The Impact of Modern Genetics

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I. GENETIC ENGINEERING: ITS PROMISE AND PROBLEMS

The discovery of the double helical structure of DNA in 1953 has played a role in modern genetics analogous to the impact that the discovery of the atom had for nuclear physics during the first half of this century. Prior to 1953, genes were defined as elements that obeyed the well-known Mendelian rules of heredity. A defective gene was often recognized by a change in some visible trait. Among the earliest known examples of genes that obey Mendel's laws are those whose mutations cause certain diseases such as hemophilia and sickle cell anemia. It has been known for a long time that the occurrence of these diseases in affected families is predictable. The advent of genetic chemistry made us consider for the first time how the DNA molecule could encode such complex traits. Discoveries since 1953 have taught us how to work with the genetic material as a chemical reagent. We have learned about the structure of DNA and its chemistry. This information explains well-known biological functions of genes such as their ability to reproduce themselves exactly and how information is encoded and expressed by genes.

It is not possible to talk reasonably about the impact of these discoveries without understanding something about the science of genetic biochemistry. I propose to explain it as briefly as possible before evaluating the risks and benefits of genetic engineering.

Genetic biochemistry

DNA has two important functions that it must carry out in living cells. It duplicates itself, and it encodes protein. Inherent in the structure of DNA are explanations for both functions.

DNA consists of four building blocks called nucleotides or "bases," designated by the letters A, G, C, and T, that are linked together to form very long molecules. Two of these long chains are intertwined to form a double helix. The key rule used by Watson and Crick to account for how the two strands are held together is called base complementarity.[1] Wherever there is an A in one strand there must be a T opposite it in the second strand. Likewise, G residues face C's. A is complementary to T, and G is complementary to C. Thus, the exact order of bases in one strand precisely specifies the order of bases in the other by the rules of base complementarity. This is a way to form two identical DNA molecules where only one had existed previously. Not only does this explain self-duplication, but the phenomenon of base complementarity is at the heart of genetic engineering and biotechnology. All methods used to find genes and manipulate them depend upon base complementarity.

Proteins are polymers consisting of twenty building blocks called amino acids linked together. The linear order of the DNA bases in a gene specifies the exact linear order of amino acids in proteins. It is a very simple code in which each arrangement of three bases is translated into only one amino acid. DNA also contains signals for the control of gene expression. The expression of a gene leads ultimately to the synthesis of the protein that it encodes. In a skin cell, the genes for skin proteins (keratin) are actively expressed, but the genes for blood proteins (globin) are silent. This differential gene expression is influenced by signals in DNA near and within genes. The understanding of how genes work in cells is one of the most exciting and fundamental unsolved problems of biology. I will use it as an example in the second lecture to show how modern methods are resolving important biological questions.

[1] J. D. Watson and F. H. C. Crick. A Structure for deoxyribose nuclecic acids. *Nature* 171 (1953): 737-38.

This is a great oversimplification of the majesty of DNA and genetic biochemistry, but it contains the salient facts to help us understand what genetic engineering is likely to be used for. To summarize:

DNA is a template for its own duplication.

DNA encodes proteins and the code is universal in all organisms studied to date from bacteria and viruses to man.

DNA has signals for the control of its expression, but these signals are not universal.

Advances in genetic biochemistry since the discovery of DNA structure in 1953 have elucidated how living cells carry out these processes. It is these details and the methods devised to study them that have made genetic engineering possible. The revolution in genetic biochemistry following that first great theoretical discovery has been marked by practicality, not theory; the development of new methods plays the greatest role in answering what are really very old questions.

Working with genes

It has been a surprise to scientists that modern methods have made DNA the easiest of all biological macromolecules to study and manipulate. It was not so long ago that DNA was a sticky mess and just about intractable for analysis. Now there are many methods available for isolating a gene of interest by recombinant DNA techniques.[2] Reagents cleave DNA at specified bases, ligate pieces of DNA together regardless of their origin, replicate DNA, and make precise mutations in genes that can then be perpetuated by recombinant DNA methods. The exact order of bases in long stretches of DNA can be determined with ease. The sequence of bases of the entire genome of the simplest viruses has been determined. In the old days, we would discover a new protein and

[2] S. N. Cohen. The manipulation of genes. *Sci. Am.* 233 (1975): 24-33.

then know that there must be a gene for it. Nowadays, we sequence DNA, enter it into a computer, and are told that it encodes a protein and the exact amino acid sequence of that protein. If the protein, or one that resembles it, has been discovered already by someone else, the computer will tell us so. Another very important advance for genetic engineering is transformation, in which pure genes are introduced into living cells or organisms in such a way that they function.[3]

Genes encode proteins. Insulin is a protein, but penicillin is not. Penicillin does not have a gene; it is made step by step in mold cells by a group of protein catalysts called enzymes. Therefore, it would take many genes to instruct a living organism to make penicillin, but only one gene for insulin. Consider also genetic diseases. Hemophilia, sickle cell anemia, and cystic fibrosis are examples of simple genetic diseases. They obey Mendel's rules of inheritance since each is caused by a defect in a single gene. In the case of hemophilia, it is the gene for a protein involved in blood clotting. Sickle cell anemia is the result of a mutation wherein one of the 438 bases encoding the blood protein globin has been changed, resulting in a replacement of one of the 146 amino acids of globin; the altered protein cannot bind oxygen as well as the normal globin, which causes severe consequences for the patient. The basic defect of cystic fibrosis is not known, but it can be predicted confidently that the gene encoding some essential but, as yet, unidentified protein is mutated.

Contrast these diseases with diabetes and certain kinds of heart disease. If your parents have either of these diseases, then you will have an increased chance to have them as well. These are complex genetic diseases with more than one gene involved, so they are not inherited in a simple manner.

