SOME RECENT DEVELOPMENTS IN GENETICS

RICHARD ROBLIN

Massachusetts General Hospital, Boston

INFORMED DISCUSSION of the ethical aspects of the application of discoveries in molecular genetics to human society requires a reasonably detailed understanding of the current and future technical possibilities. Yet, understandable descriptions of the technical possibilities are frequently difficult to come by, especially in an area which is changing as fast as molecular genetics. While newspaper reports of the latest scientific "break-throughs" are common, they are not always accompanied by sufficient information to enable the reader to put this latest "revolutionary" development in perspective. This paper is an attempt to describe in simplified terms some recent developments in the areas of molecular and human genetics, with special emphasis on developments which are already finding medical application, or which seem to me to be likely to do so in the near future. In order to focus this paper specifically on recent developments in genetics, I have not described work in other important areas such as *in vitro* fertilization and cloning. A review of recent developments in these areas, as well as an ethical critique of the experiments, can be found in the recent paper by Kass.[1]

While I have attempted to sketch the realm of the currently possible, and to extrapolate current trends a little into the future, nothing could be more deceptive than to assume that this is anything more than a highly selective "snapshot" as of early 1972. The pace of discoveries in the area of molecular genetics and the rate of development of our ability to synthesize, alter, and manipulate genetic elements *in vitro* is currently very rapid, and problems which looked like barriers to further development of genetic technology are rapidly disappearing.

BACKGROUND

Almost twenty years have elapsed since Watson and Crick's solution of the structure of the genetic material, DNA.[2] This period has seen the development of detailed understanding of many biological processes at the molecular level. For example, the details of how genes are duplicated exactly for transmission to daughter cells, how genes function to control cellular processes, and how alterations in the chemical structure of genes (mutations) can result in loss of function and genetic disease, are now reasonably well understood.

[1] L. R. Kass, in *New England Jour. Med.* 285 (1971) 1174.

[2] J. D. Watson and F. H. C. Crick, in *Nature* 171 (1953) 737, 964.

These new understandings are summarized in the so-called central dogma of molecular biology, which can be written in shorthand form as:

DNA (genes) → RNA (messages) → PROTEINS (functions)

That is, cellular genetic information is stored in the chemical structure of the DNA, and it controls cellular metabolism by first being "transcribed" into molecules of RNA which serve as messages from the genes in the cell nucleus to the rest of the cell. These messenger RNA molecules are subsequently used to order a unique linear arrangement of specific amino acids to yield individual unique proteins. This unique linear arrangement of amino acids is sufficient to determine the three-dimensional structure of the protein, and hence its biological properties. This process of reading the genetic messages encoded in RNA molecules is conveniently termed "translation."

The protein products of the translation process are many and diverse. They range from enzymes, which serve as catalysts for cellular metabolic reactions, to vital hormones such as insulin, to oxygen-carrying proteins like the hemoglobin of red blood cells, to important structural proteins like collagen. In addition, some genes appear to code for specific proteins, called repressors, which can bind to specific sites on DNA, and thus block the transcription of RNA messages from other specific genes.[3] Thus, the protein products of some genes can turn other genes "on" or "off." Although protein repressors have to date only been conclusively demonstrated in bacteria and bacterial viruses,[4] similar molecules may play important roles in higher organisms, since cellular differentiation in mammalian cells may be largely a matter of selectively turning genes "on" and "off."

This picture of the way in which genetic information encoded in DNA determines the structure and hence the functions of proteins has already had a profound impact on our conception of the nature of human genetic diseases. Human genetic diseases are now seen as the result of changes in the chemical structure or arrangement of DNA. These genetic changes (mutations) cause alterations in the function of the particular protein product of that gene. Loss of the ability to produce a vital cellular enzyme can cause genetic diseases in several ways. One of the most common appears to be the accumulation of "undigestible" products, either inside the cells or in the blood. Such accumulation can block the normal functioning of brain cells, for example, and lead to mental retardation.

[3] W. Gilbert and B. Muller-Hill, in *Proc. Nat. Acad. Sci.* 56 (1966) 1891.

[4] M. Ptashne, in *Nature* 214 (1967) 232.

