Chapter 4

The State-of-the-Art in Genetics

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Molecular biology is being integrated into genetics and medicine at a rapid pace. In the laboratory, more than 300 human conditions can be analyzed with today's molecular genetic technology (3 1). With clinical application of these discoveries comes public expectations for new means of diagnosis, screening, and even cure. This chapter provides an overview of the genetic principles important to understanding the issues involved in carrier screening for cystic fibrosis (CF). It outlines basic tenets of human genetics and molecular biology and summarizes the technical aspects of CF carrier screening, Finally, this chapter looks forward, exploring how advances in automation could affect both carrier screening for CF and testing and screening for additional genetic disorders. Earlier OTA reports provide further background in human genetics and biotechnology (128-134).

BASIC GENETIC PRINCIPLES

Genetics explores how specific traits are passed from one generation to the next. Except for certain specialized cells, each of the trillions of cells in a human being contains a complete set of genetic instructions-the genome—for the individual. A person's genome governs everything from the structure of a single molecule, such as a protein, to the expression of identifiable traits, such as eye color. An intricate hierarchy of instructions determines which cells act on this genetic information, as well as when they do so.

Scientists study human genetics on many levels. They assess, for example, the molecular basis for inheritance by examining the specific structure and function of the genetic material, DNA. Geneticists also observe how the environment influences the expression of genetic traits, and trace the clustering of biological characteristics in populations.

Function and Organization of DNA

As in all higher organisms, genetic information is stored in humans in DNA, a double stranded structure resembling a twisted ladder. This double helix consists of a genetic alphabet of four different nucleic acid molecules, or bases—adenine (A), thymine (T), guanine (G), and cytosine (C)---each of which is attached to a deoxyribose sugar group and a phosphate molecule (figure 4-l). The bases pair predictably—A with T, and G with C—to form the DNA double helix structure.

Lengths of DNA ranging from 1,000 to 2 million base pairs comprise a gene, the fundamental physical and functional unit of heredity. About 50,000 to 100,000 structural genes, spread over 3,3 billion base pairs, make up the human genome. DNA associates with proteins to form chromosomes, tightly coiled structures located in the cell nucleus.

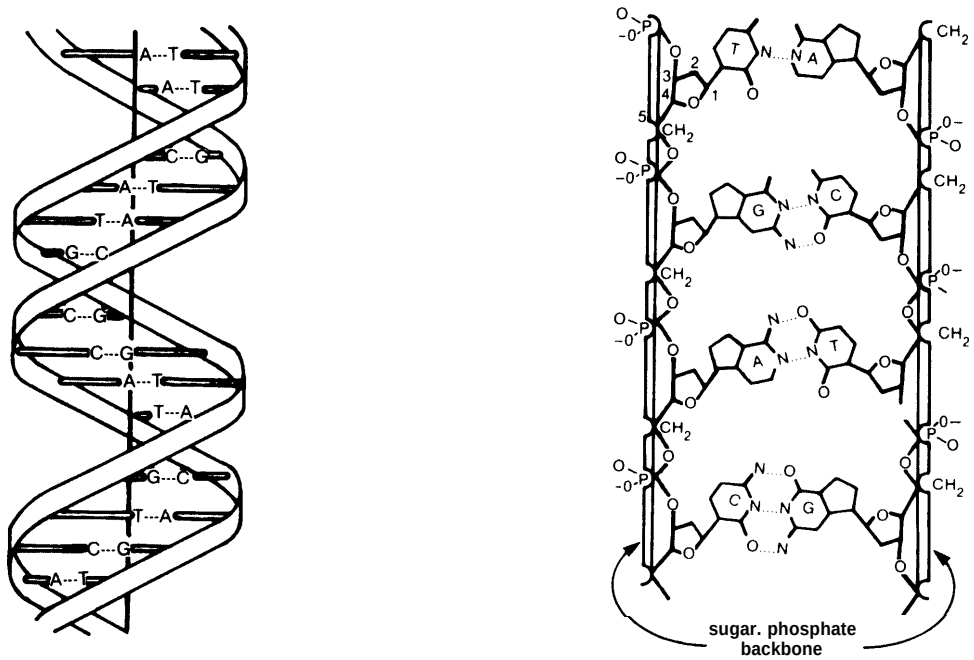
In humans, genes are arrayed on 46 chromosomes-22 pairs of autosomes and 1 pair of sex chromosomes (figure 4-2). Females have two X chromosomes, and males have one X and one Y chromosome. Egg and sperm cells contain just one copy of each chromosome; fusion of the sperm and egg at fertilization creates a full genetic complement.

The physical location of a gene on a chromosome is called its locus. Some genes have been mapped—plotted at a specific locus on a chromosome--and cloned-generated in multiple copies in the laboratory. Alternative forms of a gene at a particular locus are called alleles. At each locus along pairs of autosomes, an individual can have two identical or two different alleles, one copy inherited from the mother and one from the father. If the alleles are the same, the person is said to be homozygous for that particular locus. If the versions differ, the person is said to be heterozygous. Even though a normal individual has at most two alleles at a given locus-again, one copy inherited from the mother and one from the father-additional alleles can exist in other individuals.

DNA Replication

Through DNA replication, a full genome of DNA is regenerated each time a cell undergoes division to yield two daughter cells. In DNA replication, each chain of the double helix is used as a template to synthesize copies of the original DNA (figure 4-3). During cell division, the DNA double helix unwinds, the weak bonds between base pairs break, and the DNA strands separate. A series of enzymes insert a complementary base opposite each base in the original strand-A opposite T, and G opposite

Figure 4-I—The Structure of DNA



Left: A schematic diagram of the DNA double helix. Right: The four bases form the letters in the alphabet of the genetic code. The sequence of the bases along the sugar-phosphate backbone encodes the genetic information.
SOURCE: Office of Technology Assessment, 1992.

C--creating two identical copies of the original DNA.

Proteins

The bridge between DNA's chemical information and physical realization of its instructions consists of steps that convert the DNA code into biologically active products. Through a process known as gene expression, a DNA sequence for a structural gene ultimately results in formation of a molecule called a protein (figure 4-4). Proteins are required for the structure, function, and regulation of all cells, tissues, and organs in the body.

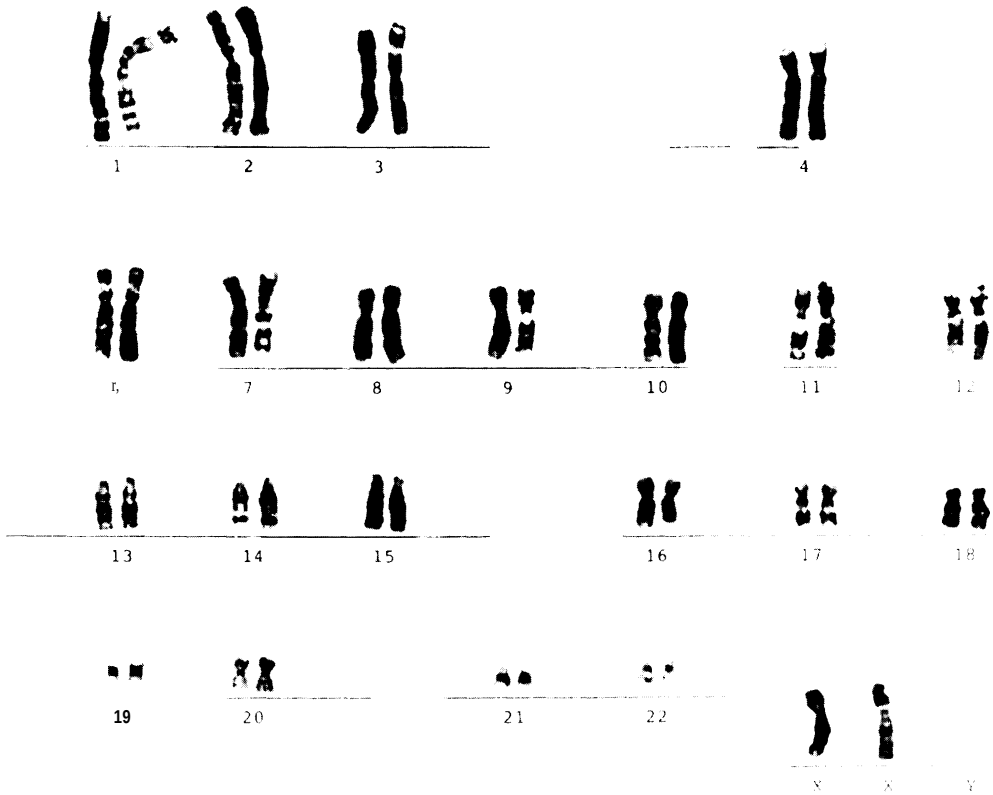
First, the bases in the DNA sequence are copied, or transcribed, into messenger ribonucleic acid (mRNA), a single-stranded molecule that carries the genetic information out of the nucleus. The bases in the mRNA are read as triplets, or codons, that specify 1 of 20 different amino acids, the building blocks of a protein molecule. Then, in accordance with the instructions in the mRNA, amino acids are assembled into a specific protein molecule. Thus, the information encoded by DNA is transcribed to mRNA and translated from the DNA code into a protein that has a particular function in a cell.

Genetics and Disease

Hereditary variation is the result of changes-or mutations—in DNA. Mutations present in germ cells (egg or sperm) are inherited by offspring, whereas those that occur in somatic cells (other body cells) are not passed on to future generations. Most mutations exist in both cell types. Mutations arise from the substitution of one DNA base for another, from rearrangements (e.g., small insertions or deletions) within the gene, or from duplication or deletion of the entire gene. Approximately 4,000 known human disorders result from genetic causes (88). Disorders arising from a mutation in only one gene are known as monogenic. CF is a monogenic condition.

Since proteins are produced from the instructions in genes, a mutation in a gene that codes for a specific protein can affect the structure, regulation, function, or synthesis of the protein. A particular mutation in a gene can produce a benign or mild effect, while a different mutation in the same gene

Figure 4-2-Chromosome Complement of a Normal Human Female



SOURCE: Genetics & IVF Institute, Fairfax, VA, 1990.

can result in gross reduction or complete loss of activity of the resulting protein. The genetic constitution of an individual is its genotype, while the observable expression of the genes is its phenotype.

Modes of Inheritance

Although DNA's structure was first determined in 1953, the inheritance of genetic traits was studied long before then. In 1865, Austrian monk Gregor Mendel postulated that discrete biological units—later named genes—were responsible for both the maintenance and variation of certain characteristics from one generation to the next. Since then, understanding of the processes underlying inheritance has been refined.

