Committee II
Agricultural Genetic Engineering
And Society

DRAFT--6/15/91
For Conference Distribution Only

2-8

THE HISTORY AND LIMITS OF GENETIC ENGINEERING

by

Jonathan Wells
Department of Molecular and Cell Biology
University of California
Berkeley, California, USA

The Eighteenth International Conference on the Unity of the Sciences
Seoul, Korea August 23-26, 1991

THE HISTORY AND LIMITS OF GENETIC ENGINEERING

Jonathan Wells
Department of Molecular & Cell Biology
University of California, Berkeley

This paper is intended to set genetic engineering in context by surveying the history of biological theories and discoveries which have led up to it and by analyzing some biological factors which set limits to what it can accomplish.

History

A history of genetic engineering, narrowly conceived, could probably begin with experiments performed in the 1940's. That history, however, occurs in a broader context which reaches at least as far back as 1859, when Charles Darwin published On the Origin of Species. Darwin's theory of "descent with modification," as he called evolution, was characterized by three concepts: (1) species can change over time; (2) such changes are due to the operation of natural laws rather than divine intervention; and (3) the mechanism responsible for such change is natural selection acting on random variations.

The first two concepts, of course, did not originate with Darwin. His unique contribution was the third, emphasizing (a) the primary role of the struggle for existence, which "selects" only the fittest organisms for survival, and (b) the secondary role of small heritable variations, which appear spontaneously in a population without any relation to the needs of the organisms or the direction of evolution. According to Darwin, this mechanism accounted for the whole of evolution. Furthermore, it not only rendered divine intervention unnecessary (at least, after the initial creation of life), but also excluded the notion that God designed particular products of the evolutionary process (such as the eye, or the human species).[1]

Although the inheritance of selected variations was essential to Darwin's theory, he confessed ignorance about the nature of heredity. His notion of "pangenesis," according to which an organism's characteristics were preserved and passed on by tiny "gemmules" produced by each cell in the body, implied that an organism could respond to its environment through the use or disuse of parts, and that this could lead to heritable variations which were blended together in the offspring. This notion, however, was never more than a speculation.[2]

Unknown to Darwin, Gregor Mendel published an alternative theory of heredity in 1865, based on his experiments with peas. Mendel's work focused on traits which seemed to be inherited in an all-or-none fashion (for example, green or yellow seed color).

He concluded that the factor determining each trait could exist in the form of one or more variants, and that each pea plant carried two variants (which could be identical or different) for each factor. To use modern terminology, Mendel concluded that each plant carried two alleles of the same gene. These were distributed separately to the sex cells (which thus contained one allele apiece), so that upon fertilization the zygote obtained a second allele from the other parent. The offspring thus inherited one allele of each gene from each parent. Mendel summarized his conclusions in two fundamental laws of genetics: (1) the Law of Independent Assortment (in modern terminology, the two alleles of the same gene are distributed independently to the sex cells); and (2) the Law of Independent Segregation (in modern terminology, as different alleles of the same gene are transmitted through succeeding generations they are not blended together, but retain their original character).[3]

In 1879, Walter Flemming observed that cell division involves the duplication and distribution of tiny, thread-like organelles called "chromosomes." Since the distribution of chromosomes to sex cells and offspring parallels the assortment and segregation of Mendelian genes, Walter Sutton postulated in 1903 that chromosomes were the carriers of Mendel's genes. At about the same time, Hugo DeVries observed that new alleles occasionally appear spontaneously, by a process he called "mutation." These and similar advances in genetics led, during the first few decades of the twentieth century, to the "modern synthesis" of Darwinism

and Mendelism, according to which new traits arise in genes by spontaneous mutation, are subjected to natural selection, and (if they survive) are then passed on to succeeding generations in a Mendelian fashion via chromosomes.[4]

Many questions, however, remained. One of them concerned the chemical nature of the genetic material. Cells were known to consist primarily of water, protein, carbohydrate, lipid, and nucleic acid. Of these, only protein seemed chemically complex enough to carry the information necessary for heredity, but direct evidence was lacking. Since bacteria are much simpler and easier to grow than plants or animals, biologists turned to them for an answer to the question.