[3] A. Pellicer, M. Wigler, R. Axel, and S. Silverstein. The transfer and stable integration of the HSV thymidine kinase gene into mouse cells. *Cell* 14 (1978): 133-41.

These simple precepts can help us to distinguish what genetic engineering can do with reasonable certainty soon or in the future from what it is unlikely ever to be able to perform. Proteins can be made by genetic engineering — certain hormones, vaccines, clotting factors, perhaps silk and wool. Defects in specific proteins caused by simple genetic mutations might be treated by gene replacement therapy with the gene for that protein. The cure of diseases or alteration of traits that are the result of the interactions of many genes will not be candidates for simple genetic engineering as we know it today. Organ transplantation would seem to be more promising. These concepts are important for an understanding of what biotechnology can do, and they are also the basis for a rational discussion of the hazards of these methods. I now wish to enumerate some kinds of genetic engineering starting with schemes already in practice, progressing toward those more in the realm of science fiction.

The microorganism as a factory

There has been a worldwide shortage of the protein insulin, which traditionally has been purified from the pancreases of cows and pigs. These animal forms of insulin also differ from human insulin in two amino acid residues. This seemingly small difference occasionally results in an adverse reaction in diabetics who receive the insulin. Their systems reject bovine insulin as a foreign protein by making antibodies against it. Eli Lilly now markets human insulin that is synthesized by microorganisms genetically engineered to contain the gene for human insulin. This is the first practical commercial application of genetic engineering in which microorganisms (bacteria or yeast) are used as living factories for making large amounts of one particular protein (or gene) in great purity.

The steps required to engineer a bacteria with the gene for insulin have become routine in research laboratories. The major

technical feat was to detect the insulin gene in the presence of the thousands of other genes in a mixture of crude human DNA. The insulin gene is present in human DNA in about one part per million. The basis for this crucial assay is the base complementarity mentioned earlier. The order of bases in the insulin gene are exact and not found in any other gene. If one has a piece of DNA (called a probe) in which the order of bases is complementary to part of the insulin gene, the probe can be made to bind specifically to the insulin gene. The first step in isolating a gene is usually to break the crude DNA into smaller fragments and splice each fragment to a piece of bacterial or viral DNA (called the vector). These recombinant molecules are then introduced one per bacterium. A population of bacteria containing the entire DNA content of another species is referred to as a library. One needs only to sort through the library with the probe to find bacteria containing the gene of interest.

The bacterium containing the insulin gene will breed true, replicating the foreign gene along with the vector and its own chromosome. The insulin gene will not make insulin in the bacteria because the signals needed for its correct expression in a human are different from those in the bacterium; more genetic engineering is needed, Bacterial DNA containing signals recognized by bacteria are spliced to the insulin gene. Now the metabolic machinery of the bacteria recognizes the gene and insulin is made.

Mutations used to be introduced laboriously into a gene by mutagenizing organisms and then selecting among survivors for mutations in that particular gene. Now the gene is purified, a base change or a deletion is placed at any location desired, and then the altered gene is cloned in a bacterium.[4]

Another example of this sort of genetic engineering has been its application to a group of animal proteins called interferon.

[4] M. Smith and S. Gillam, "Constructed mutants using synthetic oligodeoxyribonucleotides as site-specific mutagens," in J. K. Setlow and A. Hollaender, eds., *Genetic Engineering*, vol. 3 (New York: Plenum Press, 1981), pp. 1–32.

These proteins have antiviral and perhaps antitumor activity — enough promise at any rate to make their production the goal of a number of biotechnology companies. Interferons are produced by cells in the tiniest amounts. The extraction of living tissues resulted over the years in the partial purification of a small amount of interferon. There was just enough material to tantalize scientists by its biological activity, but never enough to prove conclusively the value of interferons for treatment of any disease. However, now genes for many kinds of interferons have been isolated, fitted with appropriate signals to insure their expression, and grown in bacteria and yeast. Unlimited amounts of pure interferons are now available for testing. One liter of bacteria containing a gene for interferon properly engineered for expression produces far more interferon in an hour than all of the interferon ever extracted from animal tissues and cultured cells. The engineered interferon is pure — the tissue-extracted protein is impure. In addition, variants of interferon are easily made by mutating the gene and then screening the product for activity. This would have been impossible before recombinant DNA technology.

Behind the commercial applications is an extraordinary number of basic research experiments in which bacteria have been used to isolate genes from every imaginable source, plant and animal. Thanks to this recombinant DNA methodology we have learned a great deal about the structure and function of genes especially in higher organisms. Previous notions of gene structure, function, and evolution have been revised drastically because of these powerful methods.

Hazards of the microorganism as a factory

The first hazards of genetic engineering that were addressed were those stemming from the kinds of experiments I have just described. What is the likelihood that a perfectly well-meaning scientist might introduce a gene into a bacterium that would alter

the organism drastically with unpredictable results. Would the bacteria now grow in new ecological niches or produce some dangerous product that was not suspected? The uncertainty about such an incident led scientists to police themselves and set forth guidelines for their own research in 1976. These rules were institutionalized in a number of countries and exist today in the United States in modified forms.[5]

Even the most ardent critics of this work in 1976, when the debates began, now agree that potential laboratory accidents do not constitute a danger to the public. This conclusion comes from many kinds of evidence. The microorganisms used for gene cloning do not grow outside of the laboratory — and especially not in the intestinal tract of man. Pathogenicity is a highly evolved and rather fragile state. Very few microorganisms out of the enormous variety that exist in nature are pathogenic to man. These organisms are finicky in their growth requirements because of their specialized natures. A bacterium is a finely tuned organism dependent on the integrated functions of about 5,000 genes. The introduction of one foreign gene will not drastically change its general behavior. Complex traits such as the ability to grow in a new environment (the human gut, for example) are themselves the result of many genes with integrated, highly evolved functions. An analogy would be the introduction of one extra transistor at random into an AM radio that already has 5,000 of them carefully and precisely connected. Either nothing at all would happen, or the radio would work less well. The AM radio would not turn into an FM radio.