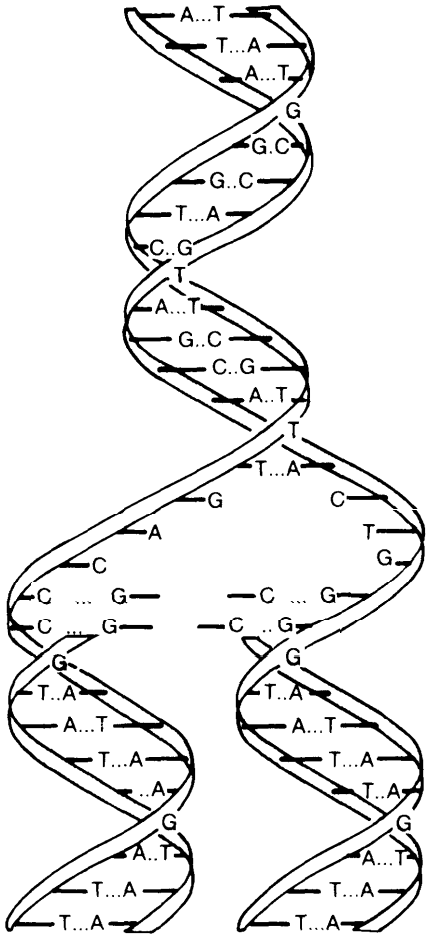
In human monogenic—i.e., single gene—disorders, the altered gene can be on any one of the 22 autosomal chromosomes or on a sex chromosome. Traditionally, modes of inheritance are classed in three basic categories:

- autosomal dominant,
- autosomal recessive, and
- X-linked.

In the past decade, several less common heredity pattems-e.g., genomic imprinting and mitochondrial inheritance-have been identified in humans and tied to specific diseases such as Fragile X syndrome and some respiratory enzyme deficiencies (55,85,104).

In an autosomal dominant disease, a single mutant allele causes the trait to be expressed, even though the corresponding allele is normal. A heterozygous individual with a mutation usually is symptomatic at some level, although many disorders, such as neurofibromatosis, vary in the severity and age of onset of the condition. Marfan syndrome, Huntington disease, and adult polycystic kidney disease are inherited in an autosomal dominant manner. Every affected individual generally has an affected parent (except for cases arising from a spontaneous, or de novo, mutation). If the affected individual has an unaffected spouse, each potential child will have a

Figure 4-3-DNA Replication



When DNA replicates, the original strands unwind and serve as templates for the building of new, complementary strands. The daughter molecules are exact copies of the parent, each daughter having one of the parent strands.

SOURCE: Office of Technology Assessment, 1992.

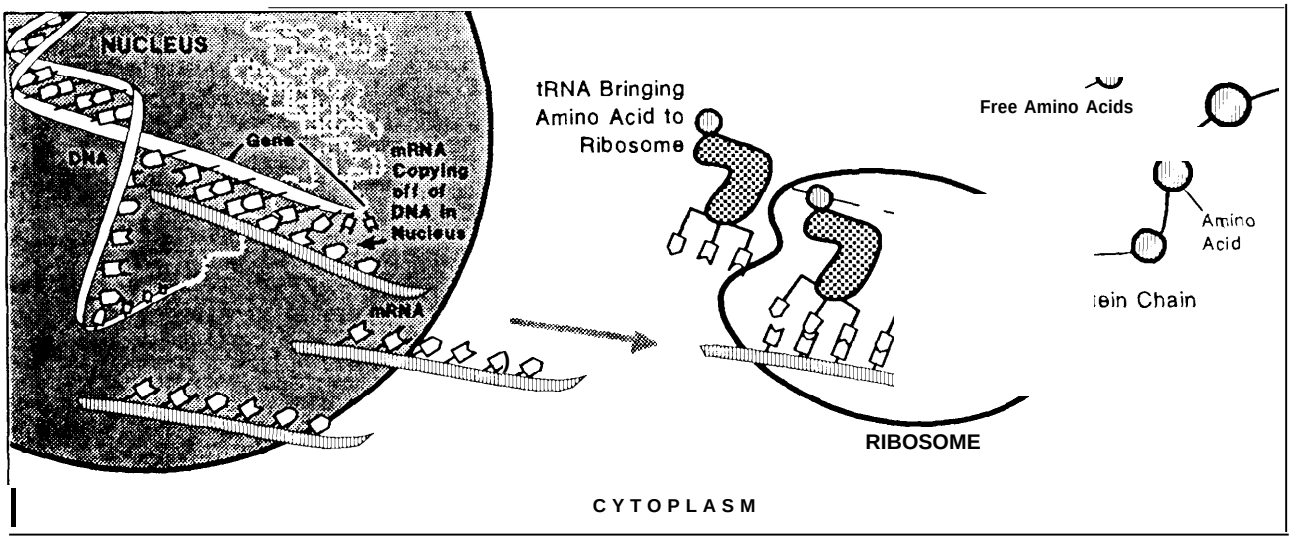
50 percent chance of inheriting the mutant allele and having the disease, and a 50 percent chance of not inheriting the mutant allele (figure 4-5). Male and female offspring are equally likely to inherit the mutation. Barring de novo mutations, children who do not receive the abnormal allele will not have the disorder and cannot pass it on to future generations.

Recessive traits result in illness only if a person has two mutant copies of the gene. That person is said to be homozygous for the gene. Heterozygous carriers of a single copy of the defective gene are usually clinically asymptomatic. CF is inherited in an autosoma1 recessive manner. Other common disorders inherited this way include sickle cell anemia, Tay-Sachs disease, phenylketonuria, and ß-thalassernia. Individuals with the disease receive one mutant allele from each parent, who are each asymptomatic carriers. When both parents are carriers of a recessive trait, each potential son or daughter has a 1 in 4 chance (25 percent) of inheriting the mutant gene from both parents, resulting in the homozygous affected state (figure 4-5). Each potential child also has a 50 percent chance of inheriting one mutation from either parent, thus being an unaffected carrier. They, in turn, can pass on the mutation to their children. Finally, each potential child of two carriers also has a 25 percent chance of inheriting the normal allele from both parents, thus being a homozygous unaffected individual who cannot pass on the mutation to future offspring.

In sex-linked disorders, the mutant gene can theoretically be on either sex chromosome, the X or the Y. In reality, the Y chromosome is small and contains few genes. To date, no known disease conditions transmit via the Y chromosome in humans. The X chromosome, however, is large and contains numerous genes for many traits that can be mutant and result in disease. Diseases caused by aberrations in genes on the X chromosome are called X-linked disorders. Duchenne muscular dystrophy and hemophilia A and B are X-linked conditions.

Genes on the X chromosome can also be dominant or recessive, but because females have two X chromosomes, and males have one X and one Y, male and female offspring show different patterns of inheritance. Sons of a carrier female-who is often asymptomatic —have a 50 percent probability of inheriting the mutant gene from their mother. These sons will be affected (figure 4-5). Sons who do not inherit the abnormal gene are unaffected and cannot transmit the gene. Daughters of a carrier mother each have a 50 percent chance of inheriting the defective gene-thus being unaffected carriers-and a 50 percent chance of not inheriting the gene and being unaffected noncarriers. A male with an X-linked recessive condition will transmit the gene to all of his daughters, who will be carriers, but to none of his sons, who will be unaffected. An X-linked dominant disease affects the mother, who can also pass it on to both her sons and daughters with a 50 percent chance. Affected males pass X-linked dominant conditions to all of their daughters, but not their sons. X-linked dominant diseases are relatively rare.

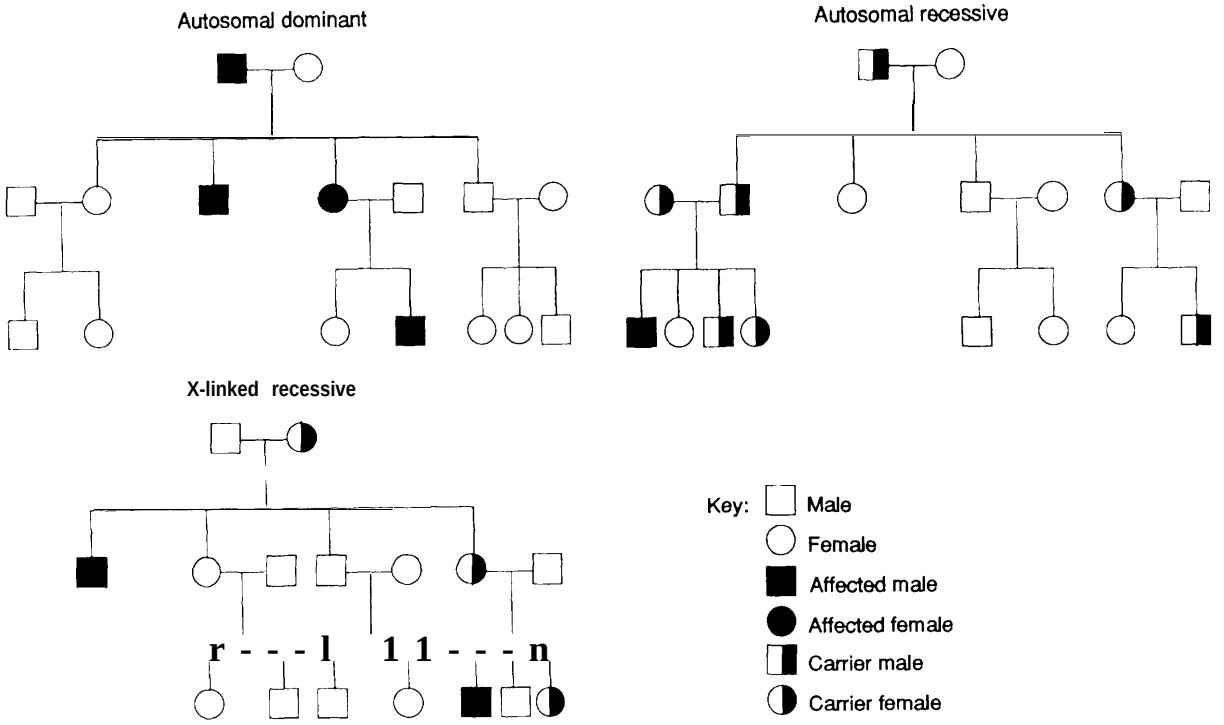
Figure 4-4-Gene Expression



In the first step of gene expression, messenger RNA (mRNA) is synthesized, or transcribed, from genes by a process somewhat similar to DNA replication. In higher organisms, this process takes place in the nucleus of a cell. In response to certain signals (e.g., association with a particular protein), sequences of DNA adjacent to, or sometimes within, genes control the synthesis of mRNA. Protein synthesis, or translation, is the second major step in gene expression. Messenger RNA molecules are known as such because they carry messages specific to each of the 20 different amino acids that make up proteins. Once synthesized, mRNAs leave the nucleus of the cell and go to another cellular compartment, the cytoplasm, where their messages are translated into the chains of amino acids that make up proteins. A single amino acid is coded by a sequence of three nucleotides in the mRNA, called a codon. The main component of the translation machinery is the ribosome—a structure composed of proteins and another class of RNAs, ribosomal RNAs. The ribosome reads the genetic code of the mRNA, while a third kind of RNA molecule, transfer RNA (tRNA), mediates protein synthesis by bringing amino acids to the ribosome for attachment to the growing amino acid chain. Transfer RNAs have three nucleotides that are complementary to the codons in the mRNA.

