A REVIEW OF THE LITERATURE ON RECOMBINANT DNA, A CONTROVERSIAL PROCESS, AND ITS APPLICATIONS

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PREFACE

This report is a review of the literature concerning recombinant DNA, the process of taking genes from one organism and splicing them into the genes of another different kind of organism. First, the mechanics of the process are explained. Then the medical, industrial, and agricultural products being synthesized by recombinant DNA are enumerated. Other areas of research which use the methodologies of recombinant DNA research are discussed.

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A very loving thanks is expressed to my husband, Bill, for the opportunity that he provided me and for his understanding and encouragement. Thanks are also extended to our daughters, Lynn and Katie, for their love and sacrifices.

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CHAPTER I

INTRODUCTION TO RECOMBINANT DNA

Recombinant DNA, gene-splicing, and genetic engineering are all terms for one controversial and exciting technology of molecular biology. Recombinant DNA is the process of taking genes from one organism and splicing them into the genes of another different kind of organism which can result in the modification of the organism.[1] Until the development of this technique, scientists were limited to working with traits found naturally in a species' genetic repertoire. Now they have domesticated a variety of life, microorganisms, that might serve humanity in efficient ways because of its simplicity and capacity for reproducing exact replicas of itself.

The work of great scientists such as Anthony von Leeuwenhoeck, inventor of the microscope; Robert Hooke, discoverer of the cell; Gregor Mendel, meticulous researcher who wrote the laws of heredity; Boodle and Tatum, who identified DNA as genetic material; Francis Crick and James Watson, discoverers of the structure of the DNA molecule; and Jacques Monod and Francois Jacob, Researchers who discovered operon genes; made contributions which led to the pioneering experiment on recombinant DNA in 1973. Stanley Cohen and Annie Chang of Stanford University, and Herbert Boyer and Robert Helling of the University of California at San Francisco inserted a gene that makes Salmonella resistant to the antibiotic streptomycin into E. coli which consequently became resistant.[2]

Recombinant DNA is not something which has never before occurred; the change of genetic material naturally occurs in sexual reproduction, transformation in bacteria, and transduction by viruses.[3] It may have even occurred naturally between certain bacteria and humans because some bacteria harbor genes similar to the human genes that regulate the production of chorionic gonadotropin.[4] But the Recombinant DNA methodology is very different in two ways from natural transplantation. One difference is that recombination may occur between widely different species, and the genetic information may be very specific and in small segments coded for a known effect. A second way that the methodologies are unlike is that the vehicle that carries the segment from the donor organism to the host allows for the donor segment to be spliced together and reassembled in a structure that can be inserted into the host.[5]

The recombinant DNA methodology allows several important opportunities to manipulate molecular genetic systems and will be explained in some detail in this report. First, it allows very long and complicated continual sets of genomes to be fractionated into much smaller functional units and placed in single cells where the study of the function controlled by one genetic expression may be done. Second, by isolating one cell in a culture with the desired fragment of genetic information, it is possible to grow colonies of cells from it, all of which will have the desired component. Therefore, the component will be greatly amplified without any deterioration in the fidelity of its expression. Third, the products of the gene fragment's expression can be separated from the natural environment where they are frequently victims of the cells own mechanisms for maintaining specified concentrations of a product. Fourth, new genetic combinations can be created which make materials advantageous to human purposes.[6]

After the process of Recombinant DNA is explained, the products being produced by it will be listed and the other areas of research which use it will be explored. The products being produced by Recombinant DNA fall into three broad categories, agricultural, industrial, and medicinal. In agriculture, one of the goals is to transfer the genes for nitrogen-fixation into the cells of plants like corn and wheat which are now consumers of fertilizers. In industry, there are many goals, such as improving the efficiency of fermentation processes, using the capacity of microorganisms to metabolize crude petroleum and metals, and using specific enzymes produced by bacteria in petrochemical syntheses and for specific bioconversion steps. In medicine, the greatest progress seems to be taking place. Substances such as human-growth hormone, insulin, interferon, and somatostatin are being produced by bacteria.[7] When products are synthesized with Recombinant DNA methodology, the cost of their production generally decreases because the amount of energy consumed in the processes is less. Also pollution is not a problem because only natural products are excreted.[8]

Other areas of research which use some of the Recombinant DNA methodology include nucleotide sequencing, evolution, physical mapping of chromosomes, gene regulation, differentiation, prenatal examination of human genes, gene therapy, and ecology. Recombinant DNA has even caused scientists to question old genetic dogmas, such as the alikeness of genes in different organisms and the shape of the DNA molecule.

FOOTNOTES

[1]S. Begley and P. Abramson, "The DNA Industry; Recombinant Technology," Newsweek (Aug. 20, 1979), p. 53.

[2]M. Clark, "The Miracles of Spliced Genes (Recombinant DNA)," Newsweek (March 17, 1980), p. 63.

[3]W. N. Hubbard, "The Industrial Potential of Recombinant DNA Technology (address, Jan. 24, 1980)," Vital Speeches (March 11, 1980), p. 342.

[4]Peter Gwynne, "Caution: Gene Transplants," Newsweek (March 21, 1977), p. 58.

[5]Hubbard, p. 342.

[6]Ibid.

[7]Ibid.

[8]M. Sheils, "How Molecular Biology is Spawning an Industry (commercial applications for Recombinant DNA)," Newsweek (March 17, 1980), p. 70.

