that determines the primary structure (that is, the amino acid sequences) of a polypeptide.

Termination codon

Codon that specifies the termination of translation (UAA, UGA and UAG).

Transcription

Formation of the RNA from the DNA template.

Transduction

The transfer of genetic material from one cell to another by means of a viral vector (for bacteria, the vector is bacteriophage).

Transfection

Infection of a cell with isolated DNA or RNA from a virus or viral vector.

Transformation

The introduction of an exogenous DNA preparation (transforming agent) into a cell.

Translation

The process in which the genetic code contained in the nucleotide sequences of mRNA directs the order of amino acids in the formation of peptide.

Transposable

Element a segment or fragment of DNA that can move from one position in the genome to another.

Transposition

Movement from one site in the genome to another.

Vector

An agent consisting of a DNA molecule known to autonomously replicate in a cell to which another DNA segment may be attached experimentally so as to bring about the replication of the attached segment.