SOURCE: Office of Technology Assessment, 1992.

Figure 4-5-Modes of Inheritance of Single Gene Disorders



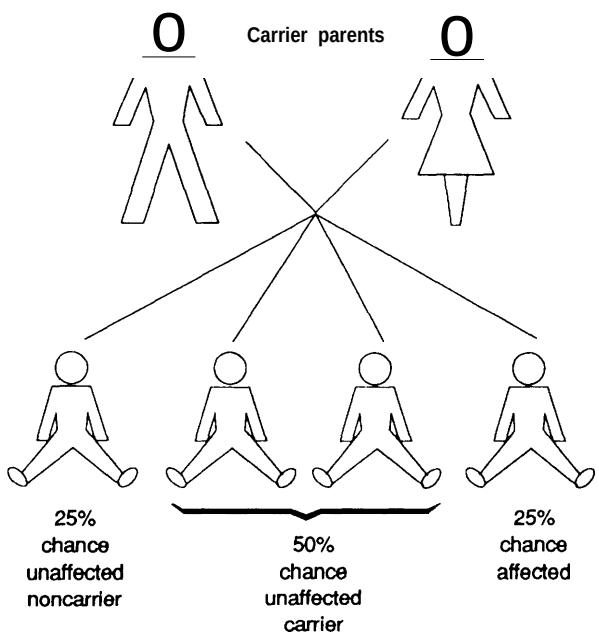
SOURCE: Office of Technology Assessment, 1992.

THE GENETICS OF CYSTIC FIBROSIS

As mentioned earlier, CF is an autosomal recessive disorder. To have CF, an individual must inherit a mutant CF gene from each parent. If a couple produces a child with CF, each parent (excluding the possibility of a new mutation, nonpatemity, or a rare genetic event called uniparental disomy) must possess one of the 170+ CF mutations, but not necessarily the same ones. Each parent, then, is an asymptornatic carrier. For these couples, the chance of having a child with CF is 1 in 4 for each pregnancy (figure 4-6). Furthermore, if two carriers have an unaffected child, there is a 2 in 3 possibility that the unaffected child is a carrier. Again, because CF is an autosomal recessive disorder, it equally affects males and females.

CF occurs in all racial and ethnic groups, although more frequently in some than in others (table 4- 1). It is the most common, life-shortening, recessive genetic disorder in Caucasians of Northern and Central European descent. In the United States, the incidence of CF in Caucasians is about 1 in 2,500 live births (17,56,81). An incidence of 1 in 2,500 implies a carrier frequency of approximately 4 percent. In other words, 1 in 25 Caucasians in the United States—about 8 million Americans—possess 1 chromosome with a CF mutation and 1 chromosome with a normal CF gene, and hence are carriers.

Figure 4-6-inheritance of Cystic Fibrosis



SOURCE: Office of Technology Assessment, 1992.

Table 4-1—incidence of Cystic Fibrosis Among Live Births in the United States

Population	Incidence (births)
Caucasian	1 in 2,500[a,b,c]
Hispanic	1 in 9,600[d]
African American	1 in 17,000[a,e] to 1 in 19,000[f]
Asian American	1 in 90,000[f]

[a]T.F. Boat, M.J. Welsh, and A.L. Beaudet, "Cystic Fibrosis," *The Metabolic Basis of Inherited Disease*, C.R. Scriver, A.L. Beaudet, W.S. Sly, et al. (eds.) (New York, NY: McGraw Hill, 1989).
[b]K.B. Hammond, S.H. Abman, R.J. Sokol, et al., "Efficacy of Statewide Neonatal Screening for Cystic Fibrosis by Assay of Trypsinogen Concentrations," *New England Journal of Medicine* 325:769-774, 1991.
[c]W.K. Lemna, G.L. Feldman, B.-S. Kerem, et al., "Mutation Analysis for Heterozygote Detection and the Prenatal Diagnosis of Cystic Fibrosis," *New England Journal of Medicine* 322:291-296, 1990.
[d]S.C. FitzSimmons, remarks at Fifth Annual North American Cystic Fibrosis Conference, Dallas, TX, October 1991.
[e]J.C. Cunningham and L.M. Taussig, *A Guide to Cystic Fibrosis for Parents and Children,* (Bethesda, MD: Cystic Fibrosis Foundation, 1989).
[f]I. MacLusky, F.J. McLaughlin, and H.R. Levinson, "Cystic Fibrosis: Part 1," *Current Problems in Pediatrics*, J.D. Lockhart (ed.) (Chicago, IL: Year Book Medical Publishers, 1985).

SOURCE: Office of Technology Assessment, 1992.

In 1989, the gene responsible for CF was isolated and precisely located among the 22 autosomes, its most common mutation identified, and its DNA sequence determined (74,101,107). Prior to this discovery, the CF gene was localized to a specific chromosom---chromosome 7-by examining markers in the area of DNA believed to surround the gene. Using a technique known as restriction fragment length polymorphism (RFLP) analysis, scientists could follow a DNA pattern of inheritance through a family. The DNA pattern itself was not the CF gene, but was close in location-r linked-to it. This method of linkage analysis enabled researchers to hone in on the exact location of the gene on chromosome 7 (77,125,126,137,143). While some of the underlying problems in CF could be studied with linkage analysis, the exact identification of the gene opened up new avenues of pursuit in understanding the nature of the biochemical defect, in elucidating possibilities for treatment and cure, and in developing assays to detect carriers of CF mutations.

The Cystic Fibrosis Gene

The CF gene is located on the long arm of chromosome 7, where it is distributed over 250,000 base pairs (250 kb) of genomic DNA (figure 4-7). At this locus, regions of the gene that code for the CF protein product are separated into 27 fragments, or exons, interspersed with portions of DNA that do not get translated-i. e., stretches, called introns, that are not decoded into proteins. During a process called transcription, introns are spliced out and the exons are pieced together into a precisely ordered string of 6,100 base pairs that codes for a protein comprised of 1,480 amino acids (74,101 ,107).

Ultimately, the CF gene product—known as the cystic fibrosis transmembrane conductance regulator (CFTR)--links DNA instructions with a critical biochemical function. When the CFTR comes from a mutant CF gene, that function is impaired and produces the medical manifestations of the disorder (box 4-A). The exact biochemical malfunction responsible for CF remains unknown, but CFTR regulates chloride ion (Cl-) conductance in affected cell types and appears to be a Cl^- channel (the structure that governs Cl^- entry and exit in the cell). Efforts to understand the underlying pathogenesis of CF, and to develop treatment for it, focus on the structure and function of the protein product (30). In particular, studies of CFTR examine its role in ion transport, the key disturbance in the disorder. One major avenue of intervention under development is gene therapy, which involves inserting DNA that codes for CFTR into cells with mutant CF alleles in order to restore physiological function (box 4-B).

Mutations in the Gene

Concomitant with the elucidation of the CF gene was the identification of a three base-pair deletion that resulted in a faulty CF gene product (i.e., a flawed CFTR). This mutation—abbreviated as DF508—results in the deletion of one amino acid, phenylalanine, at position number 508 in CFTR. Approximately 70 percent of all mutant CF genes in Caucasians in the United States and Canada exhibit this mutation (74,81). However, international studies reveal ethnic and regional variation in the distribution of this mutation (36,105). Overall, the DF508 mutation is most frequent in Northern European populations and less prevalent in persons from Southern Europe. Not surprisingly, the multicultural nature of the United States and Canada reflects this variation. The international epidemiology of the DF508 mutation is summarized in appendix A, as is the ethnic distribution of the mutation in the United States.

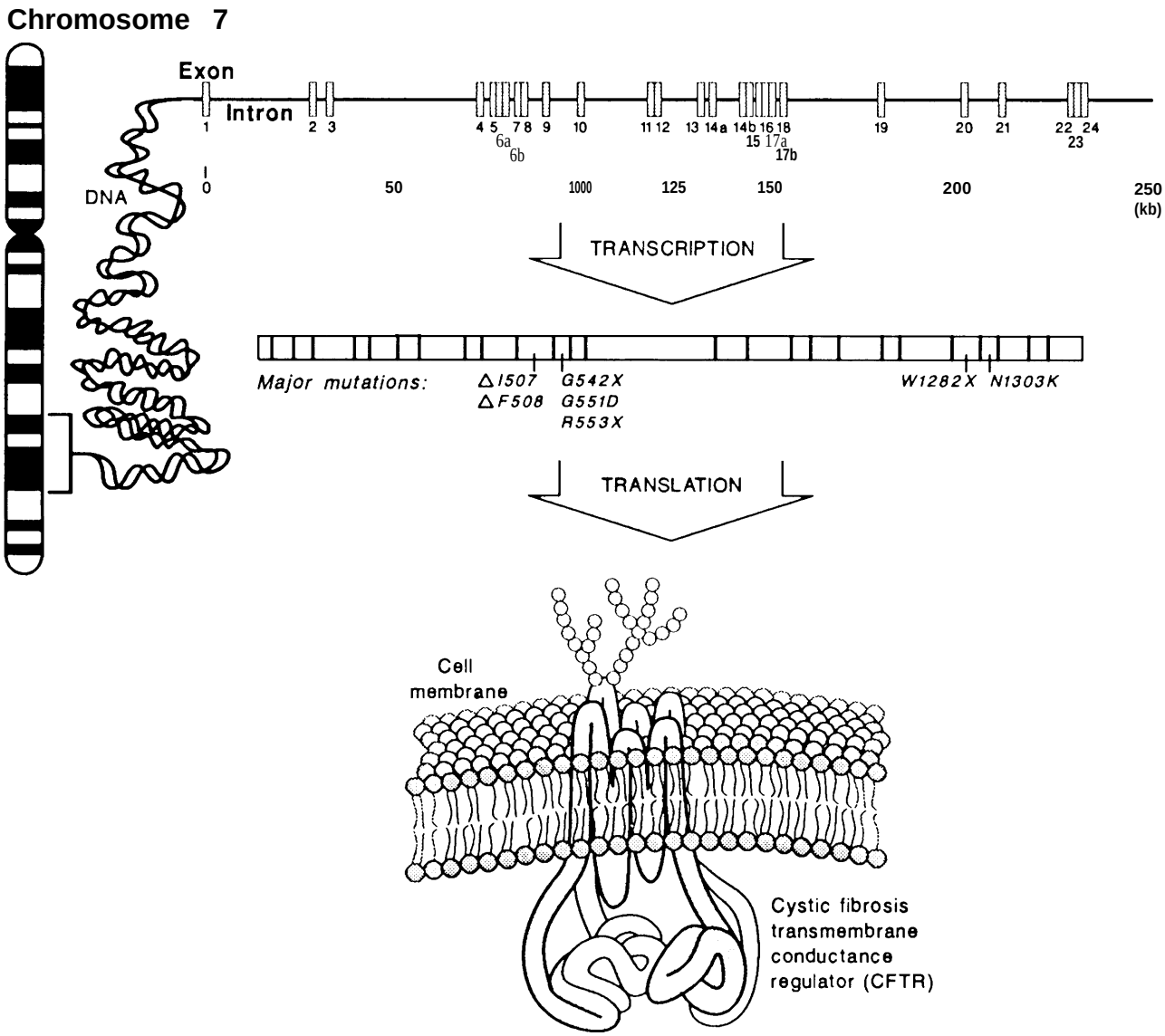
No other mutation accounts for a similarly large fraction of mutant CF genes among the remaining 30 percent of CF gene defects. To date, more than 170 additional mutations have been identified, and the number continues to grow (30). Most of these appear only in single individuals or families, although a few mutations account for 1 to 3 percent of CF mutations in the United States. The more common CF mutations and their epidemiology are also described in appendix A; some of these lesions also appear to vary among populations. Among Jewish persons of Central and Eastern European descent (Ashkenazic Jews), DF508 is relatively rare, but one mutation, W 1282X, accounts for approximately 60 percent of CF mutations in this group (1 14).