CHAPTER II

A GENERAL DESCRIPTION OF THE PROCESS OF RECOMBINANT DNA

The mechanics of the process of recombinant DNA began with the discovery of restriction enzymes in 1971.[1] In general, scientists take donor DNA, which is DNA from cells or DNA chemically synthesized in a laboratory, use restriction enzymes to cut it at specific points, insert the desired fragments into the DNA of a carrier of vehicle, which is a bacterial plasmid or a virus, and then place the carrier into a host, usually E. coli. The constellation of recombinant DNA techniques for placing and maintaining a new gene in bacteria is called cloning. It is defined as the isolation of a specific new DNA sequence in a single organism that proliferates to form a population of identical descendants, a clone.[2]

The problem, which the mechanics of this process are being developed to solve, can be broken down into three parts. First, the correct structural sequence of DNA must be located or created. Second, it must be placed in bacteria in such a way that it will be maintained as the bacteria grow and divide. Third, the surrounding information in the DNA must be manipulated so that the regulatory commands can be modified to direct the spliced sequence to produce protein. Once the spliced gene expresses itself, and protein is made, further changes in it or modifications in the bacteria are sometimes needed to obtain proten in large enough amounts to be useful.[3]

There are several ways of getting the donor DNA. Restriction endonucleases have the ability to scan a long thread of DNA and to recognize particular short sequences as landmarks at which to cut the molecule apart. Over forty of these have been discovered. Each one recognizes different landmarks, therefore, each one breaks up any given DNA into a characteristic set of short pieces which can be isolated by length.[4] Herbert Boyer found that some of these enzymes produce staggered cuts in double strands of DNA so single-stranded ends are produced at each end of the fragments. Since all the fragments produced by a given enzyme have the same self-complementary end, a single fragment can form a circle by base-pairing or can combine with another fragment to produce a dimer. If the second fragment is from a different DNA source, a recombinant molecule is produced. DNA ligase is the annealing enzyme, which glues the ends together.[5]

Even with the restriction endonucleases, there is a problem sometimes with getting a needed DNA fragment and placing only that fragment into a bacterium. Consequently, a second method for obtaining donor DNA has been developed. By knowing the amino acid sequence of a protein, the base sequence can be predicted, and a corresponding DNA molecule can be chemically synthesized.[6] Several other ways of sequencing DNA have been developed, and genes can be made in laboratories.

Structural gene information also exists in continuous form on messenger RNA (mRNA). Since different cells specialize in the synthesis of different proteins, appropriate tissues will contain the desired mRNA along with other messengers for the common proteins made by all cells. In order to convert desired structural information from a cell's mRNA into DNA to be cloned, a special enzyme, reverse transcriptase, is used

to copy a single strand of RNA to make a complementary strand of DNA (cDNA). This strand of cDNA is used to make a second strand of DNA, and the resulting double-stranded cDNA fragments are copies of the messengers that were present in the tissue. This cDNA can be cloned, but there are two problems with it, the detection of clones with the sought-after structural DNA fragment and the providing of appropriate signals.[7]

The carriers in the Recombinant DNA process must be capable of being identified as actually containing the desired DNA fragment, must have a marker that allows them to be identified after they are placed in a host cell, and must replicate in the host.[8] Bacterial plasmids and viruses are the vehicles used. They strain the definition of life because they are negentropic, which means they expand energy to keep themselves in order.[9] Plasmids carry only a few genes of their own, are separate from the main set of bacterial genes, and are maintained in several copies inside bacteria.[10] The Col El plasmid is the most popular one. If foreign DNA is inserted at the Eco RI site, the recombinant plasmid can be detected because it does not produce the antibiotic colicin.[11] Donald Helinski at the University of California at San Diego has developed two unique forms of this plasmid, Col El-kan and Col El-trp. Col El-kan confers resistance to the antibiotic kanomycin, therefore, it is less safe than Col-El-trp which has a nutritional marker. Only bacteria with it can grow in culture plates lacking the amino acid tryptophan. Neither one of these plasmids is a disabled vector.[12]

Viruses have from ten to fifty genes of their own and can often carry other new DNA segments in place of some of their own.[13] Frederick R. Blattner and Bill G. Williams of the University of Wisconsin have engineered bacteriophages, charon lambda phages, as vectors for cloning.[14]

Lambda is particularly well suited as a vehicle because a third of its DNA, a block in the middle of its genome, can be replaced without it losing its ability to grow lytically. Charon 3 and 4, both mutations of lambda, can hold large pieces of foreign DNA.[15] The success of the splicing can be monitored with dye indicator plates.[16] Cloning with lytic phages has natural advantages. One is that the phage and sensitive bacteria must repeatedly come togehter for replication. Also their chimeric DNA is delivered to the experimenter in a package. And since the phage and bacterium coexist only briefly, the cloned fragment need not be compatible with the bacterium's metabolism for an extended time period.[17] One problem with phage vectors is that they may lysogenize rather than kill the cell. The charon 3 and 4 phages have been engineered to kill cells so efficiently that only one surviving cell with the foreign gene can be found during the production of 1000 billion phages. Since large numbers of phages can be grown in a small volume of culture medium, less spillable liquid must be handled.[18]

Vector engineering has many objectives. They are to alter the distribution of restriction sites and eliminate sites in essential genome regions, to clone a variety of sizes of DNA fragments, to clone with more than one restriction enzyme, to be able to indicate by plaque type whether or not a phage has incorporated a DNA fragment, to clone with minimal manipulation, to control the transcription of cloned fragments from vector promoters, to grow vectors and clones to high yield, to recover cloned DNA readily, and to contribute to biological containment.[19]