The microorganism that is disseminated

After eight years of considering hazards posed by microorganisms used as a factory, we have now reached the next level of

[5] The Recombinant DNA Advisory Committee (RAC) supervises and approves all recombinant DNA research that is funded by the National Institutes of Health of the United States. Other agencies have chosen to abide by the RAC's oversight.

concern — the purposeful dissemination of a genetically engineered microorganism to do the work previously reserved for chemicals. An example that is being debated in the U.S. now has to do with a serious agricultural problem in California. A bacterium that colonizes certain crop plants nucleates ice crystal formation that kills the plant. Scientists have isolated a mutated strain of the bacteria which has lost this trait but, as nearly as they can tell, is identical to the parent strain in every other way. They want to field test the mutant strain by spraying it on plants in the hope that it will replace the deleterious strain. The NIH Recombinant DNA Advisory Committee have examined all of the data and concluded that it is safe to proceed. Dissidents have taken the matter to court.

Genetically engineered microorganisms are going to be used for many purposes previously reserved for chemicals. A bacterium that digests oil has been patented but not yet used or approved for use. This is a good example to help put the pros and cons of dissemination into perspective. Oil spills are now contained by pouring detergents on the oil and then trying to vacuum up the mixture. It is not terribly efficient, nor do we understand damage that may be caused by the detergent, but at least the amount of detergent added is under control. Bacteria can proliferate, and therein lies the fear. A skeptic will say that the bacteria may digest the oil spill, but what is to keep them from continuing to grow and spread? Will they infect the gastanks of our cars? There are, however, several advantages of using an engineered microorganism to do a job previously done by a detergent or a pesticide. The very methods that produce a useful bacterium can help to make it safe. Genetic engineering can introduce traits that enfeeble a bacterium so that it will self-destruct. An example in the case of the oil-eating bacterium might be mutations in life-sustaining genes that make the bacteria sensitive to high temperatures. The bacteria are sprayed over the oil and digest it until the ambient temperature rises above a certain level. Then they all die. Bacteria are biodegradable.

Each application of biotechnology must be scrutinized in every bit as much detail as new drugs and new chemicals before they are disseminated. Existing or new agencies must assume these responsibilities.

Genetic engineering of plants

In my opinion, some of the greatest societal benefits of genetic engineering will come from applications to agricultural problems. Improvement of plants has always been a highly empirical procedure. Individual plants are selected for traditional breeding because they show a bit more of some desired trait — for example size, ability to grow in poor soil, or pest resistance. Thus, a crop plant is gradually improved over a period of many years. Many of these traits are obviously the result of many genes working in an integrated fashion and thus more difficult to isolate, clone, study, and then transfer from one plant to another. However, other characteristics seem especially suited for improvement by modern genetics. An example would be the quality of some particular protein in a popular food crop. The major protein of corn, for example, is low in the amino acid lysine, an essential amino acid for humans. If the gene for that protein could be altered so that the protein contained more lysine, corn would be a more nutritional foodstuff. Plant genetics requires the patience to breed and select individual plants with very long life cycles. However, some plant cells can now be cultured *in vitro,* genetically transformed, and then grown into whole plants.

I am unaware of any new hazards posed by applications of modern plant genetics that require different supervision from what already exists.

Genetic engineering of animals

Selective breeding, freezing of sperm and embryos, artificial insemination, and surrogate motherhood are done commercially

and/or experimentally with mammals. Individual genes have been introduced into the fertilized eggs of mice and integrated permanently into their genome. These transferred genes can function as exemplified by the introduction of a gene for growth hormone into a mouse embryo, resulting in an abnormally large mouse.[6] From the standpoint of basic research, the most exciting advance in the past few years has been the genetic transformation of fruit flies *(Drosophila)*.[7] Many of the rules of animal genetics were derived over the years from studies with fruit flies because of the species' simplicity and short life cycle. Genes are injected into fertilized eggs near the region that will form future germ cells, and the genes are incorporated into egg or sperm as the cells develop. The distinction between germ cell and somatic cell transformation is the source of major controversy, and we will discuss the matter shortly. However, when used as an adjunct method for fruit fly genetics, genetic transformation provides a remarkable opportunity to study gene structure and function. Transformation of genes injected into germ cells of mice also occurs, so we can conclude that the tools are certainly at hand for introducing foreign genes into both germ cells and somatic cells of humans.

What is already done with humans

Before arguing what should or shouldn't be done with humans, it is worth summarizing what already happens either naturally or by intervention.

[6] R. D. Palmiter, R. L. Brinster, R. E. Hammer, M. E. Trumbauer, M. G. Rosenfeld, N. C. Birnberg, and R. M. Evans. Dramatic growth of mice that develop from eggs microinjected with metallothionein-growth hormone fusion gene. *Nature* 300 (1982): 611–15.

[7] A. C. Spradling and G. M. Rubin. Transposition of cloned P elements into *Drosophila* germ line chromosomes. Science 218 (1982): 341–47; G. M. Rubin and A. C. Spradling. Genetic transformation of *Drosophila* with transposable element vectors. Science 218 (1982): 348–53.

A few percent of all humans are members of clones. Of course, these are identical twins. The definition of cloning is the vegetative production of two cells (or individuals) from one cell (or individual) without sexual mating. The billions of cells in our bodies are cloned from the original fertilized egg. Identical twins occur when a fertilized egg cleaves, and the daughter cells separate to form two individuals.

Artificial insemination of fertile females using donor sperm has been an accepted procedure for a long time, when the husband is infertile. Recently, surrogate mothers have been employed by infertile couples. This arrangement seems to generate opposition more because of possible financial impropriety than for ethical reasons. *In vitro* fertilization is a relatively recent way to help some infertile couples produce children from their own egg and sperm.