Correlation Between Genotype and Phenotype

The severity of CF symptoms differs greatly among individuals. To some extent, this *is* due to DNA differences and resultant alterations in the CFTR protein. Some mutations correlate with particular symptoms—primarily status of pancreatic enzyme sufficiency—and can be considered either mild (conferring pancreatic enzyme sufficiency) or severe (conferring pancreatic enzyme insufficiency) for this criterion (7,1 1,19,20,25,38,74,76,78,110-114,1 16,124). DF508 is considered a severe mutation, generally resulting in pancreatic insufficiency in homozygotes or in conjunction with a different severe defect in the other CF gene, although some exceptions exist (20,74,76,78,1 10-1 12,124). Other mutations appear to correlate with milder clinical outcome, but more data need to be collected (11,25,78,1 13,1 16). Pancreatic sufficient patients tend to have better respiratory function.

Pulmonary function generally does not correspond to specific mutations (74,78,1 10-1 12,124). Correlations between other mutations and particular phenotypes are also being studied (1 1,33,44,91, 99,1 19,124,145). Overall the course of the disease depends on both genetic and environmental features, and complete clinical outcome cannot be predicted on the basis of DNA analysis alone.

Figure 4-7—The Cystic Fibrosis Gene



The CF gene is located on the long arm of chromosome 7, where it is spread over 250,000 base pairs (250 kb) of DNA. Coding regions of the DNA, or exons, are separated by noncoding regions, or introns. After the DNA is transcribed into messenger RNA (mRNA) comprised of all 27 exons of the gene, the mRNA is exported from the cell nucleus. Finally, instructions in the mRNA are translated, using special structures in the cell to assemble 1,480 amino acids into the final protein product.

SOURCE: Office of Technology Assessment, 1992, based on M.C. Iannuzzi and F.S. Collins, "Reverse Genetics and Cystic Fibrosis," *American Journa/ of Respiratory Cellular and Molecular Biology 2:309-316, 1990.*

Box 4-A—The Gene Product: Cystic Fibrosis Transmembrane Conductance Regulator

Since the discovery of the principal mutation responsible for cystic fibrosis (CF), scientists have begun to relate this DNA error to the defect in ion transport long known to characterize the disorder. Cells cannot pump water, but must move fluids across their membranes through osmosis, a process that depends largely on ion movement either through pores in the membrane (channels), or transport systems designed to convey ions from one side of the membrane to the other. In CF affected individuals, regulation of chloride ion (Cl-) transport is defective (46,95,140,142). Like most cellular activities, Cl^- movement requires communication among different parts of the cell. Intricate networks of messenger systems accomplish this. Two common mediators of these systems, calcium ions (Ca^{2+}) and cyclic adenosine monophosphate (cAMP), regulate Cl^- channels in epithelial cells. In individuals with CF, channels regulated by Ca^{2+} function properly, but those channels controlled by cAMP and its intermediaries do not (95).

The product of the CF gene, a protein called the cystic fibrosis transmembrane conductance regulator (CFTR), regulates this cAMP-mediated Cl^- conductance across the epithelial membrane. With current techniques, direct observation does not reveal the structure of CFTR. Rather, increasingly detailed examination of the deficits accompanying the defect, elucidation of the effect of conferring normal activity to CF cells, and speculations about both structure and function of the molecule based on predictive modeling techniques and manipulation of the gene are slowly providing insight into CFTR's nature.

Current research suggests that CITR functions as a Cl^- channel (4,13,47,49,96,98,1 18), although it may have other fictions as well (10,22,59). On the basis of predicted structure, CFTR might belong to a large family of energy-dependent membrane transport systems that consists of several membrane-sp arming regions and segments that bind the cellular energy source, adenosine triphosphate, or ATP (2,49,58,59,65,93,100,122). Activation of CFTR is mediated by a regulatory domain (3,27,98). The DF508 mutation and numerous other mutations are located in regions that code for portions of the protein likely to be of functional importance (35,38,50,71,75,92,98).

Numerous experiments have established Cl^- channel regulation by cAMP through introduction of the normal CF gene into CFTR-defective epithelial cell lines (39,52,69,96,97,102). Moreover, Cl^- conductance was induced in nonepithelial cell lines-theoretically without previously existing Cl^- channels-when the CF gene was intre-heed, suggesting that CFTR might be a channel (4,5,72,106). Conversely, Cl^- conductance was blocked in normal cells by preventing production of the CFTR product (1 15). However, recent discoveries of new types of chloride channels in CF-affected tissues complicate a straightforward interpretation that CFTR is a chloride channel (121,135).

Scientists are attempting to correlate symptoms of CF with a single molecular cause. CFTR has been demonstrated in all organs affected by the condition (123). One hypothesis to explain the multitude of CF traits attributes multiple symptoms to improper modification of a host of non-CFTR proteins due to decreased Cl- permeability in cells of CF patients (8,141).

As the workings of CFTR are better understood, new avenues for treatment open. If the underlying causes of the disease are understood therapies can be directed at correcting the molecular deficits. For example, administering large quantities of cAMP analogs elicits Cl^- conductance in cells with mutant CFTR (37,40,144). This suggests cAMP analogs might be an effective pharmacological intervention.

Elucidation of the structure and function of the CFTR protein could facilitate a means of assaying for CF mutations that would not require DNA analysis and the need to examine multiple mutations. Such a functional test theoretically would measure either the presence, absence, or altered state of the protein product. A functional test might, for example, evaluate Cl^- conductance. Indications that Cl^- conductance can be induced in cells with CF mutations, but with a recognizably different pattern than normal cells, might enable such a test (37,40). This pattern, however, appears to differ among cell types and methods of measurement (4,144). Likewise, understanding the extent to which protein processing is affected remains elusive (26,37,40,147). Although knowledge about the nature of CFTR and its structure and fiction continues to advance rapidly, answers that would render feasible a functional test for CF carriers are lacking. It might be, for example, that the more than 170 different CF mutations lead to a range of activities at the cellular level.

SOURCE: Office of Technology Assessment, 1992.

Box 4-B-Gene Therapy and Cystic Fibrosis

Recent advances have moved gene therapy from theory to clinical and therapeutic experimental application (6,57). Protocols for gene therapy in humans must be approved by the Recombinant DNA Advisory Committee (RAC) of the National Institutes of Health (NIH), the NIH director, and the Food and Drug Administration in what can be a lengthy process, although the procedure has been streamlined (48). The first human gene therapy clinical trial was approved by the RAC and the NIH director in July 1990. By June 1992, nine other protocols for human gene therapy were in various stages of approval (146). For gene transfer---experiments that mark cells to trace the course of a treatment or the disease but are not therapetic--- additional 15 protocols were in various stages of approval (146). Current human gene therapy trials include alteration of:

- white blood cells to treat a rare genetic disorder, severe combined immune deficiency due to adenosine deaminase deficiency, begun in September 1990 (84);
- immune system cells to produce an anticancer agent, begun in January **1991 (21,62); and**
- liver cells to correct hypercholesterolemia, a genetic disorder of fatal cholesterol buildup, approved in October 1991, begun in June 1992 (56 FR 58800; 146).

The theory of gene therapy is straightforward: The normal gene is inserted into the cellular DNA either to code for a functioning protein product, or, in the case of cancer thempies, to confer disease-fighting properties. Experimentally, delivering normal genes into desired cells can be accomplished through physical or chemical means that disturb the cell membrane and allow DNA to enter, including specially modified viruses, liposomes (fatty materials able to transport drugs directly into cells), and direct injection (43,136). Somatic cell therapy-the only approach approved for human trials---changes only the DNA of the person receiving the therapy and cannot be inherited by offspring. In contrast, germ line gene therapy would alter the genetic material that is passed onto future generations. To date, no germ line therapy in humans has been proposed. For most conditions, cells are removed from the patient, genetically altered, and replaced.

Correction of abnormal Cl^- transport through insertion of the normal CF gene into defective cells suggested that gene therapy was a viable consideration for treating CF (39,52,97,102); currently, the respiratory deficits of the disease are being targeted for correction by gene therapy. Unlike the RAC-approved protocols in progress, however, lung cells are generally inaccessible for removal and redelivery after gene transfer, making other means of administering the DNA necessary. Several systems are under investigation for efficacy of delivery in vivo without side effects. In one system, DNA is removed from an adenovirus, the type of virus responsible for some forms of the common cold and other respiratory ailments, and the inactivated virus shell is used as a vector to deliver the CF gene directly into the lungs of rats (108,109). The CF gene has also been delivered into cells isolated from the lungs of CF patients by bronchial brushing (32). In vivo and in vitro, significant amounts of messenger RNA for the CF gene are still present 6 weeks later, suggesting that long-term expression of the gene will be feasible. It is not yet known, however, how frequently new doses of the CF gene would have to be administered (32,109). An alternative delivery mechanism, aerosolized liposomes, has been used to deliver alpha- 1-antitrypsin genes into rabbit lungs (61). A s imilar system might be applicable to delivery of the CF gene to human lungs.