Caulimoviruses, such as CAMV Pahlia mosaic, and strawberry etched ring, are the plant viruses known to contain DNA. Therefore, they may become transducing vehicles for the introduction of foreign DNA fragments

into plant cells.[20] Defective derivatives of the DNA of the tumor viruses SV40 and polynoma are possible vehicles but are currently banned because they have been found to cause cancer in laboratory animals.[21]

Once a chosen DNA fragment has been inserted into a carrier, then the vehicle must be inserted into the host where it can replicate. The host must be capable of having its cell wall altered so the carrier can penetrate and survive the insertion.[22] This is accomplished by using a dilute solution of calcium chloride to render the bacteria permeable.[23]

E. coli is the most common host used although Saccharomyces cerevisiae, Neurospora crassa, Bacillus subtilis, and Pseudomonas have been suggested. Roy Curtiss III at the University of Alabama Medical Center disarmed E. coli to fit a pre-determined set of safety criteria. The X-1776 strain has two mutations which make it nearly impossible for the bacteria to colonize the intestinal tracts of animals. They cannot make diaminopimelic acid or colanic acid, substances required respectfully in the making of the cell wall and in holding the cell together. They are also temperature sensitive (self-destruct at 42^{o}C) and are killed when exposed to ultraviolet light (sunlight). The bacteria cannot make thymine, therefore, cannot pass genes onto healthy outsiders during conjugation. They cannot conjugate with most other bacteria or be infected by most bacterial viruses. They cannot survive in human serum including that of cancer patients and are destroyed by common household detergents.[24]

Once the vehicle has been inserted into the host, the spliced gene must be able to be turned on, a process called transformation. Bacteria have four signals which must be both efficient and optimally placed. One signal starts the synthesis of a mRNA and is located immediately in front of the segment of DNA to be transcribed. A second signal functions as

part of the mRNA to tell the bacterial translating machine where to start. The other two signals are stop signals for transcription and translation.[25] These signals can be cut out with restriction enzymes and placed wherever they are needed.

The cells which are transformed can be identified if the vehicle provides a property the bacteria need to survive.[26] The identification of the desired clone can be done after the cells have formed colonies. One way of identifying the desired colony is by doing hybridization test. If the mRNA from which the cDNA is made is pure, the host colonies are grown on a disk of cellulose nitrate paper. The bacterial cells are then broken open where they lie, and the released DNA is fixed to the paper, denatured, and reannealed to radioactive RNA. Only the DNA strands whose sequence matches the mRNA become radioactive. Since the location of the desired DNA is known, bacteria which contain the modified sequence may be obtained from replica colonies and cloned.[27]

If the mRNA is not pure, there are several ways to detect the desired colony. One way is to let antibodies detect the detailed shape of the corresponding protein molecule. Another way is to make the mRNA function in a test tube by adding the machinery needed for translation and radioactive amino acids. Then antibodies can recognize the desired radioactive protein. A third method is to take the cDNA and hybridize it to a mixture of RNA's. Only the RNA that matches the desired sequence will anneal to it, therefore, no longer functioning in translation. The desired DNA is recognized by its ability to block the synthesis of the desired protein.[28]

Several problems concerning the yield of the products occur and must be solved. Often the new proteins are broken down in the bacterial cells because their structure is such that enzymes normally present in the

bacteria can digest them. Ways must be found to stabilize the proteins either by removing these enzymes by embedding the new proteins in hybrid proteins to protect them, or by secreting them from the cell. Messenger RNA molecules are often unstable within bacterial cells. Therefore, modification in their structure and in the cells can make them more effective and lead to increased protein synthesis. Third, the number of copies of the desired gene within each cell must be increased so more product can be synthesized.[29]

FOOTNOTES

[1]"Genetic Sabotage in the Public Interest," Sci. News (March 20, 1976), p. 188.

[2]W. Gilbert and L. Vella-Komaroff, "Useful Proteins from Recombinant Bacteria," Sci. Amer. (April, 1980), p. 76.

[3]Ibid.

[4]Ibid.

[5]"Recombinant DNA: Examples of Present-Day Research (Symposium)," Sci. (April 8, 1977), p. 159.

[6]Gilbert and Vella-Komaroff, p. 85.

[7]Ibid.

[8]Hubbard, p. 343.

[9]"Genetic Sabotage in the Public Interest," p. 189.

[10]Gilbert and Vella-Komaroff, p. 76.

[11]"Recombinant DNA: Examples of Present-Day Research (Symposium)," p. 172.

[12]"Genetic Sabotage in the Public Interest," p. 190.

[13]Gilbert and Vella-Komaroff, p. 76.

[14]"Genetic Sabotage in the Public Interest," p. 190.

[15]"Recombinant DNA: Examples of Present-Day Research (Symposium)," p. 161.

[16]"Genetic Sabotage in the Public Interest," p. 190.

[17]"Recombinant DNA: Examples of Present-Day Research (Symposium)," p. 161.

[18]"Genetic Sabotage in the Public Interest," p. 190.

[19]"Recombinant DNA: Examples of Present-Day Research (Symposium)," p. 161.

[20]Ibid.

[21]Ibid.

[22]Hubbard, p. 343.

[23]Gilbert and Vella-Komaroff, p. 76.

[24]"Tinkering with Life," Time (April 18, 1977), p. 45.

[25]Gilbert and Vella-Komaroff, p. 86.

[26]Ibid.

[27]Ibid.

[28]Ibid.

[29]Ibid.