In 1928, Frederick Griffith distinguished two strains of the bacterium Streptococcus pneumoniae: a smooth (S) strain, so named because its colonies on agar exhibit smooth, glistening surfaces; and a rough (R) strain, so named because its colonies are rough and dull. Viewed under a microscope, individual organisms of the S strain are seen to be surrounded by capsules; injected into laboratory mice, these bacteria produce a virulent infection. Organisms of the R strain, by contrast, are unencapsulated and do not cause infection. Griffith observed that if he mixed heat-killed bacteria of the S strain with live bacteria of the R strain, and injected the mixture into mice, the animals developed infections and died. Griffith found live bacteria of the S strain in the blood of these animals, and concluded that

some factor had survived the heat treatment and passed from the dead S strain into the live R strain to transform the latter into virulent organisms[5].

At the Rockefeller Institute in the 1930's, Oswald Avery and his associates performed painstaking experiments to determine whether the transforming factor was protein, carbohydrate, or nucleic acid. They prepared purified extracts of heat-killed bacteria of the S strain, then treated the extract to remove or degrade proteins and carbohydrates. Antibodies against proteins and carbohydrates showed that the treated extract was, in fact, purified of these macromolecules; yet it was still capable of transforming the R strain. By contrast, if an extract was prepared in such a way as to preserve proteins and carbohydrates, but treated enzymatically to degrade deoxyribonucleic acid (DNA), it lost its transforming ability. These and other experiments convinced Avery and his co-workers that the transforming principle must be DNA.[6]

By 1944, when Avery, McCleod and McCarty published their conclusions, A.E. Garrod (studying a human genetic defect in metabolism) had already proposed that each gene specifies a particular enzyme; and George Beadle and Edward Tatum (using the bread mold Neurospora) had already provided evidence to support Garrod's suggestion: one gene, one enzyme. Salvador Luria and Max Delbruck had also performed experiments with the bacteriophage T1 (a virus that infects the common intestinal organism

Escherichia coli) to show that spontaneous mutations could arise in bacteria and be passed on to future generations. In other words, bacteria (like higher organisms such as Mendel's peas) have genes.[7]

Bacteriophages were known to consist of protein and DNA. In 1952, Alfred Hershey and Martha Chase published the results of an experiment showing that the DNA, and not the protein, carried the phage genes. Since protein contains sulfur, but no phosphorus, while DNA contains phosphorus, but no sulfur, Hershey and Chase grew one batch of T2 bacteriophage in radioactive sulfur to label the protein, and another in radioactive phosphorus to label the DNA. They then added these phage preparations to separate cultures of E. coli. After a few minutes, they interrupted the process of infection by placing each culture in a Waring blender, then centrifuged the suspensions. They found that the sediment, which contained all of the heavier bacterial cells, also contained the radioactive phosphorus; and when they resuspended this sediment in nutrient broth it produced active phage. The radioactive sulfur, however, remained in the lighter, unsedimented portion of the original preparation; and resuspending this portion in nutrient broth did not produce new phage. Hershey and Chase concluded that the protein portion of the phage remained outside the bacterial cell and did not participate in generating new phage, while the DNA was injected inside where it produced a new generation of complete and active phage.[8]

A full appreciation of DNA as the hereditary material, however, awaited the discovery of its structure. By 1950, DNA was known to be a fibrous polymer composed of four nucleotides: adenine (A), thymine (T), guanine (G), and cytosine (C). Erwin Chargaff had observed that, in samples of DNA from a wide variety of organisms, the ratios of A to T and G to C were always close to one. Then in 1950, in the laboratory of Maurice Wilkins, Rosalind Franklin used X-ray diffraction to study the crystalline structure of DNA. The data she obtained enabled James Watson and Francis Crick to construct the following model of DNA: the nucleotides were joined in a phosphate backbone winding around the outside of a double helix, in which the two strands ran in opposite directions, and in which adenines and thymines (and similarly, guanines and cytosines) in the complementary strands were hydrogen-bonded together. This 1953 Watson-Crick model accounted for the X-ray diffraction data as well as for Chargaff's ratios.[9]