An increasing number of genetic diseases and abnormalities can be diagnosed *in utero* with the option to terminate pregnancies, a decision that causes more controversy than almost anything else that occurs in medicine.

I mention these diverse natural or human interventions because they are all relevant to one or more points of controversy about genetic engineering that face us today.

Abortion

Modern biology is increasing the number of genetic conditions that can be diagnosed *in utero.* In some cases, accurate prenatal diagnosis may decrease the use of abortion where previously the threat of an abnormality was not considered worth the risk. Even the most ardent believer in free choice (as I am) realizes that the dilemma of the genetic counselor is increasing. For example, Huntington's chorea is a disease caused by a simple dominant mutation whose symptoms do not begin until middle age but can then be guaranteed to progress miserably to the victim's death.

Recent experiments show that DNA technology can diagnose potential victims *in utero* about forty years before they will display the symptoms.[8]

It is generally feared that selective abortion might be used some day on a very large scale in societies where female children are considered a liability, In my opinion, there is real potential here for misuse. Perhaps this has not been discussed as much as other matters because the methods for sex selection of embryos have been available for a long time but not abused, at least on a significant scale. Some genetic counselors refuse to divulge the sex of embryos after routine amniocentesis.

I cannot resist addressing one other issue in the abortion debate. When does life begin? This question obsesses bioethicists, theologians, and legislators. Does life begin with a living cell? The onset of brain waves? The time when a fetus can live outside the mother? Or even when a child can live without parental protection? It is not a scientific question but rather a social, political, religious, or cultural one. Even practical considerations will outweigh scientific ones. Laws must be made to protect individuals, but the decision as to when this protection should begin will be agonized over by each society with little help from scientists. The fact that scientific progress has enabled babies to survive an increasingly premature delivery will not change this basic social dilemma.

Gene therapy

We are learning more about the genetic basis of disease. For simple genetic diseases the exact gene that is defective can be determined. Precise gene therapy would be the replacement of a defective gene with a normal one. This can be done today in only one organism, yeast.[9] Many of the steps that are needed for gene

[8] J. F. Gusella et al. A polymorphic DNA marker genetically linked to Huntington's disease. *Nature* 306 (1983): 234–38.

[9] K Struhl. The new yeast genetics. *Nature* 305 (1983): 391–97.

therapy have been established already in organisms other than man; in a few years they will be applicable to humans.

Correction of a defective gene in somatic cells might be done by culturing a piece of a patient's tissue, introducing the normal gene properly engineered with the correct signals for expression into the cells in culture, selecting those cells transformed with the normal gene, growing large amounts of this transformed tissue, and then reintroducing this tissue into the patient. Most bioethicists concerned with human genetic manipulations do not seem concerned with this scheme because it would affect only individual patients. But is that the case? Here is an individual who might have died at an early age from a genetic disease but who will now be a functionally normal person with mutant germ cells capable of breeding and disseminating the defective gene to his/her progeny. Whereas, if a defect could be corrected in egg and sperm cells as well as somatic cells, the children would benefit as well as the afflicted parent.

The issue of inviolability of germ cells in humans is a vague one which is defended more from gut instincts than from genuine reason. We decided long ago to intercede on behalf of the ill and the weak. Evolution of most living things has come under the capricious influence of man. Will simple gene therapy lead to parents (or governments) controlling the physical and intellectual traits of children? This is the concern that we hear. But each complex trait is the product of many genes, so even the ability to do this is not on the horizon. Is it even a future concern? Questions of genetic manipulation of man and all other controversies raised by genetic engineering and science must be viewed in perspective.

Perspective

A favorite example of perspective goes something like this. Hundreds of thousands of Americans die each year directly or in-

directly due to cigarette smoking; there are about 50,000 deaths from automobile accidents each year. Perhaps there are one or two deaths from shark attacks world wide. On a crowded day at a southern California beach if someone yells "Shark," thousands will clear out of the water, jump into their cars, and light cigarettes.

There have been perhaps several hundred instances of *in vitro* fertilization, yet there are hundreds of thousands of infertile couples who want babies.

There is more concern about the rights of embryos than about the millions of children who starve around the world, a problem exacerbated by burgeoning populations often out of control.

At a recent meeting to discuss the advances in life sciences and the concerns that they raised, I heard the following abuse of perspective. A bioethicist predicted the impact of modern research for good and evil. On the one hand he thought it likely that modern genetics would contribute to curing diseases and to the elimination of hunger and poverty, but the price would be what he called "dehumanization," The analogy was not with Orwell but with Huxley's "Brave New World." We would be disease free, well-fed zombies enjoying the "feelies." I suspect that this man had never been really sick or poor, because *these* are conditions that are really dehumanizing. One hears this kind of vague and abstract criticism of modern genetics. It simply does not provide a useful starting point for debate, except in one respect: There is guaranteed to be some risk associated with each benefit.

The military problem

In 1969, the United States unilaterally renounced biological warfare and destroyed all its stores. In 1972, at the Biological Weapon Convention more than one hundred countries signed an international agreement outlawing all biological and toxin weapons. Recently, there have been accusations that Russia has used

mycotoxins in its struggle to subdue Afghanistan and in the so-called "yellow-rain" reported in Southeast Asia. In a series of articles in the *Wall Street Journal* during April of 1984, a writer, whose sources seem mainly to be unnamed Russian expatriates, claims that the Soviets are trying to perfect new biological weapons using modern genetic methods.[10] These are very provocative accusations in an atmosphere of international distrust. Such sensational studies can have very mischievous results. There is no undisputed example of the use of biological warfare since the Convention. The reported incidents mentioned above are highly controversial, and in the opinion of some of the most objective accounts the accusations are without merit.

