Pharmaceutical Research, Vol. 5, No. 4, 1988

Commentary

Genes and the Pharmaceutical Sciences

Wolfgang Sadée

Detailed knowledge of the structure and function of genes, and indeed the entire human genome, promises to revolutionize medicine. There are some three billion nucleotides lined up in human chromosomes, which encode for approximately 100,000 genes. Differences among individuals occur at a rate of one in one thousand nucleotides, accounting for the near infinite variation among individuals. Genes, of course, encode the body's proteins and thus determine individual characteristics, such as susceptibility or resistance to diseases. Unraveling the molecular mechanisms of pathological processes and their genetic basis will profoundly affect therapy and disease prevention. The health sciences, including the pharmaceutical sciences, thus confront a plethora of novel challenges and opportunities.

After James Watson and Frances Crick solved the double helix structure of DNA in 1953, a rapid succession of seminal discoveries led to an explosion in the field of molecular biology. Marshall Nirenberg and Heinrich Mattaei unraveled the genetic code, Hamilton Smith and coworkers discovered restriction enzymes catalyzing specific and reproducible cuts along DNA strands, and Frederick Sanger developed methods for rapid determination of the nucleotide sequence of DNA fragments. Hence, the amino acid sequence of entire proteins can be determined by inference via the genetic code. In 1973, Herbert Boyer and Stanley Cohen devised the first practical procedure for placing a piece of foreign DNA into a bacterial plasmid, which marked the birth of recombinant DNA technology. Together with the use of monoclonal antibodies obtained from cell hybridomas, this area forms a cornerstone of modern biotechnology. Among the immediate benefits, we may count the many peptides and proteins that can now be produced in large quantities and high purity. Insulin, growth hormone, interferon-α, hepatitis B vaccine, and tissue plasminogen activator are recombinant DNA products with current FDA approval. Many more are under investigation, including lymphokines such as interleukin-2, factor VIII, and erythropoietin (1).

Many of the major diseases of mankind are thought to have varying degrees of hereditary origin. This is believed to be the case with certain types of cancer, cardiovascular disease, mental illness, and the chronic diseases of old age, such as Alzheimer's disease. There are thought to be as many as 4000 loci in the human genome associated with genetic diseases. Of these, approximately 1200 have been mapped and characterized in varying degrees of detail. Examining the genes encoded for the hemoglobin protein genes, geneticists have found nearly 40 point mutations, one of which is associated with sickle cell anemia. Yet despite significant advances,the genes involved with the majority of genetic diseases remain completely unknown at this time.

To determine the causes of genetic diseases has proven extraordinarily difficult. It was first necessary to identify the mutated protein before its corresponding gene could be found. Now, however, novel techniques can lead directly to the responsible gene without any knowledge of its function. Extremely rapid sequencing procedures, employing four different fluorescent markers for the four bases of DNA, promise a theoretical capability of over 10,000 nucleotides per day (2). Furthermore, field inversion or pulsed field gradient gel electrophoresis permits the separation and analysis of large DNA fragments. Specific markers, such as restriction fragment length polymorphism markers (RFLP), are being developed to pinpoint the location of suspected genes. By linkage analysis of suitable RFLP markers and the genetic defect, one can narrow down the gene's location, until the mutated gene itself is identified (3). From here, the primary structure of the corresponding protein can be deduced and its function studied. Because the investigation proceeds in reverse of classical genetics, this approach is often referred to as reverse genetics.

The applications of reverse genetics for the understanding of human disease are immense. There is a rapidly growing list of the precise locations and even the structures of genes thought to cause Duchenne's muscular dystrophy, retinoblastoma, cystic fibrosis, Huntington's disease, polycystic kidney disease, and others. Major diseases, such as atherosclerosis, hypertension, and diabetes, are also suspected of having a genetic component as their basic cause. For example, one of the HLA class II genes (HLA-DQ_β) contributes to susceptibility and resistance to insulin-dependent diabetes mellitus (4); apparently, amino acid residue 57 of the β-chain specifies the autoimmune response against islet cells. However, these diseases often have a polygenic basis which renders the solution to their molecular nature more difficult.

Gene diagnostics exemplifies the instant technology transfer from research laboratory to clinical application, a hallmark of current biotechnology. Estimates of the diagnostics market potential in the near future range widely from $50 million to $1 billion per year, but the long-range outlook is bright with recent technical advances opening new horizons. The ability to vastly amplify specific segments of DNA with a polymerase chain reaction (PCR) has enhanced the analytical limit by two orders of magnitude over conventional Southern blots and permits the detection of gene defects with just a few cells. Genetic counseling will become a major factor in health care.