Many questions about the safety and efficacy of gene therapy for CF must be answered before it will be suitable for human trials (30,32,138,146). Scientists do not yet know how much corrected protein product is needed to restore normal function to a patient with CF. Neither do they know whether adverse health effects will result from placing too much CFTR in a patient. Further, even though the virus has been fully debilitated in theory, using a viral vector raises concerns about expression of contaminating normal virus. Crippled viruses could also join with genetic material already in the cell and allow expression of a new virus or activate cells to a cancerous state.

Ethical considerations are also raised by some (6). Because gene therapy involves altering the genetic makeup of an individual, some express concerns about eugenic overtones, although only somatic cell therapy is under consideration. The general public, however, is enthusiastic. A 1986 OTA survey found that 83 percent of the American public approved of human cell manipulation to cure usually fatal diseases, 78 percent would be willing to undergo gene therapy personally to correct a genetic proclivity to a serious or fatal disease, and 86 percent of respondents would be willing to have his or her child undergo gene therapy for a usually fatal disease (131).

Gene therapy clearly offers the promise of treatment for some disorders. On the other hand, heightened attention to genetics in general-and gene therapy in particular—in the popular press can raise false hopes for cures for diseases long before they will be feasible or readily available (53,70). For CF, critical steps have been made towards the first attempt at gene therapy in humans; clinical applications, however, are still on the horizon.

SOURCE: Office of Technology Assessment, 1992.

TESTS FOR CYSTIC FIBROSIS MUTATIONS

Localization of the CF gene and determination of its sequence enabled direct analysis of DNA for the presence of CF mutations. However, as mentioned in chapter 2, carrier screening is hindered by the multitude of mutations, particularly those too rare to be used practically in a CF carrier screening panel. Moreover, not all CF mutations have been discovered. As additional mutations are elucidated and incorporated into carrier screening protocols, the detection limitation decreases incrementally, although the inability to detect all mutations remains. This section explains the process and limits of direct DNA screening as applied to CF.

Techniques Used in Direct DNA Analysis

Multiple techniques are used to analyze DNA. Four principal processes are employed, though each technique is not performed on all samples. These methods, depicted in figure 4-8, are:

- DNA amplification;
- restriction enzyme digestion;
- gel electrophoresis; and
- Southern transfer, dot-blotting, and probe hybridization.

DNA Amplification

DNA amplification increases the amount of DNA to be analyzed by making copies of the original sequence from the sample. A process call the polymerase chain reaction (PCR) is typically used (box 4-C). Using PCR, selected areas of a gene can be amplified through repeated cycles to yield large quantities of the sample for rapid diagnosis.

While PCR amplifies a stretch of DNA between two primers, a new technique known as ligase chain reaction (LCR) amplifies only the region of DNA directly beneath the known sequence. Like PCR, millions of copies of the original sequence are made, but LCR'S advantage is that it lends itself easily to detecting mutations differing by even a single base (82, 139)---although it is not yet practical for general use.

Restriction Enzyme Digestion

Restriction enzymes act as molecular scissors, cutting the DNA into fragments at specific sequences. Different enzymes recognize and cut different sequences. Mutations in **a** gene alter the DNA sequence and sometimes create or destroy specific sequences known as restriction sites. Thus, when changes occur in the DNA, restriction enzyme digestion can yield different sizes of DNA fragments in samples taken from an affected versus an unaffected individual. To distinguish between alleles, a restriction enzyme site can be intentionally created if a mutation has not created one (45,54).

Gel Electrophoresis

In gel electrophoresis, PCR-derived or restriction enzyme digested DNA is separated into its different sized fragments. The sample is placed in a semisolid matrix, called a gel, and exposed to an electric field. In response to the electrical field, the DNA migrates toward one edge of the gel. In doing so, the gel acts as a sieve, with large DNA fragments passing through the gradient more slowly than small ones, allowing the DNA mixture to be separated according to size.

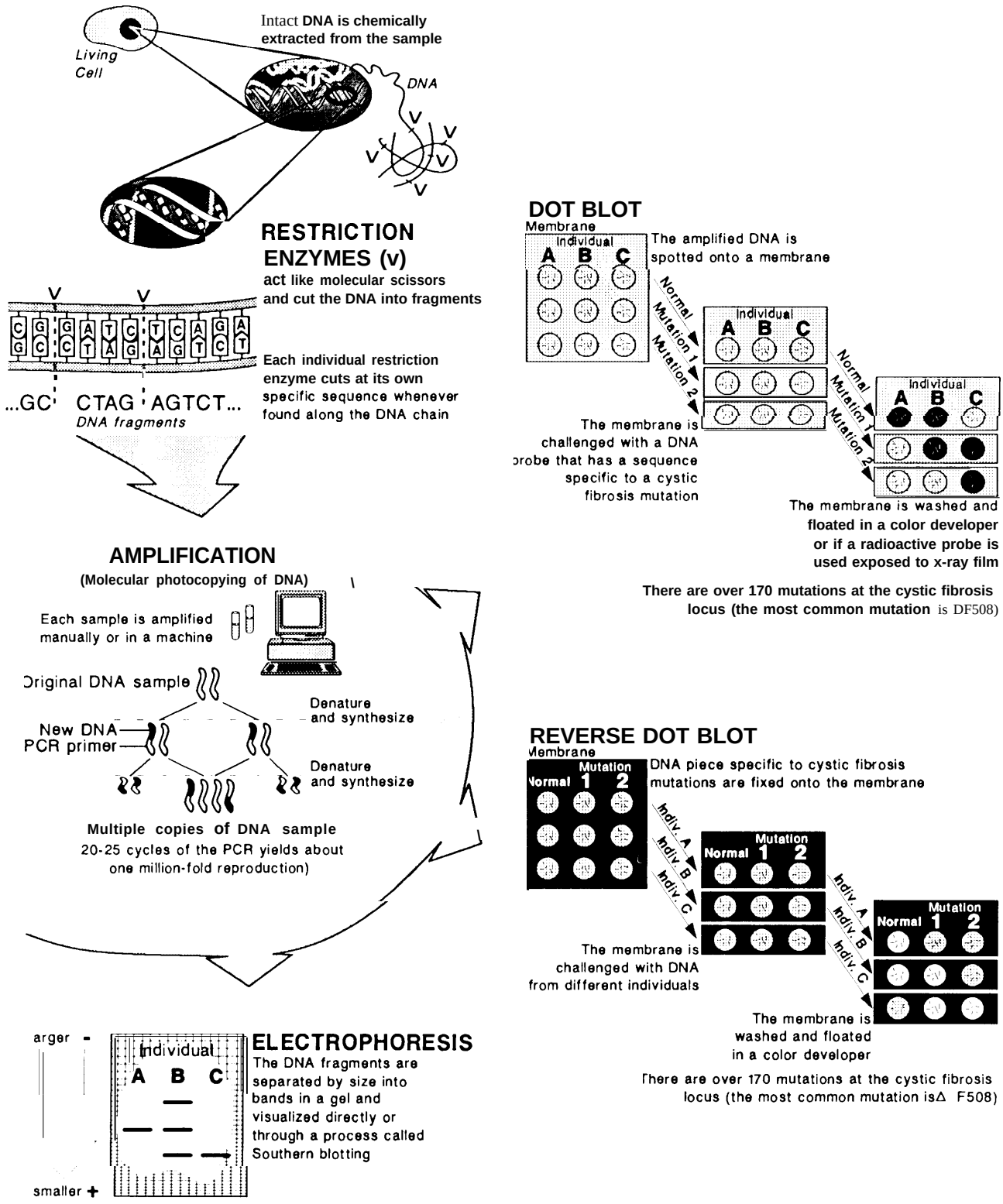
After gel electrophoresis is performed, the fragments can either be viewed directly using ultraviolet light plus a dye called ethidium bromide, or transferred onto a membrane for analysis with specific DNA probes (deseribed in the following section).

Southern Transfer, Dot Blotting, and Probe Hybridization

DNA fragments from gel electrophoresis can be transferred to a nylon or other type of membrane, forming a "Southern blot. " A specitic probe---or short sequence of single-stranded DNA complementary to the DNA sequence being sought--can be washed onto a membrane, where it will bind to complementary sequences on the membrane. Although most probes are generic and identify all alleles of a gene, allele specific oligonucleotide (ASO) probes refine diagnostic accuracy by perfectly matching the nucleotide sequence of a portion of the gene in question (24). Sequences differing by only one base can be detected. Before use, the probe is labeled with a fluorescent or radioactive marker so the region of DNA binding to the probe can be detected. The hybridized molecules can be viewed either by exposure to x-ray film for a radioactive probe or by other methods, such as colorimetric dyes.

A variation of Southern transfer, called dot blotting, involves directly spotting DNA into discrete spots on the nylon membrane. A probe, or

Figure 4-8-Techniques for DNA Analysis of Cystic Fibrosis Mutations



SOURCE: Office of Technology Assessment, 1992.

Box 4-C—The Polymerase Chain Reaction: Step-by-Step

For the most part, all body cells within an individual contain the same DNA. Thus, DNA molecules in cells must regenerate copies of themselves each time a cell divides; DNA reproduces through a process called replication. During replication, the original strands in the DNA double helix unwind and serve as templates for the building of new, complementary strands, resulting in two identical copies of the original DNA molecule.

The polymerase chain reaction (PCR) is an in vitro technology based on the principles of replication. First described in 1985, PCR is now widely performed in research and clinical laboratories, and it is a critical technology for DNA diagnostics.

PCR involves the repeated duplication of a specific area of DNA to increase the amount of that DNA available to be used for research or test purposes. For example, consider the following sequence of double-stranded DNA to be amplified using PCR:

```
                                        [-G-G-C-T]
 -T-T-C-G-A-T-G-G-A-T-A-A-A-C-C-G-A
 -A-A-G-C-T-A-  C-C-T-A-T-T-T-G-G-C-T
[-T-T-C-G-]
```

In order to perform PCR, the sequences of the DNA at both ends of the region of interest must be known, and their complementary sequences available as short pieces of purified DNA called primers. One primer must be complementary to the end of one strand, the second to the opposite end of the other strand as indicated in the diagram above. These two specific sequences flank the area the scientist wants to copy, and serve as the foundation to which bases can be added and the DNA strand copied.