The DNA in the nucleus of all human cells (except eggs and sperm) is organized into 23 pairs of chromosomes. At fertilization, each new human individual receives one complete set of 23 chromosomes from his mother and the other set of 23 chromosomes from his father. Since two copies of each human chromosome (except the sex-determining X and Y chromosomes) are present in all cells, two copies of each gene contained on the chromosomes will also be present. This redundancy of the genetic information (at least two copies of each gene per cell) has real advantages, since even if one copy of the gene contains a mutation, the other copy of the gene (on the other chromosome) frequently supplies enough of the gene product to permit essentially normal function. Thus, individuals who carry genetic mutations in one of their two gene copies usually appear normal and healthy. Genetic disease results when an individual inherits mutations *in the same gene* from both parents. Individuals with one mutant and one normal gene copy are termed "heterozygous," while those with two mutant gene copies are called "homozygous." Genetic mutations which are inapparent in heterozygous individuals are called "recessive." They are distinguished from other, "dominant" mutations which can cause genetic disease even in individuals heterozygous for that gene. Huntington's chorea is an example of a disease which appears to be due to a dominant mutation.

Incorrect balance or arrangement of human genetic information can also lead to genetic disease. Many cases of Mongolism (Down's syndrome) are caused by the presence of three copies of one human chromosome instead of the normal two copies. Other genetic diseases are due to deletions of parts of chromosomes, or to translocations of chromosomal material, in which parts of the genetic material normally found on one chromosome become stably associated with another chromosome.

LARGE-SCALE SCREENING PROGRAMS FOR VARIANT HUMAN GENES

Our understanding of the biochemical basis of some human genetic diseases is leading to an ability to test individuals for the presence of the variant genes which cause disease. For example, sickle-cell anemia is caused by a genetic mutation which leads to the production of an altered form of the protein hemoglobin. This sickle-cell hemoglobin is sufficiently different in electrical properties from the common type of hemoglobin so that the two proteins can be separated in an electric field. Thus, the diagnosis of the sickle-cell gene requires only drawing a small blood sample, a simple separation of the hemoglobin fraction from the red blood cells, and the electrical separation. If an individual is homozygous for the gene for sickle-cell hemoglobin, only that type of

hemoglobin will be observed. However, if an individual has one gene for sickle-cell hemoglobin and one gene for the common type of hemoglobin (i.e., he is heterozygous for the sickle-cell hemoglobin gene), both types of hemoglobin will be observed. Thus, the theoretical understanding and practical analytical techniques for determining the presence of the gene for sickle-cell hemoglobin are already available.

During the last several years, conversion of the scientific understanding of sickle-cell disease into a technology for testing (screening) people on a large scale has begun. Since the frequency of the gene for sickle-cell hemoglobin is particularly high among American blacks, many groups, both inside and outside urban black communities, are initiating screening programs for detection of the gene for sickle-cell hemoglobin in blacks. Since the required theoretical and practical knowledge is already available, the availability of money and trained personnel for such programs is the only real barrier to instituting large-scale, even nationwide, screening programs for the sickle-cell gene. Any large-scale screening program will require moderately large amounts of money. However, the National Institutes of Health have recently shown an increasing willingness to appropriate money for screening and treatment programs, at least for sickle-cell disease. For example, in fiscal year 1972, approximately $6,000,000 was appropriated for research and treatment of sickle-cell disease, although only a small fraction of that money has gone into screening programs. As I write this, a Congressional conference committee is trying to reconcile differing versions of a new appropriation for sickle-cell disease programs passed by the Senate and the House of Representatives. This legislation would authorize some 40–45 million dollars per year for the next three years. Should this bill pass the Congress, presidential signature, in an election year, seems likely. Thus, the amount of money available to fund genetic screening programs for the sickle-cell gene will probably increase rapidly in the near future.

Most current sickle-cell gene screening programs detect heterozygous carriers as well as a much smaller number of homozygous affected individuals. Since the heterozygous carriers of the sickle-cell hemoglobin gene are not usually sick, their major benefit from the screening program is knowledge that will help them make more informed choices about parenthood. Therefore, an essential component of any screening program which detects adult heterozygous carriers is genetic counseling. A genetic counselor is needed to explain, in an understandable way, the results of the genetic tests to those who have been screened. The news that one is the carrier of a potentially lethal gene is received very differently by different people. Since important questions regarding

sexual relations, parenthood, and marital stability may depend on understanding and use of the genetic facts, the genetic counselor must be a mature and experienced person with considerable psychological insight. How genetic counselors should be trained and what their qualifications should be are currently matters of considerable debate. What does seem clear, however, is that there may be an acute shortage of trained genetic counselors in the early stages of genetic screening programs.