0724-8741/88/0400-0199$06.00/0

Therapy of genetic diseases can proceed by replacing the defective protein, its product, or the defective gene itself. While tinkering with the human genome is considered hubris by some, it is important to recognize that the mechanisms for shuffling genes freely between individuals or species occur in nature and are thought to be one of the earliest means of genetic exchange in primitive bacteria. These natural exchanges call on the very same tools we use in recombinant DNA technology: enzymes that reproduce, splice, and recombine nucleic acids. Despite these facts, germ line gene therapy is currently off limits. However, the introduction of genes into somatic cells is presently under vigorous study and raises fewer ethical problems, since the genome of somatic cells cannot be inherited. Retroviral vectors are among the leading candidates for introducing foreign genes into somatic cells (5), and bone marrow cells are presently one of the primary targets of gene therapy (6). Combined severe immunodeficiency, caused by adenosine deaminase deficiency, may respond to somatic gene therapy. Other approaches include gene insertion into transplantable human epidermal cells (7) or fibroblasts (8), which were employed as the delivery system of human growth hormone in animal experiments. Major hurdles remain to be overcome (e.g., tissue specific gene expression at sufficiently high levels over long time periods) (9). After transducing hematopoietic cells, specific expression of the human β-globin gene in the erythroid cell lineage has been achieved (10). Although gene therapy is only in its infancy, it may become a viable alternative to other therapeutic modalities, such as bone marrow transplantation or administration of the correct gene product.

Meanwhile, the introducion of foreign genes into the germ lines of laboratory animals has become a powerful new research tool. Transgenic mice may be used to study tissue specific control of gene expression, functioning of the immune system, genetic diseases, and oncogenes (11). The correction of murine β-thalassemia by gene transfer into their germ line has been demonstrated. Further, expression of the tat gene of HTLV-I in murine germ cells could lead to a model of neurofibromatosis (van Recklinghausen's disease) (12). Scientists at Integrated Genetics (Framingham, Mass.) have successfully fused the gene that normally controls the production of mouse milk to the gene for human tissue plasminogen activator (tPA). These genetically altered mice produce human tPA in their milk. One may speculate that a small cattle herd with the tPA gene stably introduced into their germ line could satisfy the world's demand for tPA as a drug.

These few examples demonstrate the extraordinary power of the new research tools in recombinant DNA technology. Other opportunities involve the construction of viral vectors for vaccinations. One can insert genes that encode for antigenic peptides into the vaccinia virus which infects the cell and reproduces in the cytoplasm without inserting into the host genome. Ramshaw *et al.* (13) suggest the insertion of an additional gene for interleukin-2 expression in order to enhance the body's immune response and safeguard immune-deficient individuals against deleterious effects of the vaccinia virus. Such a strategy may prove successful in the design of anti-AIDS vaccines.

New therapeutic strategies are evolving at such a rapid pace that biotechnology may eventually be capable of instantly turning genes and their corresponding proteins into novel therapeutic agents. Pharmaceutical scientists must concern themselves with the production and packaging of these new agents into suitable formulations, their analysis and quality control, mode of application, delivery to target tissues, biodisposition, pharmacodynamics, and pharmacology-toxicology. Principal targets of the pharmaceutical scientist include the understanding, through the use of physicochemical methods, of both DNA and protein structure in relation to their folding into functional domains, mechanism of pharmacological action, and *in vitro* and *in vivo* stability. The possibility of employing the body's own cells as therapeutic agents, either after gene transfer or with targeting molecules placed on their surfaces, highlights the need to promote cell biology as a major discipline in the pharmaceutical sciences. And special consideration needs to be paid to the regulatory, economic, and ethical aspects of these technological advances. Within the AAPS, although each of the Sections must therefore be directly involved and take immediate advantage of the many opportunities presented to us, it falls on the new Biotechnology Section to bring these novel research directions and therapeutic strategies to our attention. The Biotechnology Section must boldly strike out into this new territory and function as a source of new ideas for all other Sections of the AAPS.

REFERENCES

1. W. Sadée. *Pharm. Res.* 3:3–6 (1986).
2. J. M. Prober *et al. Science* 238:336–341 (1987).
3. D. Drayna. *BioTechniques* 4:412–418 (1986).
4. J. A. Todd *et al. Nature* 329:599–604 (1987).
5. E. Gilboa *et al. Biotechniques* 4:504–511 (1987).
6. R. Parkman. *Science* 232:1373–1378 (1986).
7. J. R. Morgan *et al. Science* 237:1476–1479 (1987).
8. R. F. Selden *et al. Science* 236:714–718 (1987).
9. S. A. Camper. *BioTechniques* 5:638–650 (1987).
10. D. J. Weatherall. *Nature* 331:13–14 (1988).
11. E. A. Dzierzak, T. Papayannopoulou, and R. C. Mulligan. *Nature* 331:35–38 (1988).
12. S. H. Hinrichs *et al. Science* 237:1340–1343 (1987).
13. I. A. Ramshaw *et al. Nature* 329:545–546 (1987).