In PCR, the temperature of the solution containing the DNA to be amplified is raised to about 95 'C, which results in the separation, or melting, of the double helix to yield single-stranded pieces:

```
-T-T-C-G-A-T- G-G-A-T-A-A-A-C- C-G-A-

-A-A-G-C-T-A-C-C-T-A-T-T-T-G-G-C-T-
```

Copies of the primers are then added to the solution and allowed to hybridize to the DNA of interest, by lowering the temperature:

```
-T-T-C-G-A-T-G-G-A-T-A-A-A-C-C-G-A-
                          ←[-G-G-C-T]

[-T-T-C-G-]→
 -A-A-G-C-T-A-C-C-T-A-T-T-T-G-G-C-T-
```

The scientist then sets conditions in the reaction that allow new copies of the DNA of interest to be synthesized from the ends of the primers (referred to as primer elongation). That is, DNA polymerase (a heat-stable version of an enzyme from *Thermus aquaticus,* a thermophilic microorganism isolated from a hot spring in Yellowstone National Park) starts at the end of the primers and, using bases (G, A,T,C) that are part of the reaction mixture, synthesizes complementary strands of each of the two single strands to yield two strands from the original one:

```
 -T-T-C-G-A-T-   G-G-A-T-A-A-A-C-C-G-A
 -A-A-G-C-T-A-C- C-T-A-T-T-T-G-G-C-T---

---T-T-C-G-A-T-G-  G-A-T-A-A-A-C-C-G-A-
   -A-A-G-C-T-A-C-C-T-A-T-T-T-G-G-C-T-
```

Thus one cycle of PCR occurs, doubling the number of DNA copies from the original area of interest. After this frost round of synthesis, and for each subsequent cycle, the temperature of the reaction is raised to approximately 95 °C to separate the DNA strands. Primers are again allowed to hybridize to the strands, and DNA synthesis allowed to occur. After a second cycle of PCR, the two strands become four, and after 20 to 25 cycles of PCR, the original DNA area of interest has been amplified about a millionfold.

SOURCE: Office of Techology Assessment, 1992.

probes, can be hybridized to the membrane and diagnosis made rapidly (42). Reverse dot blotting is similar in principle to Southern transfer and dot blotting. However, in reverse dot blotting, the unique ASOs (which would be used as probes in conventional Southern analysis or dot blotting) are immobilized on the membrane. Key segments of the individual's uncharacterized DNA are then amplified, labeled, and hybridized to the probes on the membrane.

Carrier Assays for Cystic Fibrosis

CF carrier tests involve direct DNA analysis, and all laboratories currently performing them directly analyze the gene. In individuals with a family history of CF or an affected child, an indirect assay-ailed linkage analysis-sometimes can be used to obtain additional information in the event of a negative test result. The following sections describe the techniques used to detect CF mutations.

Direct Analysis of the Cystic Fibrosis Gene

DNA is generally obtained from white blood cells in blood samples, although it can be obtained from almost all nucleated cells in the body. Some groups in the United Kingdom and one project in the United States use buccal cells from mouthwash samples (ch. 10). In 1992, most laboratories in the United States assay DNA samples for the DF508 mutation and 6 to 12 other common mutations (DF508+6-12). Some laboratories screen for additional mutations.

After DNA is extracted from a blood sample, key segments containing mutations are amplified with PCR. When multiple segments are arnplified at once, it is referred to as multiplexing. For some of the mutations, the amplified DNA can be electrophoresed and the migration pattern-specific to each mutation—visualized directly on the gel. Digestion with restriction enzymes followed by gel electrophoresis and visualization, or blotting followed by ASO hybridization, can also be used to analyze the mutations.

Reverse dot blotting using ASOs can be used to simultaneously analyze multiple CF mutations, and test kits that do so are under development in the United States (42) (figure 4-9).

The Limits of DNA-Based Tests

DNA analysis for CF is limited by the diversity of mutations and the variation in frequency among different racial and ethnic groups (34). At present, DF508+6-12 other mutations account for approximately 85 to 95 percent of all mutant CF genes in Caucasians, depending on ethnicity (14,89), The range in detection rates presented by different commercial vendors likely results from their using tests that detect different mutations and assaying different populations (29,66).

The presence of a specific mutation establishes CF carrier status; a negative test, however, does not preclude carrier status, since not all mutations are known or assayed. About 5 to 15 percent of carriers remain undetected because their mutation is not included in the assay.

Linkage Analysis

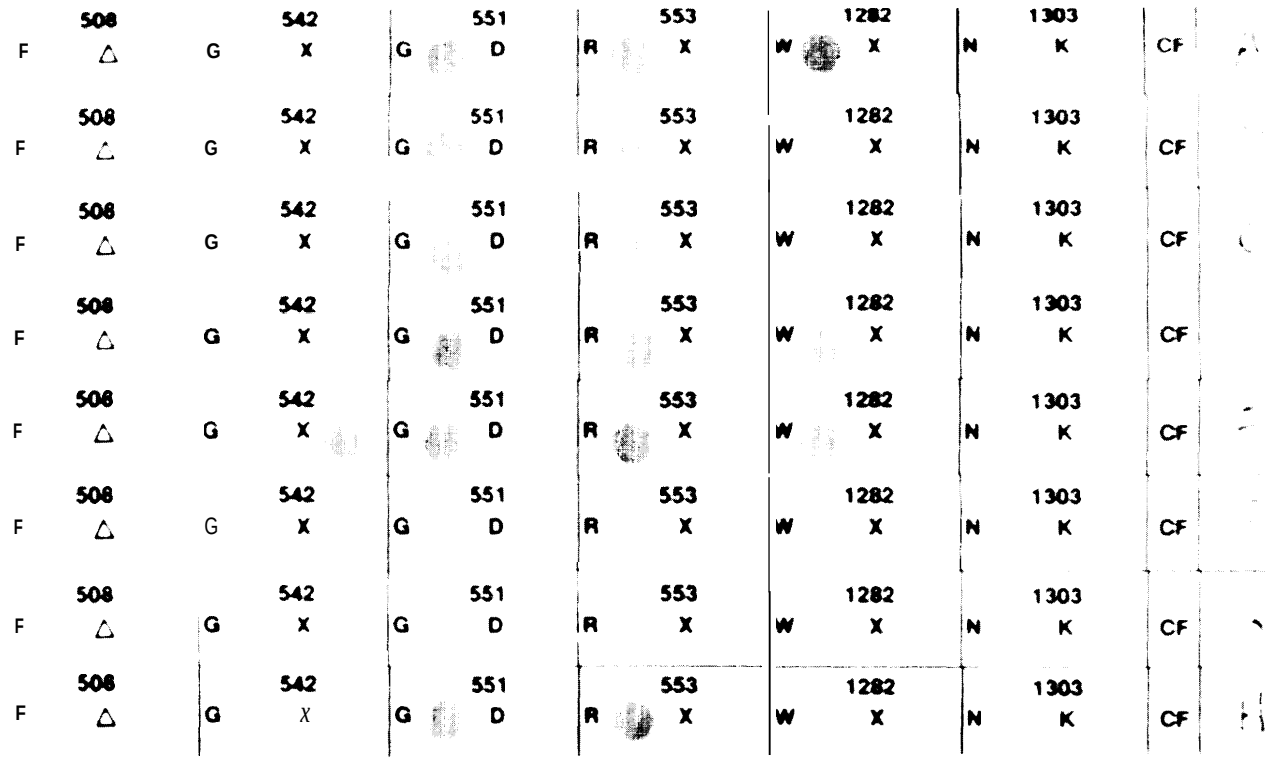
In some cases where one partner is a known carrier and the other does not test positive for the most common mutations, linkage analysis can be used to gather more information about an individual's risk. Linkage analysis can only be performed in families with living members with CF or on DNA samples stored from a deceased individual; frequently, such samples are unavailable. In this procedure, DNA markers are studied to trace the transmission pattern in a particular family of the CF gene and a specific mutation. For families with the necessary DNA samples, linkage analysis is generally informative. In rare instances, recombination events, which can alter marker patterns, can lead to erroneous conclusions from linkage results.

Automation of DNA Diagnostic Procedures

The ability to test quickly and accurately will be crucial to widespread, inexpensive CF carrier screening, particularly if batteries of genetic assays are to be developed: Automation will be key. At present, the Human Genome Project serves as the primary impetus for automating DNA analysis, and the National Laboratories engaged in this aspect of the project are at the forefront in developing these technologies (1,5 1,64,103). As such, most advances in automated technology are specific to DNA sequencing, not diagnosis. Facets of the two processes overlap considerably, however, and some sequencing technologies can be made directly applicable to, or adapted for, DNA diagnosis.

Over the past few years, a number of instruments have been developed to increase the speed and volume of routine DNA diagnostic procedures (79).

Figure 4-9—Reverse Dot Blot Analysis for Six Common Mutations



Allele specific oligonucleotide probes are bound to the test strip to detect six common CF mutations; in this photograph, each individual strip runs horizontally. DNA samples from individuals of unknown CF status are PC R-amplified and hybridized to separate test strips. Here, test strips for eight different individuals are shown (rows A through H). Following hybridization and colorimetricanalysis, the patterns of dots on **the** strips are revealed-and hence the CF status of the individuals.

For each mutation on the strip (DF508, G542X, G551D, R553X, WI 282X, and N1303K) the left dot, if present, indicates the person has a normal DNA sequence at t hat part of the CF gene. The right dot, if present, indicates the person has a CF mutation at that site. Individual A, then, has no CF mutations at the loci tested, as demonstrated by single dots on the left side for all mutations. In contrast, individuals B,D, F, and H are carriers, as demonstrated by the presence of two dots for one of the CF mutations. Individual C has CF, as demonstrated by a single dot on the right side of the DF508 panel; individual E has CF, as demonstrated by the single dot on the right side of the G542X panel. Individual G also has CF, but this person's CF arises from two different mutations—DF508 and R553X—as indicated by the pairs of dots in each of these panels.

SOURCE: Roche Molecular Systems, Inc., Emeryville, CA, 1992.

To the extent that the Human Genome Project is federally funded, integration of existing technologies specifically for clinical diagnostics will depend on Federal research priorities. Given the state of already automated procedures used in diagnosis and the rapid development of new DNA technologies under the auspices of the Human Genome Project, DNA automation is advancing at a pace that could realize entirely automated DNA diagnosis in the next few years.

Already, a nonelectrophoretic method of mutation detection has been automated and applied to detection of DF508 (90). Most DNA diagnostic techniques, however, still depend on electrophoresis, especially in the early stages of gene identification. Currently, instrumentation for such DNA diagnosis, except probe hybridization to a membrane, are automated or being automated. At a minimum, these steps include:

- DNA isolation from source material (e.g., blood, mucosal, or buccal cells),
- DNA amplification,
- gel preparation and loading for electrophoresis, and
- Visualization and interpretation of results.