The model suggested a mode of replication which Gunther Stent called "semi-conservative:" that is, the original double helix separates into two strands which then serve as hydrogen-bonding templates for the synthesis of complementary strands. Each double-helical daughter molecule would then contain one original strand and one newly synthesized strand. If, on the other hand, replication were "conservative," the original double helix would remain intact and the one daughter molecule would contain two new strands; while if it were "dispersive," each of the four

strands in the two daughter molecules would contain some of the original nucleotides and some new ones. In 1957, to distinguish between these three possibilities, Matthew Meselson and Franklin Stahl grew E. coli bacteria in heavy nitrogen, then transferred them to ordinary nitrogen for one or two rounds of replication and analyzed the density of the resulting DNA by centrifuging it in a cesium chloride gradient. The data they obtained were inconsistent with both conservative replication and dispersive replication, but were consistent with the semi-conservative replication suggested by the Watson-Crick model.[10]

The Watson-Crick model, then, successfully accounted for the faithful transmission of the genetic message from one generation to the next. But could it explain how DNA controls the composition and structure of enzymes? All known enzymes were proteins, and it had already been shown that the sequence of amino acids in a protein controls its final form, so all that was necessary was to show that DNA controlled the sequence of amino acids. According to Watson and Crick, this sequence was determined by the sequence of nucleotides in the DNA, the "genetic code."

In Paris, Arthur Pardee, Francois Jacob and Jacques Monod had been studying the way E. coli regulates genes involved in lactose metabolism. They discovered that such regulation depends on a specific region of the DNA (the "operon"), and also that an unstable intermediate carried the coded message between the DNA and its final protein product. In 1956, Elliot Volkin and

Lazarus Astrachan at Oak Ridge, Tennessee, reported that cells contain a minor species of unstable ribonucleic acid (RNA). Watson and Crick proposed that the DNA transfers its message to RNA (a process called "transcription"), which then serves as the template for protein synthesis (a process called "translation"). In other words: "DNA makes RNA makes protein."[11]

This "messenger RNA" presumably duplicates the code written in the DNA. But what is the code? With four different nucleotides, a pair could specify 16 different amino acids, while a triplet could specify 64. Since proteins were known to consist of 20 different amino acids, the code must be based on triplets, with some amino acids presumably being specified by more than one triplet. In 1961, Heinrich Matthaei and Marshall Nirenberg discovered that RNA made entirely of uracil (which corresponds to thymine in DNA), when mixed with appropriate enzymes and a mixture of various amino acids, produces a protein made entirely of phenylalanine. Similarly, they found that RNA composed only of cytosine produces a protein made entirely of proline. Matthaei and Nirenberg thus identified two "words" of the genetic code: UUU = phenylalanine, and CCC = proline. During the next five years, the rest of the code was solved.[12]

So the Watson-Crick model succeeded in explaining at the molecular level not only how genes replicate, but also how genes program the synthesis of proteins. In addition, it explained mutations, attributing them to changes in the sequence of

nucleotides due to mistakes in replication or damage from toxic chemicals or radiation. The picture seemed so complete that Crick concluded: "We have discovered the secret of life." Monod (who, like Darwin and Crick, was committed to purging biology of all vestiges of vitalism and teleology) echoed Crick's sentiment in 1970, and went on to claim that "the mechanism of Darwinism is at last securely founded." In fact (according to Monod) "man has to understand that he is a mere accident. Not only is man not the center of creation; he is not even the heir to a sort of predetermined evolution that would have produced either man or something very like him in any case."[13]

If molecular biology completed the Darwinian account of how evolution has occurred, it might also hold the key to controlling how further evolution will occur. By 1970, various enzymes had been discovered which appeared to make the artificial manipulation of life a practical possibility. These enzymes included restriction endonucleases (which cleave DNA at specific sequences), DNA ligase (which re-attaches severed pieces of DNA), and terminal transferase (which catalyzes the addition of new nucleotides to the end of a strand of DNA). In the early 1970's, Paul Berg and others at Stanford used these enzymes to join DNA from different sources. Stanley Cohen and his co-workers then took the next step in 1973 by combining DNA from various sources (including frogs) and placing the recombined DNA in E. coli, where it not only replicated but also produced new proteins. Although the idea of "genetic engineering" was

already several years old, the advent of recombinant DNA technology made genetic engineering a reality.[14]