CHAPTER III

THE PRODUCTS BEING SYNTHESIZED BY RECOMBINANT DNA

Agricultural, industrial, and medical researchers are investigating the use of DNA techniques to synthesize valuable products. Anything that is basically a protein will be makable in unlimited quantities. Especially important will be the production of proteins which cannot be obtained in useful forms from animals. Recombinant DNA microorganisms may eventually produce 10 to 20 percent of all the organic compounds required by industry, such as petrochemical derivates from plastics to pigments. Genex, a Bethesda, Maryland, firm has identified 107 existing products that can be manufactured in 5 to 20 years with recombinant DNA.[1] Five products--human growth hormone, somatostatin, and A and B chains of human insulin, human proinsulin, and thymosinalpha-1--have been approved for large-scale culture by Genentech.[2] Only one artificially altered genetic material, a corn variety, has been approved to be released into the environment.[3] DNA ligase is produced by N. E. BioLabs of Beverly, Massachusetts, and is being sold to scientists.[4] Many restriction enzymes are also available.

More medically important products are being synthesized than ones in the other two research areas. The first protein made was somatostatin. It was followed by insulin, human growth hormone, and interferon.[5]

Somatostatin is a mammalian neurohormone secreted by the hypothalamus in trace amounts. It inhibits the release by the pituitary gland of

hormones that regulate body growth and glucagon and insulin production. Therefore, it may be useful in the treatment of diabetes, pancreatitis, and acromegaly, a disease of abnormal bone growth.[6] When somatostatin was first isolated, it took half a million sheep brains to produce five milligrams of it. With recombinant DNA, Herbert Boyer, Aruther Riggs of the City of Hope Medical Center, and Wylie Vale of the Salk Institute, respectively, required only two gallons of bacterial culture to produce five milligrams of it.[7] The researchers choose somatostatin as the first substance to produce with recombinant DNA because of its small size (fourteen known amino acids), sensitive radioimmune and biological assays, and intrinsic biological interest.[8] They synthesized the gene for somatostatin from its known amino acid sequence and linked it to the bacterial beta-galactosidase gene which can be recognized by E. coli. The genes were bridged by the nucleotides that code for methionine. The long chain was spliced into a plasmid vector that was picked up by E. coli and introduced into colonies. The chain was isolated from a colony and treated with cyanogen bromide which breaks it at methionine, therefore, freeing somatostatin.[9]

Insulin is produced by the cell in the Isles of Langerhans in the pancreas and regulates the metabolism. Five percent of diabetics have allergic reactions to the currently used cattle and swine insulin.[10] If human insulin can be produced commercially with recombinant DNA, the risk of allergy or antibody-rejection by diabetics will be eliminated, the cost for diabetics will be less, and the problem of the shortage of animal insulin will be solved.[11] Insulin is a small hormone made of two chains, chain A, twenty amino acids long, and chain B, thirty amino acids long. These are initially part of a chain of 109 amino acids,

proinsulin. This chain folds up bringing the first and last segments together, and the central portion is cut out by an enzyme. The purpose of this central portion is to align the two chains correctly.[12] Two teams, one at the City of Hope Medical Center under the direction of Dr. Keirehi Itakura and the other at Genentech, Inc., under the direction of David Goeddell have produced insulin with recombinant DNA. An artificial gene for each chain of insulin was spliced into a plasmid among a group of genes that bacteria switch on in the presence of lactose. Each bacterium was induced to produce 100,000 molecules of either chain, then the chains were purified and combined. Ten to forty percent of the material formed the two appropriate chemical bonds and became complete insulin.[13] Prior to this final experiment, the chains were produced in separate bacteria and then combined, proinsulin was produced, and rat insulin was produced.

Human growth hormone is produced by the pituitary gland. It stimulates the natural healing process and is used to treat pituitary dwarfism, stomach ulcers, and burn injuries. Currently it takes fifty pituitary glands from cadavers to supply enough hormone for one child suffering from dwarfism for one year.[14] Both human and rat growth hormone have been produced with recombinant DNA. Eighty percent of the gene was copied from mRNA, and the rest of it, a restriction enzyme site, was synthesized. The gene was linked to a fragment of the trp D gene of E. coli in a plasmid vehicle. The fusion protein was synthesized at a high level, reacted specifically with antibodies to human growth hormone, and was stable in E. coli.[15] There were three problems concerning the production of it by bacteria. One problem was finding a method to trim the hybrid protein into the desired hormone; another problem was handling large volumes of hormone-producing bacteria; and the third problem was getting FDA to use it clinically.[16]

Interferon was accidentally discovered in England in 1957. It is a protein possessing both antiviral and antitumor properties. In large doses, it is effective against chicken pox, shingles, and German measles viruses. It is used in cancer treatment because it seems to block cell division and in research on flu and colds. Currently it takes 65,000 pints of blood to get 100 mg of it, and it costs $75 per shot.[17] The main problem that had to be overcome before it could be produced with recombinant DNA was getting enough mRNA for it since this is very rare. Human white blood cells were exposed to a virus, and the mRNA was extracted from them and used to generate the gene. The gene was spliced into a plasmid which was taken up by E. coli. The bacteria produced human fibroblast interferon with all its normal properties. It is a protein, withstands acid treatment, reacts with antibodies that bind it, and is species specific. But it also had some properties that were different from human interferon. It was a larger molecule and did not have the sugars found on human interferon. The larger size may be due to a signal sequence that animal cells can cleave, but bacterial cells cannot.[18] Since this experiment, Biogen's Charles Weissman of the University of Zurich and Walter Gilbert of Harvard have produced human leucocyte interferon.[19]