It should be noted that it would be hard to construct a virus more deadly than some of the ones that occur naturally, or to make in the laboratory a toxin more dangerous than some well-known examples.

Dealing with the risks and benefits of biology

Many of the benefits of modern biology are easy to assess. They include the detection and cure of many diseases. For the first time, in my opinion, we have real insight into the cause of cancer. There are normal genes that can cause cancer when they mutate. We don't know yet how these genes work, but techniques of modern genetics which led to the discovery of these genes in the first place will provide these insights. In the next ten years we are going to understand better and perhaps even cure some of the most serious diseases that afflict mankind, such as diabetes, arteriosclerosis, parasitic diseases, the common cold, cystic fibrosis, certain kinds of arthritis, immune diseases, and infectious diseases, just to name a few. A molecular basis for at least some kinds of schizophrenia will be found. We will learn about the biochemistry of the ageing process, which also has a strong genetic com-

[10] W. Kucewicz in The *Wall Street Journal*, April 23, 25 and 27, 1984.

ponent. This doesn't guarantee prolongation of life, but rather an improvement of the quality of life in old age. W e need sensitive assays for the effects of chemicals, pollutants, and drugs as causative agents of birth defects like those developed to determine carcinogenic potential. There have yet to be developed simple, safe, and reversible contraceptives for males.

I have already mentioned the influence that modern genetics will have on agriculture and the use of biology to meet needs previously served by chemicals. This could have a salutary effect on the environment. Indeed, modern genetics is expected to help clean up the environment.

Every powerful new technology brings potential for misuse. Biotechnology is often compared with nuclear physics, which brought us the Bomb. Perhaps a fairer comparison is with the computer or electronics industries. These are both nonpolluting industries with many applications, yet we can list misuses of these technologies.

So it will be with biotechnology. If a new drug or chemical is produced by these methods it must be tested for safety with rigor and intelligence. Genetic manipulations on humans can proceed if testing for safety is thorough and objective, and if all experimentation and applications require the strictest control and especially the informed consent of human subjects. There are existing rules and agencies, boards, and committees that are empowered to protect the public against new products and individuals against capricious experimentation by scientists.

If we are to have the benefits with minimal risks, we must prepare to review each problem as it arises. There is no simple solution. Much rests with the quality of our governmental institutions. Enforcement of individual rights and safety has always been their responsibility. They must be kept current with the progress of biotechnology by informed scientists. Science is as sensitive to interference as any art form, so its regulation is a delicate matter. A fine balance needs to be struck between regulation of science

and nourishment of the scientific enterprise. One need only glance at the magnitude of society's unsolved problems that this technology might help to solve to conclude that we must take the risk.

II. HOW NEW METHODS IN BIOLOGY ARE SOLVING OLD PROBLEMS

From information stored in eggs and sperm, a developmental timetable is established that precisely determines the formation of about one- to two hundred different cell types in an adult organism. The timing of gene expression must be flawless, otherwise a birth defect will ensue. About 5 percent of all live human births have some sort of congenital anomaly. Half of these malformations are genetic in origin.

Embryogenesis is so complex that most researchers study some tiny aspect, preferably a simple contained system, with the hope that answers can be generalized. In the first lecture, I mentioned how modern methods are playing a role in our understanding of gene control in development. I will summarize here some of the varied ways by which living cells control the expression of their genes during development." It is an area of current research so active that this list will be incomplete; new mechanisms are being uncovered weekly.

In order to put this problem in perspective, I begin with a brief and rather biased history of the field of embryology or developmental biology, as it is now called. At the beginning of this century embryology flourished closely coupled with genetics. The great early embryologists, such as Boveri and Morgan, were geneticists. In the 1920s "experimental embryology" was the popular way to study the field. This approach used animals like chickens and frogs that are not suited for classical genetics. Experimental

[11] D. D. Brown. Gene expression in eukaryotes. *Science* 211 (1981): 667-74.

embryology mapped the fate of cells and tissues and their changing capacity to develop. Surgical manipulations transplanted pieces of embryonic material. This led to ideas about how cells moved during embryogenesis and how one tissue could instruct another one ("embryonic induction"). Despite the importance of concepts raised during the thirties and forties, experimental embryology had, in one sense, a detrimental effect on the field because it split embryology from the burgeoning fields of genetics and biochemistry. Experimental embryologists considered themselves members of a special discipline when, in fact, they were studying a set of questions. They needed the concepts and methods that could be provided by other areas of biology. For fifty years genetics and embryology were separate.

Another feature which emerged in those days of experimental embryology was the overwhelming complexity of embryogenesis. It had a stultifying effect on the field. Young scientists were frightened off, and embryologists seemed to be proud of the impenetrability of their subject. They advanced the notion that answers would only come from studying an entire organism, the whole being greater than the sum of its parts.

Biochemical embryology followed experimental embryology. Biochemists homogenized embryos and measured the changes in various molecules with developmental stages, They confirmed what anyone could deduce, namely, changes occurred not only in visible structures but in molecules as well. There was little intellectual communication between "biochemical embryologists" of the forties and fifties and the authors of the truly innovative advances in biochemistry that were occurring at the same time. Biochemical embryologists studied chickens, frogs, and sea urchins; geneticists studied fruit flies *(Drosophila)* and to some extent the mouse. There were no known mutants in chickens, frogs, and sea urchins, and no biochemistry carried out with fruit flies. In fact, up until five or ten years ago, there was not a single mutant identified in *Drosophila* that encoded a specific protein.

Most mutants invariably caused some morphological defect. Nowadays, there are many genes encoding proteins in fruit flies that have been identified and characterized, but this is the result of modern genetic biochemistry.