Of course, manipulating genes is much more easily done in relatively simple organisms such as bacteria than it is in complex multicellular organisms such as mammals. Even now, almost two decades after the advent of recombinant DNA techniques, human gene therapy is still a distant hope rather than a clinical reality.[15] Nevertheless, if Crick and Monod were correct in their claim to have discovered the secret of life, it might eventually be possible to genetically engineer completely new species. Thus it is not surprising that critics warn: "If genetic engineering were allowed to continue at its current pace, the Homo sapiens species would experience no more than five or six more generations before being irreversibly replaced by a new, artificially engineered organism ...[which] would in many ways be as different from us as we are from our closest relatives, the primates."[16]

But were Crick and Monod correct? The main issue is not Monod's sweeping conclusion about teleology, which might be disputed on philosophical grounds, but their claim to have discovered the molecular mechanism of life itself. Is this claim biologically justified?

Limits

Crick's and Monod's claim to have discovered "the secret of life" implicitly relies on two assumptions: (1) all significant features of living organisms are ultimately derivable from the amino acid sequences of their proteins, which are encoded by DNA, and (2) DNA is the sole (or at least the primary) carrier of biologically significant information from one generation to the next. These assumptions are certainly questionable, and probably false.

(1) Although molecular biology has demonstrated conclusively that DNA carries the genetic code for the amino acid sequence of proteins, and thus the complex three-dimensional structure of protein molecules, this is not sufficient to specify a whole organism. Combining E. coli DNA with all the chemicals necessary for protein synthesis does not make a bacterial cell. The transforming factor discovered by Griffith and identified as DNA by Avery enabled living bacteria to synthesize a capsule necessary for virulence, but it did not revive dead bacteria or produce cells de novo.

Why not? By way of analogy, one might imagine the process of building a house. One needs to specify the building materials: the sizes and shapes of boards, nails, insulation, shingles, windows, doors, electrical wires, and pipes. One also needs to specify the intended floor plan, because the individual

materials could be combined in a number of different ways. Furthermore, one needs to specify the order in which components should be assembled, because the foundation must be completed before the walls and roof are erected, the walls must be erected before the pipes and electrical wires are installed, and so on. Molecular biology has shown that in a living organism the DNA-makes-RNA-makes-protein machinery provides the building materials, the specifications for which are encoded in the sequence of nucleotides in the DNA. Molecular biology has not shown, however, that the DNA contains either the floor plan or the assembly instructions.

Conceptually, it would be difficult to imagine how DNA could encode the floor plan, at least in eukaryotes. The DNA, in the nucleus, directs the production of proteins which are then transported throughout the cell. In order to perform their functions, many of these proteins must become localized in specific regions of the cell, and this depends on the prior localization of receptor molecules for which the proteins have specific affinities.[17] The spatial patterning of these receptor molecules (located in the membrane or the cytoskeleton) must precede the production of transported proteins by the DNA-RNA apparatus.

Of course, the receptor molecules themselves are specified by DNA, as are the constituent molecules of the membrane and cytoskeleton. But the three-dimensional structure of large

aggregates of these molecules cannot be derived just from the structure of the individual subunits. Consider, for example, the cytoskeleton. The cytoskeleton consists of microtubules, microfilaments, and intermediate filaments, all of which are made up of protein subunits. The assembly of these subunits into complex three-dimensional structures is a dynamic process which responds to the changing needs of the cell and which depends on intracellular nucleating sites and environmental cues. DNA determines the amino acid sequence, and thus the molecular structure, of each protein subunit. Subunit structure can affect large-scale structure, but it cannot determine it: for any given subunit structure, the cytoskeleton can be assembled in a great many different forms, as evidenced by the shape changes produced by an amoeba as it moves, or by the morphological diversity seen in the cells of a single multicellular organism.[18] To claim that DNA controls the large-scale structure of the cytoskeleton is analogous to claiming that the shape of the bricks controls the floor plan of a house.