Several other medically important substances have been produced with recombinant DNA. John D. Baxter of the University of California at San Francisco engineered the production of the mammalian pain-countering chemical beta-endorphin. It is naturally produced by the pituitary gland. Currently, it is being tested as a treatment for both depression and schizophrenia. The gene for mouse beta-endorphin was modified and connected to a portion of the bacterial gene, beta-galactosidase, in a plasmid.[20]

Thymosin alpha-1, a hormone that stimulates the human immune system, is being produced by bacteria. It has shown promise in clinical trials for the treatment of brain and lung cancer.[21]

Bethesda Research Labs has produced proline, an amino acid, in the first formal application of recombinant DNA to nutrition. It is used in livestock feed and intravenous feedings and may become a food supplement, therefore, decreasing the costly world food supply. A bacterial virus was the vehicle in this experiment.[22]

The largest protein that has been successfully expressed and recovered is ovalbumin from fowl.[23]

The recombinant DNA techniques hold promise for producing materials for vaccines against infectious diseases. They provide new opportunities for making large amounts of pure viral components and for redesigning viruses for use in live vaccines. Some advances have already occurred. Bacterial components implicated in diarrheal diseases and genes from influenza and hepatitis viruses have been produced. The G. D. Searle Company produces the flu virus antigen hemagglutinin with bacteria.[24] Kenneth Murray of Edinburgh University has cloned the coat protein of the hepatitis B virus,[25] and an antibody against one chemical on the surface of the malaria parasite has been produced.[26] Genentech and the Department of Agriculture have cloned viruses needed to produce a vaccine against hoof-and-mouth disease.[27] Bacteria are also making individual subunits of disease-causing toxins. Toxins have been found to have two subunits, one is the enzyme that produces the detrimental effect and the other is the delivery component. The delivery subunit may be a valuable vaccine material because it should induce an immune response without harming the patient. Some bacteria are making the enzyme subunits so the toxins can be deactivated.[28]

Recombinant DNA is also used in antibiotic research. Low producing organisms can be amplified so their product is in sufficient quantity to be examined as a possible medicine. Antibiotics can be produced in a non-destructive environment, and their concentrations in the culture medium can be increased, therefore, decreasing their cost and widening their availability.[29]

There are four speculative possibilities for the future use of recombinant DNA in medicine. It can be used to identify very specific action sites within parasitic organisms that cause tropical diseases. Then specific antibodies can be produced by transformed organisms. It could also be used to make highly specific antigens to protect whole populations. A male antifertility agent could be made. It would be an antibody against the contractile protein of the sperm tail that would not interfere with sperm maturation or affect the genetic information in the head. An antidote to drunkenness could be developed by providing medicinally the alcohol dehydrogenase exhausted from the liver.[30]

Biotechnology or industrial processes based on biological systems is the growth business of the future.[31] It was started when General Electric's Ananda M. Charkrabarty created a multiplasmid super bug which can digest about two-thirds of the hydrocarbons in crude oil much faster and more efficiently than other biodegradation systems. The genetic ability to digest petroleum is transmitted on plasmids. He induced different strains of Pseudomonas to conjugate in such a way that a recipient strain ended up with the hydrocarbon breakdown plasmids from three other strains. The by-products of the breakdown are the useful metabolic products, carbon dioxide, water, and protein which can become very useful.[32]

Other current experiments are projects to make alcohol from biomass

more economically or from manioc or cellulose, therefore, boosting its potential as a partial substitute for gasoline.[33] Two approaches can be taken to this project. Either develop more efficient yeasts, which has been done, or use microorganisms as enzyme factories and then add enzymes directly to the substrate to produce the desired effect.[34] Another project is to cut the production costs for chemicals like antifreeze by controlling reactions with bacterial enzymes.[35] This would save energy, increase the efficiency of the process, and decrease the need for oil.[36] Bacterial factories can be created for specific bioconversion steps such as those in steroid production that now are unobtainable for lack of bacteria with specific enzyme capacity.[37] Another project is to use the capacity of microorganisms to metabolize metals to increase the recovery of metals from lean ores by leaching with them. Industrialists also want to convert ethylene and propylene, basic petrochemical feedstocks, into their oxides and glycols for half price. Ethylene glycol is the main ingredient in antifreeze and propylene oxide is used in the production of plastics. Bacteria can also be used to make specialty chemicals needed to produce dyes, detergents, flavor and perfume agents, lubricants, fertilizers, synthetic rubber and pesticides, to produce enzymes for food and animal feed, to produce gums to aid in the recovery of oil from played-out wells, to convert coal to natural gas, to distill whisky, and to metabolize margarine so that it will taste like butter.[38]

The need for a quantum increase in agricultural production has been recognized for a long time, but there is a ceiling imposed on it by the lack of genetic variation in many crop species.[39] The main objective of recombinant DNA in agriculture has been to create bacteria capable of nitrogen-fixation. Nitrogen is fixed naturally by the root nodule

bacteria, Rhizobia, of leguminous plants like soybeans. These bacteria harbor the nitrogen-fixing, NIF, genes which code for the nitrogenase proteins. A nitrogen-fixing recombinant of E. coli has been constructed by transferring the NIF segment of DNA from Klebsiella pneumoniae to E. coli on a plasmid. There may be a need for the enzyme that catalyzes the transformation of molybdenum to a form suitable for insertion into nitrogenase and for enzymes that catalyze various membrane-associated reactions. E. coli already has these support enzymes.[40] If the plasmid that carries the NIF genes can be incorporated into the cells of plants like corn and wheat, they would be able to fix nitrogen, and our dependence on the energy required to produce nitrogen fertilizers would decrease. Several techniques need to be developed before the incorporation can be done. These include developing some form of protection to prevent destruction by plant nucleases, stimulating the infected protoplast to produce a callus and subsequently sexually competent plants, and developing some form of protection to prevent oxygen inhibition of the nitrogenase activity. Once solutions have been found, utilization will be through plant breeding techniques.[41]