Screening programs for other variant human genes are likely to follow those for the sickle-cell gene. Genetic screening programs to detect carriers of the gene causing Tay-Sachs disease, modeled on Kaback's pioneering program in the Baltimore-Washington area,[5] are being initiated in other major cities in the United States. There are already at least 20 other genetic diseases in which adult heterozygotes can be identified by an appropriate biochemical test. As the biochemical basis for other common genetic diseases (i.e., cystic fibrosis) becomes known, screening programs for these diseases will doubtless be proposed.

The fact that current genetic screening programs have been initiated for single genetic diseases should not blind us to a possible future trend toward simultaneous testing for more and more variant genes. Cost-effectiveness calculations ("get the most genetic information for your tax dollar"), so popular as a yardstick for evaluating government programs, would favor such a trend. Would new ethical and social issues be raised if our paradigm genetic screening program was a government-funded, data-banked, multifactorial testing program for *all* adults of childbearing age? Would such programs have a tendency to become compulsory, either explicitly by law or implicitly through social pressure?

AMNIOCENTESIS AND ABORTION

In addition to screening parents before conception, it is now technically possible to test fetuses *in utero* for some specific genetic and chromosomal defects. In this technique, called amniocentesis,[6] a small sample of the amniotic fluid which surrounds the fetus is withdrawn through a needle. This fluid contains fetal cells which are constantly being sloughed off as the fetus grows. For many human genetic diseases, fetal amniotic fluid cells reflect the genetic disorder; that is,

[5] M. M. Kaback, personal communication; *Medical World News*, May 14, 1971.

[6] A. Milunsky, J. W. Littlefield, J. N. Kanfer, E. H. Kolodney, V. E. Shih, and L. Atkins, in *New England Jour. Med.* 283 (1970) 1370, 1141, 1498; T. Friedmann, in *Scientific American* 225 (1971) 34.

if an enzyme is deficient in adult cells, it is also found to be deficient in the amniotic fluid cells. There are now 25–30 different human genetic diseases in which the unaffected, heterozygous, and homozygous affected fetal conditions may be distinguished by biochemical testing of amniotic fluid cells.

As noted above, chromosome abnormalities are also responsible for many types of human genetic defects. It is now a routine matter to examine the chromosome complement of an individual's cells, by staining the chromosomes of the white cells from a blood sample. Amniocentesis permits isolation and examination of the chromosome complement of cells from the fetus *in utero*. Until recently, the available techniques for staining human chromosomes had considerable limitations. It was not even possible to unambiguously distinguish all the human chromosomes from one another. In the last two years, however, chromosome-staining techniques have undergone a major revolution.[7] New techniques, which involve staining the chromosomes with fluorescent dyes,[8] now permit all 23 different pairs of human chromosomes to be distinguished from each other. Other new staining techniques can show up chromosome deletions and rearrangements in the chromosome structure that were not detectable before.[9] These new chromosome-staining techniques may reveal a whole new set of human chromosome abnormalities.

The amniocentesis procedure currently carries with it a small degree of risk to the health of the mother and fetus.[10] Because of this, it has been suggested that amniocentesis is indicated only when the couple appears to be at significantly higher risk for birth of a child with a genetic defect.[11] (For example, a couple in which both prospective parents were carriers of the gene for Tay-Sachs disease would probably have amniocentesis recommended to them, since they have a 25% chance of having a child with Tay-Sachs disease.) But surely, in the future, increased familiarity with amniocentesis will decrease the risk to mother and fetus, more and more chromosomal and enzymatic defects will become diagnosable through amniocentesis, and more couples will learn about amniocentesis and ask for it. Thus, if only a comparative-risk criterion is used, will there not come a time when amniocente-

[7] F. Hecht, H. E. Wyandt, and R. W. Erbe, in *New England Jour. Med.* 285 (1971) 1483.

[8] T. Caspersson, G. Lomakka, and L. Zech, in *Hereditas* 67 (1971) 89.

[9] B. Dutrillaux and J. Lejune, in *Compt. rend. Acad. sci.* (Paris) 272 (1971) 2638.