There is evidence that a certain amount of patterning in embryos is controlled by the mother's genome: for example, the shells of Limnaea snails form a right-handed spiral unless the mother carries a genetic mutation producing a left-handed spiral.[19] Thus DNA can clearly affect morphology; but can it determine it? The simple choice between right- and left-handed coiling in a shell (which could easily be due to a change in a single molecule) falls far short of accounting for the three-dimensional

patterning of the snail's body as a whole. As one developmental biologist recently put it, "genes are responsible for determining which molecules an organism can produce," but this fact "fails to address the basic problem posed by morphogenesis, namely, how distinctive spatial order arises in embryos over characteristic distances." He concludes that "the molecular composition of organisms does not, in general, determine their form."[20]

Shape is not the only variable unspecified by the DNA. In multicellular organisms, cell types differ not only in their shape but also in their physiology. For example, liver cells, muscle cells, and nerve cells differ not only in the three-dimensional structure of their cytoskeletons, but they also manufacture different proteins. Yet they contain the same DNA. Evidence obtained from nuclear transplantation experiments and DNA hybridization studies indicates that the differentiated cells of multicellular organisms almost all contain the same complement of DNA as the original egg cell from which they developed. There are minor exceptions to this generalization: in some invertebrates, the somatic cells (i.e., those not developing into sperm or egg cells) lose a portion of their genes during early development; in some organisms, multiple copies of certain genes may be selectively replicated in specialized cells; and in vertebrates, cells of the immune system rearrange genetic elements to generate a diversity of antibodies. These exceptions, however, do not account for

differentiation; in fact, they are more likely to be consequences of differentiation than causes of it.[21]

In other words, although liver cells, muscle cells, and nerve cells exhibit striking differences in the proteins they produce, they all contain the same genes. Different genes are transcribed and translated into protein in each cell type, but the "code" for these differences must reside in some factor(s) other than the DNA. Since different regions of the original egg cell give rise to different cell types, it is likely that these factors are regionally localized. In fact, there is evidence that this is the case in some organisms.[22] Once again, the floor plan seems to be imprinted in spatial patterning which precedes transcription of the organism's DNA.

The assembly instructions are also a problem. Once the building materials are provided and the floor plan is specified, it is still necessary to assemble the materials in the proper order. Indeed, the development of multicellular organisms is a very ordered process, the disruption of which generally results in serious abnormalities or even death. Yet there is, as yet, no evidence from any organism that DNA contains a program specifying the temporal sequence of development.[23]

(2) None of this implies that mystical forces are involved in development. The fact is that DNA is not the only material passed from one generation to the next which carries significant

biological information. Hershey and Chase showed that DNA alone carried the information for the replication of a virus, but few biologists would consider viruses living organisms, since they can only reproduce inside a living cell. Avery, McCleod and McCarty showed that DNA could transmit information necessary to encode a capsule needed for virulence, but only to living bacteria. And even when simple bacteria reproduce, their descendants inherit more than their DNA.

When a bacterial cell divides, it passes on a membrane and some cytoplasm to its progeny; and these features are not unstructured. When a eukaryotic cell divides, its descendants inherit highly structured portions of its membrane, cytoplasm, and cytoskeleton. Even when a sperm cell fertilizes an egg, in most cases it transmits not only DNA but also a nucleating center for the embryonic cytoskeleton. And an egg cell is highly structured, in ways that determine important features of future development. In many species, an enucleated egg which is activated artificially will proceed through the early stages of development and morphogenesis without any DNA at all, until it eventually dies from lack of new proteins.[24]

In ciliated protozoa (single-celled eukaryotes), evidence indicates that the cortex (membrane and associated cytoskeleton and cytoplasm) carries developmental information which is independent of the organism's DNA. The complex organization of cilia on the cell surface of such protozoa can be surgically modified,

and the modified organization is transmitted indefinitely by direct cortical replication during cell division, regardless of genotype.[25]

Therefore, it appears likely that significant biological information is transmitted from one generation to the next independently of the DNA. The transforming factor is not the whole story, and DNA-makes-RNA-makes-protein is is only a small part of the secret of life. As one recent (1988) textbook in developmental biology puts it: "One can no longer retain the view (which once prevailed in molecular biology) that molecular biology had solved all the major problems of biology and that the molecular analysis of embryogenesis is merely the application of existing knowledge." Furthermore, "the analysis of macroevolutionary trends (above the species level) demands knowledge of developmental laws.... These laws of development await their discoverer."[26]

Genetic engineering may still be a powerful, useful, and even potentially dangerous tool.[27] The development and evolution of living organisms, however, appear to involve significant factors in addition to those discovered so far by molecular biology, and these factors will limit the potential of genetic engineering to modify existing life forms.