Automated instruments for DNA extraction, gene amplification by PCR, sequence gel loading, and visualization of sequence gel results already exist

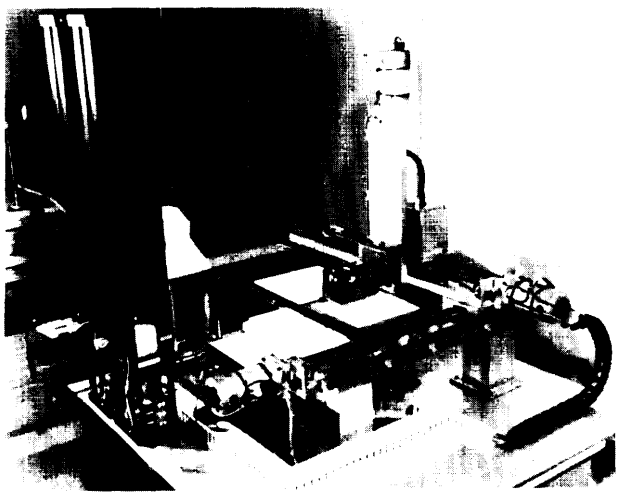


Photo credit: Tony J. Beugeisdijk, Los Alamos National Laboratory

Automated robotic system used in DNA analysis at Los Alarms National Laboratory.

(42,51,64,79,103). While not faster than humans, these machines are designed to standardize procedures and decrease human error. They are not, however, always reliable (18,73,103). At Lawrence Berkeley Laboratory, scientists are creating separate robotic systems to allow simultaneous amplification and analysis of 96 different samples (94,127). At Los Alamos National Laboratory, a robotic system can produce high-density falters that increase the amount of information on one 96-sample filter by a factor of 16-i.e., 1,536 results can be obtained from one filter (15). To visualize and interpret results, scientists have designed an image plate reader that eliminates x-ray film (51,94,127).

Many new techniques for automated DNA analyses have been proposed, although at present they are not viable for large-scale clinical application (51,60, 64). One new procedure, DNA sequencing by hybridization, involves coating a DNA chip with short pieces of known DNA to which DNA of unknown sequence can be hybridized. Based on the hybridization pattern, mathematical algorithms can be used to decode the unknown sequence (923,51,64). A technique known as flow cytometry sequences DNA one molecule at a time by identifying individual, consecutive bases through base-specilic fluorescence (67,68,83). Several other approaches to increasing sequencing speed are under development, including mass spectroscopy, atomic probe microscopy, and x-ray diffraction (28,51 ,64,79,103). As DNA sequencing becomes more rapid and less expensive, the prospect of determining CF (and other) mutations by direct DNA sequence analysis might someday be realized (86,87).

At present, most components of DNA analysis necessary for DNA-based genetic tests are automated as individual units, though some scientists are working on coordinating sequential steps into one system (15,94,127). In Japan, HUGA-1, a fully integrated robotic system that automates DNA sequencing from purification of DNA to interpretation of the final sequence, was to begin operating in April 1992 (41,64,1 17). Research into automation is also being carried out in Europe (63,120).

Clearly, crucial steps in DNA-based carrier screening assays for CF are automated now in research settings: DNA extraction, amplification, gel preparation, loading for electrophoresis, and visualization and interpretation of results. Only the step where a probe is hybridized to the membrane remains to be automated. The availability of high density filters means numerous DNA samples can be stored and analyzed concurrently, suggesting improved methods for handling the quantity of samples in high-volume screening. While currently the procedures are automated as single units, integrated robotic systems are being developed. Taken together, these advances in instrumentation indicate that automating the components of rapid carrier screening for CF is already technologically feasible, although an integrated system incorporating all of the processes has yet to be created.

RESEARCH FUNDING

Federal finding for CF research is principally through the National Institutes of Health (NIH). Unlike most biomedical research, private-sector funds, which are made available through grants by the Cystic Fibrosis Foundation (CFF) and direct conduct of research by Howard Hughes Medical Institute (HHMI), account for a large portion of CF-related research. Some investigators receive funding through one or more of these sources. All three sources fund basic biomedical research. NIH and CFF support specific projects, while HHMI awards salaries and research support to individual investigators.

Federal Efforts

In fiscal year 1991, NIH allocated $46,937,000 for all research related to CF. Of that, $20,662,724 was provided for intramural research. Extramurally,

$19,753,956 was granted to investigators for whom CF is the primary focus of laboratory or clinical research and an additional $6,520,320 to those for whom CF research is a secondary component. Nine of 12 institutes of the NIH and 3 of 4 research centers apportion these monies. In fiscal year 1991, two institutes-the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and the National Heart, Lung, and Blood Institute (NHLBI)--accounted for 73 percent of Federal funding for CF-related research: NIDDK disbursed $10,226,070 (39 percent) and NHLBI disbursed $8,847,051 (34 percent). One institute of the Alcohol, Drug Abuse, and Mental Health Administration provided $66,236 in fiscal year 1991 for CF-related research as a secondary research focus. Table 4-2 lists funding by institute or center. The U.S. Department of Energy (DOE) appropriated $48 million in fiscal year 1991 and $59 million in fiscal year 1992 for the human genome initiative. Indirectly, technology development---e.g., in the form of automation of DNA diagnostic technologies-horn DOE's funding of the Human Genome Project could affect DNA diagnostics if it is determined to be a program priority.

Beginning in fiscal year 1991, eight coordinated CF carrier screening pilot studies were funded by NIH at a total of $1,657,086 for the first fiscal year and $4,442,568 over a 3-year period (80). Six of these NIH pilots were funded by the National Center for Human Genome Research's Ethical, Legal, and Social Issues (ELSI) program (ch. 2), one by the National Center for Nursing Research, and one by the National Institute of Child Health and Human Development, although all eight are considered a single consortium (ch. 6). ELSI alone targeted $1,340,963 in fiscal year 1991 and $3,200,178 through the end of fiscal year 1993 for these pilots (80).

Private Efforts

The Cystic Fibrosis Foundation is a nonprofit organization whose mission is to further research, medical care, public policy, and education for CF. It dedicates 33 percent of its annual budget to research towards a cure. CFF maintains research centers at medical schools and universities throughout the United States and provides grants to individual researchers. For calendar years 1991 and 1992, CFF earmarked $20 million per year for biomedical research, nearly equivalent to NIH's extramural

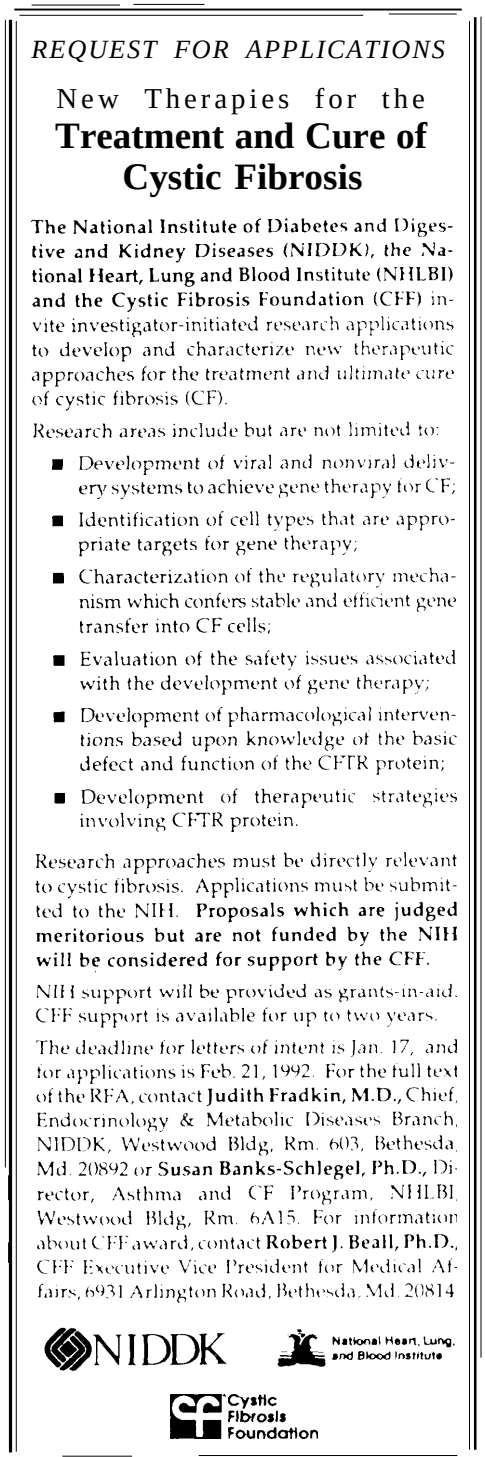


Photo *credit: Cystic Fibrosis* **Foundation**

Advertisement seeking grant proposals for CF-related research. The Cystic Fibrosis Foundation funds proposals that are deemed meritorious by the National Institutes of Health peer review panels, but that do not receive support due to lack of funds.

Table 4-2—Federal Extramural Funding for Cystic Fibrosis Research, Fiscal Year 1991

Institute or center[a]	CF-related research funding (dollars)	Number of projects	Total budget (dollars)	Percent of budget for CF-related research
NIDDK	8,413,847[b]	66		
	1,812,223[c]	12		
	10,226,070[d]	78	615,300,000	1.7
NHLBI	7,620,247	45		
	1,226,804	9		
	8,847,051	54	1,126,900,000	0.8
NIAID	1,106,789	6		
	753,823	3		
	1,860,612	9	907,300,000	0.2
NCHGR	1,340,963	6		
	449,074	3		
	1,790,037	9	87,400,000	2.0
NCRR	773,673	37		
	208,347	3		
	982,020	40	335,800,000	0.3
NICHD	361,381	2		
	254,018	1		
	615,399	3	479,000,000	0.1
NIDR	0	0		
	558,351	4		
	558,351	4	148,900,000	0.4
NEI	0	0		
	478,044	2		
	478,044	2	253,200,000	0.2
NIGMS	0	0		
	330,200	3		
	330,200	3	760,000,000	0.04
NCNR	274,110	1		
	0	0		
	274,110	1	39,900,000	0.7
NIAMS	0	0		
	220,862	1		
	220,862	1	193,200,000	0.1
NINDS	0	0		
	162,338	1		
	162,338	1	541,700,000	0.03
NIH	19,753,956	163		
	6,520,320	43		
	26,274,276	206	8,276,700,000	0.3
ADAMHA				
NIMH	0	0		
	66,236	1		
	66,236	1	622,714,000	0.01

a Institute and Center abbreviations refer, in order, to the following: NIDDK, National Institute of Diabetes and Digestive and Kidney Diseases; NHLBI, National Heart, Lung, and Blood Institute; NIAID, National Institute of Allergy and Infectious Diseases; NCHGR, National Center for Human Genome Research; NCRR, National Center for Research Resources; NICHD, National Institute of Child Health and Human Development; NIDR, National Institute of Dental Research; NEI, National Eye Institute; NIGMS, National Institute of General Medical Sciences; NCNR, National Center for Nursing Research; NIAMS, National Institute of Arthritis and Musculoskeletal and Skin Diseases; NINDS, National Institute of Neurological Disorders and Stroke; NIH, National Institutes of Health; ADAMHA, Alcohol, Drug Abuse, and Mental Health Administration; NIMH, National Institute of Mental Health.
b CF-related research is primary focus of grant.
c CF-related research is secondary focus of grant.
d Total CF-related research (primary plus secondary).