[10] H. M. Nadler and A. B. Gerbie, in *New England Jour. Med.* 282 (1970) 596.

[11] J. W. Littlefield, in *New England Jour. Med.* 282 (1970) 627.

sis and multifactorial testing will be considered to be "indicated" for every pregnancy?

The technical capability of detecting specific genetic defects in fetuses seems likely to add new dimensions to the current debate about whether abortion is ever justified, and if so, under what conditions.[12] For some, the knowledge that a given fetus *in utero* is *proven* to be Mongoloid or afflicted with Tay-Sachs disease is an important factor for making abortion decisions. For others, this certainty about the condition of the fetus, available through amniocentesis, would not be a determining factor. However, as citizens, all of us share a responsibility to decide upon an appropriate legal policy on abortion.

GENETIC ENGINEERING

Although a specific, genetically determined enzyme defect has already been identified in 92 different human genetic diseases,[13] treatment with dietary therapy, drug therapy, or enzyme therapy has been successful for only a few diseases. The limitations of current therapies and enthusiasm in some quarters about the technical prospects have led to proposals for using DNA therapy to attempt to cure human genetic defects.[14] Since cells in tissue culture (either isolated from individuals with genetic diseases or produced artificially) can reflect genetic disorders, much experimental work is currently under way which involves adding isolated DNA segments carrying the gene for the missing enzyme to mammalian cells in culture. The hope is that the isolated DNA segments can become permanently associated with the cells in some way, and thus supply the genetic information required to produce the missing enzyme. Thus, demonstration of DNA-directed genetic changes of mutant human cells in tissue culture would be a first step toward potential techniques for genetic engineering in man.

At least three technical difficulties currently appear to limit the prospects for DNA-directed genetic changes using isolated DNA.[15] They are: (*a*) the low specificity of natural DNA in terms of isolated specific gene sequences, (*b*) intracellular degradation of isolated DNA molecules taken up by cells, and (*c*) failure of the added DNA to become permanently associated with the existing DNA of the recipient cells. However, there are recent scientific developments which suggest

[12] Cf. D. Callahan, *Abortion: Law, Choice and Morality* (New York, 1970).

[13] V. A. McKusick, in *Annual Review of Genetics* 4 (1970) 1.

[14] H. V. Aposhian, in *Perspectives in Biology and Medicine* 14 (1970) 98; S. Rogers, in *New Scientist*, Jan. 29, 1970, p. 194.

[15] T. Friedmann and R. Roblin, in *Science* 175 (1972) 949.

ways of solving all these problems, and which, if combined in a single approach, might render DNA-mediated genetic modification of human cells technically feasible.

The problem posed by the low specificity of isolated cellular DNA can be illustrated by the following rough calculation. The molecular weight of the DNA from a normal human cell is about 4×10^{12} daltons. If a representative gene contains genetic information sufficient to code for a protein containing 200 amino acids, then the DNA segment for that gene would have a molecular weight of 4×10^5 daltons. Assuming that there are two copies of each gene per cell (one on each chromosome), the fraction of the total cellular DNA *specific* for the representative gene would be $(4 \times 10^5) \times 2/4 \times 10^{12} = 2 \times 10^{-7}$. This means that when whole cellular DNA is extracted and added to mutant cells, only one DNA molecule in five million carries the desired genetic information. Any biological process that depends on one molecule among five million reaching a specific target is not likely to be very efficient.

However, imagine the increases in efficiency possible if *every* DNA molecule added to the cells contains the desired genetic information. There are several recent scientific reports which suggest ways to approach this condition. First, techniques now exist which permit the isolation of specific bacterial genes.[16] These techniques are quite general and could, in theory, be applied to the isolation of any desired bacterial gene. Also, specific genes may be synthesized chemically in the test tube, if the genetic information of their transcribed RNA is known.[17] Finally, research on the mechanism of cancer-causing RNA viruses recently led to the discovery of a virus-associated enzyme which could make DNA copies of RNA molecules.[18] This enzyme has been dubbed "reverse transcriptase" because it reverses the usual direction of transcription of DNA into RNA. Its importance to the present discussion is that it may be able to make DNA copies of *any* RNA molecule which can be isolated in pure form. Preliminary experimental demonstrations that the reverse transcriptase can indeed be used in this way have come from the near-simultaneous publications from three different laboratories.[19]

[16] J. Shapiro, L. McHattie, L. Eron, G. Ihler, K. Ippen, and J. Beckwith, in *Nature* 224 (1969) 768.