NOTES

1. For extended discussions of these issues see C.C. Gillispie, Genesis and Geology (New York: Harper & Row, 1959); P.J. Bowler, Evolution: The History of an Idea (Berkeley: University of California Press, 1984); N.C. Gillepsie, Charles Darwin and the Problem of Creation (Chicago: University of Chicago Press, 1979); and J. Wells, Charles Hodge's Critique of Darwinism: An Historical-Critical Analysis of Concepts Basic to the 19th Century Debate (Lewiston, NY: Edwin Mellen Press, 1988).

2. See Bowler, op. cit., 197-198; Gillespie, op. cit., 122-123.

3. J.G. Mendel, "Versuche uber Planzen-hybriden," Verh. Natur. Vereins Brunn 4 (1865): 3-57; Bowler, op. cit., 256-60; J.D. Watson et al., Molecular Biology of the Gene, Fourth Edition (Menlo Park, CA: Benjamin/Cummings Publishing Company, 1987), 8-11.

4. See Bowler, op. cit., 260-265; Watson et al., op. cit., 12-22.

5. F. Griffith, "The Significance of Pneumococcal Types," Journal of Hygiene 27 (1928): 141-144.

6. O.T. Avery, C.M. McCleod, & M. McCarty, "Induction of Transformation by a Desoxyribonucleic Acid Fraction Isolated from Pneumococcus Type III," Journal of Experimental Medicine 79

(1944): 137-158. See also H.F. Judson, The Eighth Day of Creation (New York: Simon & Schuster, 1980), 30-39.

7. G.W. Beadle & E.L. Tatum, "Genetic Control of Biochemical Reactions in Neurospora," Proc. Natl. Acad. Sci. (USA) 27 (1941): 499-506; S.E. Luria & M. Delbruck, "Mutations of Bacteria from Virus Sensitivity to Virus Resistance," Genetics 28 (1943): 491-511. See also H.F. Judson, op. cit., 55-56, 351.

8. A.D. Hershey & M. Chase, "Independent Functions of Viral Protein and Nucleic Acid in Growth of Bacteriophage," Journal of General Physiology 36 (1952): 39-56.

9. E. Chargaff, "Chemical Specificity of Nucleic Acids and Mechanisms of Their Enzymatic Degradation," Experientia 6 (1950): 201- 209; J.D. Watson & F.H.C. Crick, "A Structure for Deoxyribose Nucleic Acid," Nature 171 (1953): 737-738; idem, "Genetical Implications of the Structure of Deoxyribonucleic Acid," Nature 171 (1953): 964-967; idem, "The Structure of DNA," Cold Spring Harbor Symp. Quant. Biol. 18 (1953): 123-131. See also Judson, op. cit., 59-60, 96-107, 119-124, 133-177, 608-611; Watson et al., op. cit., 65, 73.

10. M. Meselson & F.W. Stahl, "The Replication of DNA in Escherichia coli," Proc. Natl. Acad. Sci. (USA) 44 (1958): 671-682. See also Watson et al., op. cit., 76-78; Judson, op. cit., 188-192.

11. A.B. Pardee, F. Jacob, & J. Monod, "The Genetic Control and Cytoplasmic Expression of 'Inducibility' in the Synthesis of B-galactosidase by E. coli," Journal of Molecular Biology 1 (1959): 165-178; L. Astrachan & E. Volkin, "Properties of Ribonucleic Acid Turnover in T2-infected Escherichia coli," Biochimica et Biophysica Acta 29 (1958): 536-544. See also Judson, op. cit., 313-337, 400-433.