Immunologists studied rabbits, went to their own meetings, and spoke a language all their own. Immunology was a self-contained discipline. The effect of having scientists outside the field apply modern molecular methods to immunology is instructive for a student of science history. In the past ten years the most fundamental problems of immunology have been solved by outsiders applying new methods previously alien to the field.

Plant biology is another example of the segregation of science. Many universities still have separate departments for plant and animal biology. The American Society of Developmental Biology attempts to integrate botany and zoology by alternating its presidency each year between plant and animal scientists. However, modern methods are accomplishing a less artificial integration as techniques learned from studying one kind of organism can now be applied to all.

New and powerful methods are bringing biologists together. We can now perform genetics at will on frogs and chickens, biochemistry on fruit flies and mice, and both genetics and biochemistry on plants.

The summary of known developmental control mechanisms that follows will give some idea of how organisms control the expression of their genes. I divide these two into categories — gene alteration and gene activation. In the former, the genes are literally changed, that is, lost, amplified, or rearranged. By the later, I mean that the genes are unaltered, but their expression is controlled.

Gene alteration

The earliest known example of gene alteration is called chromosome diminution. In 1903, the great embryologist and geneti-

cist Theodore Boveri showed that the parasitic worm *Ascaris* loses genetic material from most of its cells during early cleavage stages of the embryo.[12] This also occurs in some, but not all, crustaceans, insects, and worms. It is clearly not a general phenomenon of all embryos. The only cells in *Ascaris* embryos that do not lose genes are those that give rise to the sperm and egg (germ) cells of the adult. All of the body (somatic) cells are affected. Most embryos actually set aside their future germ cells in early development even though they do not undergo chromosome diminution of somatic cells. The germ cells do not divide during embryogenesis when the animal's tissues are forming. Only when this process is completed will the germ cells then find their place in the embryo and divide to form a functional gonad.

Some years ago, Igor Dawid and I[13] and, independently, Joseph Gall[14] at Yale discovered another mechanism of gene alteration which we called "gene amplification." In this case, a specific gene is actually duplicated. The example that we studied occurs in the growing egg cell (oocyte) of frogs, fish, and many (but not all) other animals. Oocytes grow to enormous size and synthesize certain cytoplasmic constituents in huge amounts. One of these is an essential component for protein synthesis called the ribosome. The oocyte, which is a single cell, can synthesize as many ribosomes in a unit of time as many thousands of the most active body cells of a frog. Each ribosome consists of three RNA molecules and about one hundred different proteins. More than one genetic mechanism enables an oocyte to make such a huge amount of ribosomes. Two of the RNA molecules are encoded for by genes that are amplified more than one thousand-fold early in the growth of the oocyte. This gene amplification is a mechanism by

[12] T. Boveri.Die Entwicklung von *Ascaris megalocephala* mit besonderer Rucksicht auf die Kernverhaltnisse: Festschr. f. C. von Kupffer XIII (1899).

[13] D. D. Brown and I. B. Dawid. Specific gene amplification in oocytes. *Science* 160 (1968) : 272–80.

[14] J. G. Gall. Differential synthesis of the genes for ribosomal RNA during amphibian oogenesis. *Proc. Nat. Acad. Sci. U.S.A.* 60 (1968): 553–60.

which the cell produces more of a given product. It makes more genes first and with these extra genes it can then synthesize more RNA. There is, however, a third RNA molecule in each ribosome called 5S ribosomal RNA, which we have also studied, There is an auxiliary set of genes for 5S RNA that are present in the chromosomes of all cells of the frog, but these genes are only expressed in the oocyte where the demand for 5S RNA is so great.[15] In all other cells, the "oocyte"-specific genes are present, but they are silent.

Recently, gene amplification has been extended to other systems. There are now instances of genes other than those for ribosomes that have been shown to be amplified as part of the developmental program. A related phenomenon, which I refer to as "forced gene amplification" has medical implicaions.[16] When a cell (animal or bacterial) is challenged with a drug that can kill it, the cell can escape the effects of the drug if it can metabolize the drug. Often this metabolic machinery exists in a cell, but not in large enough amounts to cope with high doses of the drug. Resistant cells can emerge. For example, tumors will be suppressed for a time by chemotherapy, but resistant cells will often grow. A cell learns to overcome the drug by increasing the amount of its metabolic machinery through making more genes for that machinery. Another important example of gene amplification occurs in some cancerous cells in which gene amplification has occurred. Apparently too much of certain gene products will lead to cancerous changes.

About forty years ago, Barbara McClintock, studying at the Carnegie Institution's Department of Genetics in Cold Spring Harbor, New York, discovered transposable genetic elements in

[15] D. D. Brown. How a simple animal gene works. *The Harvey Lectures* 76 (1982): 27–44.

[16] R. T. Schimke, F. W. Alt, R. E. Kellems, R. J. Kaufman, and J. R. Bertino. Amplification of folate reductase genes in methotrexate-resistant cultured mouse cells. *Cold Spring Harbor Symp. Quant. Biol.* 42 (1977): 649–58.

maize (corn).[17] She noted that certain kinds of genetic mutations were unstable and the very characteristic of instability itself was genetically inherited. Through cytogenetics and the breeding of mutant plants, she concluded that the instability was due to genes that were able to move around the chromosomes, entering and leaving other stationary genes. When a transposable element moved into another gene, the activity of the stationary gene was often abolished. When the element moved out again, the gene could once again function normally. This is one of the great stories of unrewarded and unrecognized science. In retrospect, however, it is not surprising that scientists could not understand the significance of these bizarre findings. (It was fortunate that she worked at a research institution where applying for grants was not required.) The ramifications of transposable elements grow daily, as well as the realization of their importance. I will give some examples of genetic rearrangement.