Another use of recombinant DNA in agriculture is to transfer characteristics which determine resistance to pathogens into cells of otherwise successful varieties of crop plants.[42] If this can be done, the problems of toxicity and the use of pesticides and herbicides can be avoided.[43] Recombinant DNA is also being used to increase the efficiency of photosynthesis in order to get larger crop yields, to change the husks of grain plants to make them more resistant to cold or drought, thereby, increasing their geographical range,[44] and to circumvent the mating barrier between different species so that novel genetic systems can be explored.[45]

FOOTNOTES

[1]"New Life Forms: A Clear Road Ahead?" U.S. News (June 20, 1980), p. 34.

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[5]W. Stockton, "On the Brink of Altering Life (Recombinant DNA Research)" N.Y. Times Mag. (February 17, 1980), p. 64.

[6]"Bacteria Synthesize Brain Hormone," Sci. News (November 12, 1977), p. 310.

[7]"E. coli at Work. Scientists Use Bacteria to Make Human Hormone," Time (November 14, 1977), p. 56.

[8]K. Itakuna "Expression in Escheuchra coli of a Chemically Synthesized Gene for the Hormone Somatastatin," Sci. (December 9, 1977), p. 1056.

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[12]Gilbert and Vella-Komaroff, p. 88.

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[14]J. Baslough, "Gene Splicing: Key to Miracles in Science," U.S. News (December 31, 1979), p. 48.

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[16]"Gene Splicing Gives Growth Hormone," Sci. News (January 20, 1979), p. 39.

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[20]"Bacteria Make Brain Opiate (beta-endorphin)," Sci. News (May 17, 1980), p. 309.

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[22]"Gene-Spliced Amino Acids (work at Bethesda Research Labs, Inc.)," Sci. News (April 5, 1980), p. 216.

[23]Hubbard, p. 343.

[24]N. Wade, "Recombinant DNA: Warming up for Big Payoff," Sci. (November 9, 1979), p. 665.

[25]Ibid.

[26]H. M. Schmeck, "Toward a New Scientific-Industrial Revolution," Current (March/April, 1980), p. 43.

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[28]"Gene Research Opens Vaccine Possibilities," Sci. News (October 27, 1979), p. 279.

[29]Hubbard, p. 343.

[30]Ibid.

[31]"Where Genetic Engineering will Change Industry," Bus. Week (October 22, 1979), 160.

[32]"G. E. Super Bug Created to Clean Up Oil Spills," Sci. News (September 20, 1975), p. 180.

[33]"Where Genetic Engineering will Change Industry," p. 160.

[34]Hubbard, p. 345.

[35]"Where Genetic Engineering will Change Industry," p. 160.

[36]Hubbard, p. 346.

[37]Ibid.

[38]"Where Genetic Engineering will Change Industry," pp. 160-172.

[39]P. S. Carlson and J. C. Polacco, "Plant Cell Cultures: Genetic Aspects of Crop Improvement," Sci. (May 9, 1975), p. 622.

[40]K. T. Shanmugam and R. C. Valentine, "Molecular Biology of Nitrogen Fixation," Sci. (March 14, 1975), pp. 919-923.

[41]G. F. Sprague, "Plant Breeding and Genetic Engineering: A Perspective," BioSci (January, 1980), p. 20.

[42]B. B. Haskins, "Applications of Genetic and Cellular Manipulations to Agricultural and Industrial Problems," BioSci (March, 1977), p. 188.

[43]Hubbard, p. 346.

[44]Schmeck, p. 42.

[45]"Recombinant DNA: Examples of Present-Day Research (Symposium), p. 210.

CHAPTER IV

OTHER AREAS OF RESEARCH WHICH USE THE RECOMBINANT DNA TECHNOLOGY

Because of the opportunities to manipulate molecular genetic systems, recombinant DNA is allowing scientists to re-examine old genetic dogmas. For example, bacterial genes have been found to be different from those of higher organisms. All the nitrogenous bases in their DNA are read by enzymes three by three and translated directly into amino acids. But in viral and mammalian DNA, elements of DNA that code for amino acids are separated by base sequences (introns) that do not seem to get translated into any protein. The DNA bases are copied into a molecule of RNA which enzymes process. These enzymes cut the introns out of the RNA and splice the remaining coding segments together. It is thought that these piece-meal genes may have helped humans evolve since they can be shuffled more easily into new combinations which may change the character of a cell and give the organism a selective advantage.[1]

A second dogma that has been re-examined is that of the shape of the DNA molecule. Its shape, a small percent of the time, is that of a zig-zag that twists to the left instead of a smooth curve that twists to the right. The smooth spiral changes into this zigzag form at special base sequences. This change may occur when genes turn themselves off, and some of these transformations may attract carcinogens and trigger cancer.[2]

The recombinant DNA methodology is currently leading to discoveries

in still more areas of scientific research. For instance, by being able to reproduce isolated DNA segments in bacteria, scientists are being provided with enough DNA to determine the exact nucleotide sequence. Being able to do this increases the knowledge of the language of the genetic code and provides instructions for chemists interested in synthesizing specific genes. The information provided is also useful in studying evolution because theories about mutation and selection can be explored by comparing the chromosomal sequences of closely and distantly related species.[3] Since scientists can synthesize DNA, they may create mutations and may eventually design specific mutations to be inserted into organisms so they will continually exist.[4]