[17] K. L. Agarwal, H. Buchi, M. H. Caruthers, N. Gupta, H. G. Khorana, K. Kleppe, A. Kumar, E. Ohtsuka, U. L. Rajbhandary, J. H. Van De Sande, V. Sgaramella, H. Weber, and T. Yamada, in *Nature* 227 (1970) 27.

[18] H. M. Temin and S. Mizutani, in *Nature* 226 (1970) 1211; D. Baltimore, *ibid.* 1209.

[19] J. Ross, H. Aviv, E. Scolnick, and P. Leder, in *Proc. Nat. Acad. Sci.* 69 (1972) 264; I. M. Verma, G. Temple, H. Fan, and D. Baltimore, in *Nature* 235 (1972) 163; D. L. Kacian, S. Spiegelman, A. Bank, M. Terada, S. Metafora, L. Dow, and P. A. Marks, *ibid.* 167.

All three groups took advantage of the fact that a large proportion of the messenger RNA molecules of red blood cells code for the oxygen-carrying protein hemoglobin. By adding reverse transcriptase to a purified RNA fraction from red blood cells, all three groups achieved *in vitro* synthesis of DNA components of the genes for hemoglobin. In one case, DNA components of the gene for *human* hemoglobin were produced. Although the published work has not yet demonstrated the complete synthesis of the hemoglobin gene, this may be only a small extension of work already accomplished. I believe that application of molecular genetic techniques like these will soon overcome the problems posed by lack of specificity of natural DNA mixtures, thus enhancing the technical prospects for successful DNA-mediated genetic modification.

Intracellular degradation of added DNA molecules may also not remain a problem for very long. Protection of the DNA with specific substances like DEAE-dextran[20] or by coating it with reassembled viral protein coats may prevent intracellular degradation of the DNA long enough to give it a good chance to become permanently associated with the DNA of the cell.

Even if specific segments of added DNA can be delivered intact to nuclei of mammalian cells, their ability to function there may only be transient unless they become permanently associated with the chromosomal or other DNA of the cell. While this may, at first, sound like an unlikely event, there is highly suggestive evidence that the DNA of certain cancer viruses can become permanently associated with the DNA of cells made malignant by infection with these viruses.[21] It seems likely that these viruses possess the genetic information for special enzymes which are required to integrate the viral DNA into the cellular, chromosomal DNA. Some investigators are currently trying to take advantage of this integrating property of viral DNA. They plan to chemically link the specific DNA segment which they wish to introduce into the cells to pieces of viral DNA. This will create man-made, hybrid DNA molecules containing the integrating properties of the viral DNA, which might carry the specific DNA segments into the cellular DNA. This kind of genetic technology, feasible at the current stage of our knowledge, may represent an orders-of-magnitude increase in the prospects for successful DNA-mediated genetic modification.

Ironically, there are some recently published experiments which, if confirmed in other laboratories, would demonstrate that introducing foreign genes into human cells may not require the elaborate genetic

[20] J. H. McCutchan and J. S. Pagano, in *Jour. Nat. Cancer Inst.* 41 (1968) 351.

[21] J. Sambrook, H. Westphal, P. R. Srinivasan, and R. Dulbecco, in *Proc. Nat. Acad. Sci.* 60 (1968) 1288.

manipulations described above. I refer to the report by Merril and his coworkers,[22] which describes reacquisition of the ability to produce the missing enzyme in human cells from galactosemic patients. The point is that the DNA used to effect this change was extracted from a virus which grows in bacteria, and the virus apparently had picked up the gene for the missing enzyme from the bacterial DNA. Moreover, other recent experiments suggest that this same virus may be used to acquire almost any desired bacterial gene.[23]

Only time and further experiments will tell whether DNA-directed genetic modification of mammalian cells will be technically simple or difficult. Whichever, it is surely not too early to start to examine the complex ethical and social issues that will be raised by this potential to predictably alter human heredity.

[22] C. R. Merril, M. R. Geier, and J. C. Petricciani, in *Nature* 233 (1971) 398.

[23] K. Shimada, R. A. Weisberg, and M. E. Gottesman, in *Jour. Molecular Biology* 63 (1972) 483.