The movement of genes from one part of the genome to another can be divided into those events not programmed into the developmental timetable and those that are an integral part of the life cycle of an organism. The best-known example of the latter type is the immune system.[18] In sperm and eggs, the functional genes for antibodies are not found next to each other. During the development of the cells that make antibodies, the genes for antibodies become rearranged so that they can then function. If gene amplification is the cell's way of making large amounts of a few kinds of molecules, then genetic rearrangement is a way that a population of cells can make many closely related molecules. Gene amplification fulfills a need for quantity, while rearrangement provides diversity of gene expression.

[17] B. McClintock. Controlling elements and the gene. *Cold Spring Harbor Symp. Quant. Biol.* 21 (1956) : 197–216.

[16] S. Tonegawa, C. Brack, N. Hozumi, and V. Pirotta. Organization of immunoglobulin genes. *Cold Spring Harbor Symp. Quant. Biol.* 42 (1977): 921–31.

Certain parasites escape their host's defenses by rearranging their genes.[19] Trypanosomes have surface proteins. An infected host raises an immune reaction that kills most of the parasites. The infection seems to have died down, but then it reappears. Each cycle of the disease is due to the emergence of a population of parasites with a new surface protein. This is accomplished by a rearrangement of just those genes for surface proteins. The genetic change is a rare event, but the individual parasite that undergoes the change has such an enormous advantage over the others that it will reproduce in the host until the host responds with immunity to the new surface protein.

We know now that many genetic mutations are not due to simple base changes in the DNA, as is the case with sickle cell anemia. It is common for long pieces of DNA to be found interrupting genes, presumably the result of a transposable genetic element.

Using modern molecular methods, transposable genetic elements have been isolated and characterized. Their ability to move about the genome has been the basis for their use as vectors to introduce foreign genes into fruit flies.[20] The transposable element is spliced to the gene of interest and the recombinant molecule injected into an early embryo. The movable element helps the other gene jump into the fly's chromosomes and thus become an integral and permanent part of the animal's genes.

Certain viruses resemble transposable elements, and almost certainly the two are related in evolution. These viruses transform cells the way that transposable elements enter chromosomes.

When one considers the plasticity of genes exemplified by movable elements, it is not surprising that evolution of organisms could occur either in great leaps (called punctuated evolution) or gradually as Darwin first envisaged.

[19] P. T. Englund, S. L. Hajduk, and J. C. Marini. The molecular biology of trypanosomes. *Ann. Rev. Biochem.* 51 (1982): 695–726.

[20] See note 7, above.

Differential gene expression (gene activation)

The direct gene product is actually RNA, not protein as I implied in the first lecture. One of the great discoveries in the past ten years has been the elucidation of the chemistry and many of the molecular details of gene expression. In a eukaryotic cell, the genes are in the nucleus where they synthesize RNA, a process called transcription. The RNA is then processed by several steps before it moves to the cytoplasm to impart genetic instructions for protein synthesis. The ultimate expression of a gene can be controlled at any one of the many steps between formation of the gene product RNA and the final production of a functional protein molecule in the cytoplasm.[21] For example, RNA is synthesized from certain genes at extremely high rates. Many RNA copies are made from these genes while even neighboring genes might be entirely silent. This is called transcriptional control. For example, in blood cells the genes for the protein globin are actively transcribed into RNA; in skin cells these same genes are silent.

The RNA that is transcribed from a gene directly is not itself usable for the formation of protein; it must be processed. The molecule requires essential modifications at each end (a cap and a tail). In addition, the RNA molecule must have certain regions removed from inside it. This is because the gene itself is interrupted by long stretches of DNA, called intervening sequences or "introns," which have no known function. These extra DNA stretches interrupt the coding order of DNA bases. In order to translate the resultant RNA into protein, these extra bases must be removed and the adjoining parts of the molecule rejoined. In some genes, there are as many as fifty interruptions of the gene, all of which must be repaired in the RNA copy. It is perhaps a testimony to the limitations of traditional genetics that scientists had no hint whatsoever of this striking and pervasive phenomenon

[21] J. E. Darnell, Jr. The processing of RNA. *Sci. Am.* 249 (1983): 90-100.

until the advent of molecular genetics. The discovery was completely empirical; it was not predicted, and we are now about eight years after the discovery with no adequate explanation for why most genes in animal and plant cells should be split by what appears to be extraneous DNA.[22] Biology is surely an experimental, not a theoretical science. Even though we do not know the purpose of what seems to be a gratuitous phenomenon, each added complexity of a system represents yet another step at which a gene might be controlled. In a few cases, RNA splicing has already been implicated in gene control. For example, two different proteins can be fashioned from the same gene just by splicing the RNA transcript differently.[23] Alternatively, synthesis of RNA can proceed from two different starting sites next to the gene with quite different efficiencies.[24] This can happen in different tissues. There are regions at the ends of genes that influence RNA synthesis. These are all control sites for differential gene function that have already been shown to play some role in the control of gene action.

The RNA message that carries genetic instructions to the cytoplasm of cells can undergo controlled metabolism. In some cases, RNA stability depends upon the presence of hormones. We began studying an exaggerated example of gene control in the silk worm about twelve years ago. Silk consists essentially of two kinds of protein molecules that are synthesized at the end of larval development by an enormous gland. The silk protein itself is virtually the only protein made by the posterior end of the silk

[22] R. Breathnach, P. Chambon, L. A. Klobutcher, and F. H. Ruddle. Organization and expression of eucaryotic split genes coding for proteins. *Ann. Rev. Biochem.* 50 (1981) : 349–83.

[23] T. R. Broker, L. T. Chow, A. R. Dunn, R. E. Gelinas, J. A. Hassell, D. F. Klessig, J. B. Lewis, R. J. Roberts, and B. S. Zain. Adenovirus-2 messengers — an example of baroque molecular architecture, *Cold Spring Harbor Symp. Quant. Biol.* 42 (1977): 531–53.