Isolated DNA segments can be used to locate genes on original chromosomes, therefore, providing a physical map. A map can help scientists understand the coordination of gene expression by learning more about the interaction of adjacent regions and the meaning of repeated sequences.[5] The understanding of the regulation of gene expression is very important in research having to do with cancer and differentiation. To find the DNA region that is necessary to start the expression of a gene, the DNA can be trimmed until the gene no longer functions.[6] In one study with the lambda virus, the behavior of the genes was shown to depend on a repressor molecule. Normally DNA sends instruction for protein synthesis with the aid of a transcribing enzyme. Depending on how the repressor is positioned within the control region of the DNA, it can either attract the transcribing enzyme, thereby, turning on the genes for viral reproduction in E. coli or deflect the transcribing enzyme, thereby, keeping the genes turned off.[7]

Recombinant DNA methodology is valuable in testing hypotheses about

how genes control development. Scientists try to distinguish the control of protein production at the DNA-to-RNA step and at the RNA-to-protein step in order to tell when a gene is first expressed.[8] In this research, techniques from cancer studies are used because genes of tumor causing viruses that invade cells are virtually the same as genes that already inhabit the cell, and these viral genes have been cloned.[9]

The prenatal examination of human genes in amniotic fluid cells requires the use of restriction enzymes. Delta beta-thalassemia disease and trait can be identified by a special technique requiring them. After they fragment the DNA, and the segments are separated according to size, probes of radioactively labelled DNA segments of the same sequence as the beta-globin gene being studied are added. The probes bind specifically to the DNA segments containing pieces of the beta-globin gene DNA. Then the DNA-containing material is exposed to photographic paper. Since only the beta-globin bands appear, scientists can compare normal patterns with those from fetuses suspected of having delta beta-thalassemia known to be due to deletions of the beta-globin gene. Scientists have found that in delta beta-thalassemia, there is an absence of certain DNA segments that hydridize with the beta-globin gene and that if the beta-globin gene bands are present in a smaller number than normal in the material, the fetus is a trait carrier. In alpha-thalassemia fetuses, there is an absence of alpha-globin genes. Sickle-cell anemia is the next most likely candidate for prenatal detection because its DNA abnormality is known.[10]

Gene therapy which is the insertion of a properly functioning new gene into a cell or the repair of a defective gene, is also an offshoot of recombinant DNA methodology. W. French Anderson of the National Institute of Health injected genes prepared with recombinant DNA methods with

a micropipette directly into specific mouse cells growing in a laboratory tissue culture. In several instances, the cells accepted the foreign material, either a viral gene for the enzyme thymidine kinase or the human beta-globin gene, and thirty generations later the genes were still functioning in the descendants. This type of research will allow scientists to compare under identical conditions the operation of a gene when it is transplanted into different types of mammalian cells or the operation of different genes in one cell type.[11]

The first successful insertion of a selected gene into a living animal was done by Martin J. Cline, Howard Stang, Karen Mercoli, and Winston Salser. Using recombinant DNA methods, they isolated genetic material containing the gene that confers resistance to an anti-cancer drug from mice and inserted it into the bone marrow cells of other mice. The first spinoff of their research will probably come in cancer therapy. Cancer patients may be given the gene for resistance to the chemotherapy drug, methotrexate, in their bone marrow cells. Consequently, the drug will not harm healthy tissue or impair blood production. There are many problems with gene insertion that need to be solved, but thalassemia and sickle-cell anemia are targets for gene therapy.[12]

Ecologists may use recombinant DNA methodology to improve the ecological balance of species. They would be able to do this because recombinant DNA would enable humans to decrease their reliance on land devoted to crops and herds. This could be done if scientists can manipulate the genes of higher plants and animals.[13]

FOOTNOTES

[1]Clark, p. 69.

[2]Ibid.

[3]"Recombinant DNA Research. What are Those Molecular Biologists so Excited About?" Sci. News (April 2, 1977), pp. 216-217.

[4]I. Asimov, "Gene Scene," Saturday Evening Post (September, 1977), p. 12.

[5]"Recombinant DNA Research. What are Those Molecular Biologists so Excited About?" pp. 216-217.

[6]"Gene Injection Remedies Cell Defect; Use of Gene Transplant Technique (work of W. F. French)," Sci. News (October 20, 1979), p. 260.

[7]Clark, p. 64.

[8]"Recombinant DNA Research. What are Those Molecular Biologists so Excited About?" pp. 216-217.

[9]Clark, p. 69.

[10]"Prenatal Examination of Human Genes; Clinical Application of Recombinant DNA Research," Sci. News (August 5, 1978), p. 83.

[11]"Gene Injection Remedies Cell Defect; Use of Gene Transplant Technique (work of W. F. French)," p. 260.

[12]S. Begley, "Curing Diseases with Genes (UCLA experiment in gene therapy)," Newsweek (April 21, 1980), p. 80.

[13]Asimov, p. 12.