12. J.H. Matthaei & M.W. Nirenberg, "Characteristics and Stabilization of DNAase-Sensitive Protein Synthesis in E. coli Extracts," Proc. Natl. Acad. Sci. (USA) 47 (1961): 1580-1588; M.W. Nirenberg & J.H. Matthaei, "The Dependence of Cell-Free Protein Synthesis in E. coli upon Naturally Occurring or Synthetic Polyribonucleotides," ibid., 1588-1602. See also Judson, op. cit., 470-489.

13. Judson, op. cit., 22, 109, 175, 214-217, 353, 447-449, 576, 613.

14. D. Jackson, R. Symons, & P. Berg, "Biochemical Method for Inserting New Genetic Information into DNA of Simian Virus 40: Circular SV40 DNA Molecules Containing Lambda Phage Genes and the Galactose Operon of E. coli," Proc. Natl. Acad. Sci. (USA) 69 (1972): 2904-2909; S.N. Cohen, A.C.Y. Chang, H.W. Boyer, & R.B. Helling, "Construction of Biologically Functional Bacterial Plasmids In Vitro," Proc. Natl. Acad. Sci. (USA) 70 (1973): 3240-3244; J.F. Morrow, S.N. Cohen, A.C.Y. Chang, H.W. Boyer,

H.M. Goodman, & R.B. Helling, "Replication and Transcription of Eukaryotic DNA in Escherichia coli," Proc. Natl. Acad. Sci. (USA) 71 (1974): 1743-1747. See also S.N. Cohen, "The Manipulation of Genes," Scientific American 233 (July, 1975): 24-33; and R. Hutton, Bio-Revolution: DNA and the Ethics of Man-Made Life (New York: New American Library, 1978), 30-88.

15. J.L. Marx, "Gene Therapy - So Near and Yet So Far Away," Science 232 (1986): 824-825; H. Muller, "Human Gene Therapy: Possibilities and Limitations," Experientia 43 (1987): 375-378; T. Friedmann, "Progress Toward Human Gene Therapy," Science 244 (1989): 1275-1281.

16. T. Howard & J. Rifkin, Who Should Play God? (New York: Delacourt Press, 1977), 224.

17. B. Alberts, D. Bray, J. Lewis, M. Raff, K. Roberts, J.D. Watson, Molecular Biology of the Cell, 2d edition (New York: Garland Publishing, 1989), 414-415.

18. ibid., 629-644, 650-675.

19. S.F. Gilbert, Developmental Biology, 2d edition (Sunderland, Massachusetts: Sinauer Associates, 1988), 85-86.

20. B.C. Goodwin, "What Are the Causes of Morphogenesis?" Bioessays 3 (1985): 32-36.

21. Gilbert, op. cit., 314-328; L.W. Browder, Developmental Biology, 2d edition (Philadelphia: Saunders, 1984), 136-137.

22. W.R. Jeffery, "Spatial Distribution of Messenger RNA in the Cytoskeletal Framework of Ascidian Eggs," Developmental Biology 103 (1984): 482-492; J.R. Whittaker, "Cell Lineages and Determinants of Cell Fate in Development," American Zoologist 27 (1987): 607-622. See also Gilbert, op. cit., 249-278.

23. A.S. Wilkins, Genetic Analysis of Development (New York: John Wiley & Sons, 1986), 484-486; H.F. Nijhout, "Metaphors and the Role of Genes in Development," Bioessays 12 (1990): 441-446.

24. Gilbert, op. cit., 474-475.

25. D.L. Nanney, "The Ciliates and The Cytoplasm," Journal of Heredity 74 (1983): 163-170; E.M. Nelsen, J. Frankel, & L.M. Jenkins, "Non-Genic Inheritance of Cellular Handedness," Development 105 (1989): 447-456.

26. Gilbert, op. cit., 813-814.

27. See, for example, D. Pimentel, M.S. Hunter, J.A. LaGro, R.A. Efroymson, J.C. Landers, F.T. Mervis, C.A. McCarthy, & A.E. Boyd, "Benefits and Risks of Genetic Engineering in Agriculture," BioScience 39 (1989): 606-614.