[24] O. Hagenbuchle, M. Tosi, U. Schibler, R. Bovey, P. K. Wellauer, and R. A. Young. Mouse liver and salivary gland a-amylase mRNAs differ only in 5′ non-translated sequences. *Nature* 289 (1981) : 643–46.

gland in the last several days of larval life. Each silk gene makes about 10^4 molecules of RNA during this period, and each molecule of RNA then is responsible for the synthesis of about 10^5 molecules of silk protein.[25] That is, one gene, 10^4 RNAs, 10^9 protein molecules. We refer to this as "translational amplification" to distinguish it from gene amplification; it happens when one gene takes over the cell's metabolism, These exaggerated examples of specialized gene expression often result in the death of the cell.

There are other examples of control at the level of protein synthesis and degradation. For example, some proteins are first synthesized in a precursor form called "polyproteins."[26] Some of these polyproteins are precursor to more than one functional protein. Metabolized in one way a polyprotein yields a hormone, and in another way a different protein.

Some genes are organized into multigene families comprising more than one copy of a gene per cell. In the case, mentioned earlier, of the 5S RNA genes that encode a ribosome component, there are tens of thousands of copies of the same gene in each cell.[27] The purpose of having many identical genes is to make more of one product.

Another kind of multigene family includes genes for related but not identical genes. Well-studied examples include transfer RNA genes and histone genes. The related genes are often clustered on chromosomes. Clustered related genes occur often enough that one is led to suspect some important functional reason for the clustering. Alternatively, clustering may just mark the evolutionary origin of a gene family by a process of gene duplication and divergence.

[25] Y. Suzuki, L. P. Gage, and D. D. Brown. The genes for silk fibroin in *Bombyx mori*. *J. Mol. Biol.* 70 (1972): 637–49.

[26] D. F. Steiner, W. Kemmler, H. S. Tager, and J. D. Peterson. Proteolytic processing in the biosynthesis of insulin and other proteins. *Fed. Proc.* 33 (1974): 2105–15.

[27] See note 15, above.

Another example of a multigene family is the genes for blood proteins in humans. Closely related but different genes are expressed in blood cells of embryos, fetuses and adults.[28] In humans, these genes are located next to each other on one chromosome in the exact order that they are expressed chronologically in human development. We do not know if this is a coincidence or has some regulatory purpose.

DNA signals

Consider that tens of thousands of different genes are linked together in gigantic continuous molecules of DNA called chromosomes. As many as 10^8 pairs of the four bases comprise each of these giant DNA molecules. It is evident that there must be signals that identify the beginning and end of each gene. Molecules must be present in cells that will recognize these signals and instruct specific genes to make RNA while other genes remain silent. The situation is not unlike an intercontinental highway with exits and entrances for each city along the way, A road map is needed and some identifications to aid the driver who has a specific destination. Otherwise, the pavement looks the same from beginning to end. The order of the four bases in DNA not only encodes the exact order of amino acids in protein but also contains these signals. The signals are read by molecules in the cell, probably proteins. Modern molecular methods have provided enormously powerful tools to identify the DNA signals as well as the molecules that interact with the signals. This kind of research is aptly called biochemical, *in vitro,* or surrogate genetics. It is genetics that avoids the traditional methods of breeding organisms and selecting progeny for inherited traits. Instead, the purified gene is isolated by recombinant DNA methods, mutated in the test tube, and each mutant gene reisolated in pure form. One needs an

[28] E. F. Fritsch, R. M. Lawn, and T. Maniatis. Molecular cloning and characterization of the human □ß-like globin gene cluster. *Cell* 19 (1983): 959-72.

assay for gene function that also works in the test tube. Alternatively, the mutated gene is reintroduced into a living cell or even into a developing embryo to test whether the gene can still function properly, By systematic mutation and testing, the DNA signals of many genes have been discovered and some of the regulatory molecules in cells have been isolated.

I cannot go into detail here about these kinds of experiments. My purpose is to emphasize what can now be done with new genetic methods. These kinds of experiments were unimaginable even a few years ago, and now they are routine.

What are we going to learn about gene expression through these very powerful methods? As I mentioned, we are already learning about the exact signals in and around genes that control them and about the molecules that interact with these signals. We are beginning to understand the basis of hormone action as it affects genes. It is almost certain that many hormones work by turning genes on or off. We will learn how multiple genes are coordinated in their activity. It is evident that many genes must function together to enable a complex cell to fulfill its exact physiological roles. We are going to understand how molecules are placed in space; for example, the structure of an egg is important because molecules placed in exact locations will end up in specific regions of a developing embryo and influence development of just those cells. These carefully localized molecules commit a region of the embryo to develop in a certain way, presumably by interacting specifically with certain genes according to a precise timetable. We will learn about this in very great detail. We can expect to understand how one cell in an embryo, or in a tissue, instructs a neighboring cell and the molecular basis of such intercellular communication,

However, there are many problems we are not going to be able to solve using this methodology. Although we may understand how genes are controlled in development, paradoxically this will not tell us exactly what they do. For example, there are genes

that control other genes. These genes account for the very complicated, integrated patterns that we see in tissues — the formation of wings, legs, and other complex body parts. Mutations in these genes can cause whole tissues to change. An antenna becomes a leg. One body segment changes into another. Such genes, called homeotic genes, can and have been isolated, sequenced, and characterized. We will learn about the RNA molecules which are made from these genes and even about the proteins encoded by them. We can determine many things about a protein, such as its physical characteristics, its cellular location, and the other molecules in cells that the protein interacts with. But how do these facts explain how an antenna or a leg is made? What is "legness" anyway? These are concepts which elude us. In our pursuit of them we are going to be helped immeasurably by modern genetic methods, but they are not going to be elucidated entirely by existing methods. We will need more biology, biophysics, and biochemistry and a whole new set of principles and methods before we discover these greater global concepts and learn how embryos develop.