CHAPTER V

ASPECTS OF THE CONTROVERSY CONCERNING RECOMBINANT DNA

The debate over recombinant DNA started in 1971 when Paul Berg at the Stanford Medical Center decided not to do an experiment in which he would insert the monkey virus SV40 into *E. coli*. SV40 has a few genes, one of which has the ability to turn normal cells into cancerous ones in laboratory animals and human cell cultures. He wanted to unlock the mystery of this lethal gene which would have been a major step toward understanding the mechanism of cancer.[1]

As a result of the discovery of the restriction enzyme that leaves a bit of sticky single-stranded DNA at each end and the pSC 101 plasmid which can take on a new gene and slip into *E. coli*, the number of planned experiments using recombinant DNA increased. Consequently, an investigatory committee of molecular biologists met in 1974 and asked all researchers to honor a temporary ban on certain types of recombinant DNA experiments deemed potentially the most dangerous: those involving animal tumor viruses, and those increasing toxicity in bacteria or drug resistance. In 1975, another committee of scientists met at Asilomar and voted to continue the ban on the worrisome experiments and to get the National Institute of Health to establish safety levels, including both physical and biological containment, that should be required for different experiments. The NIH had written the safety guidelines by the summer of

1976.[2] Government research agencies and universities receiving government funds have had to abide by the guidelines, but private industry has not, although, in general, it has voluntarily compiled.

The guidelines were followed until 1979 when a reversal in policy for recombinant DNA technology was initiated by the Recombinant DNA Advisory Committee to NIH. It recommended that 80 to 85 percent of the work with recombinant DNA be exempted from the guidelines. It qualified its recommendation in four ways. It said that only work using vectors that would not easily transfer DNA to other bacteria could be done. The worrisome experiments and work with more than ten liters of culture are prohibited. Researchers must still notify the biohazard committees at their institutions of their experiments. All work must be conducted under PI safety conditions with an additional ban on mouth pipetting and a requirement that biological material must be sterilized before its disposal.[3]

On June 16, 1980, the United States Supreme Court in five to four decision stated that life forms can be patented if there is a man-made element to them. This decision was a reversal of the Court's opinion from 1978 when it sent General Electric's patent application for its oil-eating microorganism back to the Court of Customs and Patent Appeals. General Electric filed its application in 1972. Congress extended patent rights to plants but specifically excluded bacteria in 1970. So the question of patenting man-made organisms is still unresolved.[4] Approximately 114 patent applications, most of them for processes, are pending.[5]

The history of recombinant DNA demonstrates that it is controversial. The main objections to it have centered around it leading to a disruption of the evolutionary process and to the creation of new, lethal organisms. Specifically the reasons for anxiety are fear of the unknown, new types

of life; cancer spread by bacteria into which cancer viruses have been transplanted; fear that humans may become susceptible to new diseases caused by organisms with genes from extremely diverse species, and fear that efforts to create new microbes for man's good may backfire and change the balance of nature.[6]

The arguments put forth to continue the NIH guidelines included the fact that researchers should not assume that microorganisms are always handled safely, the possibility of transfer to strains indigenous in populations or already epidemic, the hazards to technical workers, the creation of a new route of exposure to cancer, and the possibility that bacteria making a biologically active protein might disrupt the normal physiological processes in a human or animal host (i.e., Bacteria making the enzyme cellulase might destroy roughage in the digestive tract, therefore, producing chronic diarrhea.).[7]

On the other side of the controversy are the reasons for doing recombinant DNA. This report has already covered the products it is producing, the areas of research benefitting from it, and the tremendous possibilities of what it can do in the future. The arguments for doing recombinant DNA with reduced guidelines can be understood if the epidemic pathogen scenario is explained. In this scenario, large numbers of E. coli bearing the foreign gene would have to escape from a laboratory. They would have to have a sufficient selective advantage over other organisms so that they can establish themselves in an ecological niche. Finally they would have to have some detrimental effect on some other organism as a result of the presence of the foreign gene.[8] The mutated E. coli used in the experiments could not fit into the scenario. Even if the bacteria died and released their recombinant DNA, the DNA has been

shown to be rapidly degraded when added to low concentrations of rat intestinal contents.

Researchers know that the probability of contaminating a natural population with recombinant DNA is low if the correct materials are used and procedures are followed. But there are two difficulties with estimating the probability. One is the failure of estimation procedures to consider the population dynamics of the contamination process. The other is the inability to get accurate estimates of some parameters necessary to compute the contamination probability. The probability of the establishment of altered bacteria is directly proportional to the fertility of the host-plasmid combination and to the number of plasmid-carrying bacteria released into the environment. It is inversely proportional to the host cell's death rate. Also the rate of transfer of a nonconjugative plasmid is proportional to the density of bacteria carrying a mobilizing, conjugative plasmid.[9] All these factors need to be considered when estimating risks.

The question has been raised as to whether or not the creation of a new organism to be used as a biological weapon is prohibited by the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological and Toxin Weapons and on Their Destruction. The general opinion is that no treaty signatory will use recombinant DNA to create a weapon because any new pathogens and toxins created will not differ in a militarily significant way from natural ones. Even if genotype-targetable bioweapons (viruses or infectious nucleic acids) are created, they would be subject to the usual problems of operational stability and controllability, and host specificity probably could not be restricted to a targeted group or military force.[10]

As can be concluded from this report, recombinant DNA is a marvelous technology which has created a controversy in every realm of life, moral, physical, and spiritual. It will be many years before the final verdict on it can be passed.

FOOTNOTES

[1]"Tinkering with Life," pp. 34 and 39.

[2]Ibid.

[3]S. Wright, "Recombinant DNA Policy: From Prevention to Crisis Intervention," Envir. (November, 1979), p. 34.

[4]N. Wade, "Court Says Lab-Made Life can be Patented," Sci. (June 27, 1980), p. 1445.

[5]"New Life Forms: A Clear Road Ahead?" p. 34.

[6]Gwynne, p. 57.

[7]Wright, pp. 35-37.

[8]Ibid.

[9]"Recombinant DNA: Examples of Present-Day Research (Symposium)," pp. 218-220.

[10]"DNA Technology Unlikely to Develop Biological Weapons," BioSci (November, 1978), p. 736.

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