SOURCE: Office of Technology Assessment, 1992.

funding level (12). Approximately 30 to 40 percent of the research budget supports studies in gene therapy, and the balance is spent on research into the pathophysiological basis of the disease and other therapies (12). In collaboration with NIH, CFF funds projects deemed meritorious by the NIH peer review process but not supported because of lack of funds. A total of $2 million in calendar year 1991 and $3.6 million in calendar year 1992 has been designated under this program (12). CFF does not support research into carrier screening.

HEMI is a philanthropic organization that sponsors biomedical research through support of individual investigators. For fiscal year 1992, $68.9 million, or 34 percent of its research budget, has been dedicated towards this purpose (16). No breakdown was available for the amount of spending specifically for CF, but six HHMI investigators who carry out such research as all or part of their activities receive a total of $5,743,093 to cover salary, research materials, and all operative costs (16).

SUMMARY AND CONCLUSIONS

The 1989 isolation of the CF gene-and a single mutation responsible for about 70 percent of mutations in CF patients and families-opened new possibilities for understanding the basic defect, finding a cure, and testing and screening for carriers. Since then, more than 170 mutations of the CF gene have been identified. Approximately 13 mutations account for 85 to 95 percent of CF mutations in the United States in Caucasians.

One hallmark of CF—its varied symptoms and severity-sometimes correlates with differences in genetic mutations. Pancreatic insufficiency, for example, appears to be associated with the most common mutation (DF508), although other aspects of the disease have not yet been shown to correspond to specific mutations.

One outgrowth of identifying the CF gene has been the ability to directly analyze DNA to detect carriers of the condition. Although the presence of multitudinous mutations that vary in frequency among ethnic and racial groups confounds screening and DNA diagnosis on clinical, ethical, and legal levels, new technologies promise to surmount at least some of the technical difficulties. Coordinating existing automation and developing new automated techniques would facilitate rapid, large-scale detection of CF mutations. DNA automation is advancing at a pace that would enable entirely automated DNA diagnosis to be realized in the next few years, if this should be deemed a priority.

Another significant research result has been elucidation and understanding of the CF gene product, cystic fibrosis transmembrane conductance regulator, which could lead to improved diagnosis and treatment. As research in the underlying mechanism of CF progresses, new molecular-based treatments could further improve the health and quality of life of affected individuals. Future therapies, for example, might be targeted at correcting the deficits in Cl^- flow or overcoming the defects in the gene through gene therapy.

CHAPTER 4 REFERENCES

1. American Society of Human Genetics, Human Genome Committee Project Report, "The Human Genome Project: Implications for Human Genetics," *American Journal of Human Genetics L19:687-691, 1991.*
2. *Ames,* G.F.-L., Mimura, C. S., and Shyamala, V., "Bacterial Periplasmic Permeases Belong to a Family of Transport Proteins Operating From *Escherichia coli* to Human: Traffic ATPases,'' *FEMS Microbiology Reviews* 75:429-226,1990.
3. Anderson, M. P., Berger, H. A., Rich, D. P., et al., ''Nucleoside Triphosphates Are Required to Open the CFTR Chloride Channel, " *Cell* 67:775-784, 1991.
4. Anderson, M. P., Gregory, R. J., Thompson, S., et al., "Demonstration that CFTR Is a Chloride Channel by Alteration of Its Anion Selectivity, " *Science* 253:202-205, 1991.
5. Anderson, M. P., Rich, D. P., Gregory, R.J., et al., ''Generation of cAMP-Activated Chloride Currents by Expression of CFTR,'' *Science* 251:679-682, 1991.
6. Anderson, W. F., ''Human Gene Therapy, " Science 256:808-813, 1992.
7. Bal, J., Sturhman, M., Schloesser, M., et al., "A Cystic Fibrosis Patient Homozygous for the Nonsense Mutation R553X, ' *Journal of Medical Genetics* 28:715-717, 1991.
8. Barasch, J., Kiss, B., Prince, A., et al., "Defective Acidification of Intracellular Organelles in Cystic Fibrosis," *Nature* 352:70-73, *1991.*
9. Barinaga, M., [4]''Will 'DNA Chip' Speed Genome Initiative?," Science 253: 1489, 1991.
10. Barinaga, M., ''Novel Function Discovered for the Cystic Fibrosis Gene, " *Science* 256:444-445,1992.

11. Barreto, C., Pinto, L. M., Duarte, A., et al., "A Fertile Male With Cystic Fibrosis: Molecular Genetic Analysis, " *Journal of Medical Genetics* 28:420-421, 1991.
12. Bean, R., Cystic Fibrosis Foundation, Bethesda, MD, personal communication, November 1991.
13. Bear, C. E., Li., C., Kartner, N., et al., ''Purification and Functional Reconstitution of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR),'' *Cell* 68:809-818, 1992.
14. Beaudet, A. L., Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX, personal communication, February 1992.
15. Beugelsdijk, T. J., Los Alamos National Laboratory, Los Alamos, NM, personal communication, 1991.
16. Blanchard, F., Howard Hughes Medical Institute, Bethesda, MD, personal communications, November 1991, February 1992.
17. Boat, T. F., Welsh, M. J., and Beaudet, A.L., "Cystic Fibrosis," *The Metabolic Basis of Inherited Disease,* C.R. Scriver, A.L. Beaudet, W.S. Sly, et al. (eds.) (New York, NY: McGraw Hill, 1989).
18. Bobrow, M., Pediatric Research Unit, Division of Medical and Molecular Genetics, United Medical and Dental Schools of Guy's and St. Thomas's Hospital, London, England, personal communication, December 1991.
19. Bonduelle, M., Lissens, W., Liebaers, I., et al., "Mild Cystic Fibrosis in Child Homozygous for G542 Non-Sense Mutation in CF Gene," *Lancet* 338:189, 1991.
20. Borgo, G., Mastella, G., Gasparini, P., et al., "Pancreatic Function and Gene Deletion F508 in Cystic Fibrosis," *Journal of Medical Genetics* 27:665-669, 1990.
21. Borrnan, S., "Gene Therapy Eyed to Elicit Cancer Immunity,' *Chemical and Engineering News* 69:6, *1991.*
22. Bradbury, N. A., Jilling, T., Berta, G., et al., "Regulation of Plasma Membrane Recycling by CFTR,'' *Science* 256:530-532, *1992.*
23. Cantor, C., Southern, E., and Mirzabekov, A., ''Moscow Workshop on Sequencing by Hybridization," *Human Genome News* 3:12-13,16, *1992.*
24. Caskey, C.T., "Disease Diagnostics by Recombinant DNA Methods, ' *Science* 236:1223-1228, *1987.*
25. Chalkley, G., and Harris, A., "A Cystic Fibrosis Patient Who Is Homozygous for the G85E Mutation Has Very Mild Disease," *Journal of Medical Genetics* 28:875-877, *1991.*
26. Cheng, S. H., Gregory, R. J., Marshall, J., et al., ' 'Defective Intracellular Transport and Processing of CFTR is the Molecular Basis of Most Cystic Fibrosis, " *Cell* 63:827-834, 1990.
27. Cheng, S. H., Rich, D, P., Marshall, J., et al., ''Phosphorylation of the R-Domain by cAMP-Dependant Protein Kinase Regulates the CFTR Chloride Channel," *Cell* 66:1027-1036, 1991.
28. Coghlan, A., "Dyes Could Speed Up the Genome Project. . .," *New Scientist* 133:25, *1992.*
29. Collaborative Research Inc., Waltham, MA, "Now . . . A Test to Identify Greater than 90% of Cystic Fibrosis Carriers,' advertisement, 1991.
30. Collins, F.S., "Cystic Fibrosis: Molecular Biology and Therapeutic Implications," *Science 256:774-779, 1992.*
31. Cooper, D.N., and Schmidtke, J., "Diagnosis of Genetic Disease Using Recombinant DNA. Third Edition," *Human Genetics* 87:519-560, 1991.
32. Crystal, R.G., National Institutes of Health, Bethesda, MD, presentation before the U.S. Congress, Office of Technology Assessment, Advisory Panel on Cystic Fibrosis and DNA Tests: Implications of Carrier Screening, September 1991.
33. Cuppens, H., Maryen, P., De Boeck, C., et al., "A Child, Homozygous for a Stop Codon in Exon 11, Shows Milder cystic Fibrosis Symptoms Than Her Heterozygous Nephew," *Journal of Medical Genetics* 27:717-719, 1990.
34. Cutting, G.R., Curristin, S.M., Nash, E., et al., "Analysis of Four Diverse Population Groups Indicates That a Subset of Cystic Fibrosis Mutations Occur in Common Among Caucasians," *American Journal of Human Genetics* 50:1 *185-1194, 1992.*
35. Cutting, G.R., Kasch, L.M., Rosenstein, B.J., et al., ''A Cluster of Cystic Fibrosis Mutations in the First Nucleotide-Binding Fold of the Cystic Fibrosis Conductance Regulator Protein," *Nature 346:366-369, 1990.*
36. Cystic Fibrosis Genetic Analysis Consortium, "Worldwide Survey of the ΔF508 Mutation-Report from the Cystic Fibrosis Genetic Analysis Consortium,'' *American Journal of Human Genetics* 47:354-359, *1990.*
37. Dalemans, W., Barbry, P., Champigny, G., et al., ''Altered Chloride Ion Channel Kinetics Associated With the ΔF508 Cystic Fibrosis Mutation," *Nature* 354:526-528, *1991.*
38. Dean, M., White, M. B., Amos, J., et al., "Multiple Mutations in Highly Conserved Residues Are Found in Mildly Affected Cystic Fibrosis Patients, *Cell* 61:863-870, 1990.
39. Drumm, M. L., Pope, H. A., Cliff, W. H., et al., "Correction of the Cystic Fibrosis Defect In Vitro by Retrovirus-Mediated Gene Transfer," *Cell* 62:1227-1233, 1990.
40. Drumm, M. L., Wilkinson, D. J., Smit, L. S., et al., "Chloride Conductance Expressed by ΔF508 and Other Mutant CFTRs in *Xenopus* Oocytes, ' *Science* **254: 1797-1799,** 1991.

41. Endo, I., Soeda, E., Murakarni, Y., et al., "Human Genome Analysis System, " *Nature* 352:89-90, *1991.*
42. Erlich, H. A., Gelfand, D., and Sninsky, J.J., "Recent Advances in the Polymerase Chain Reaction, ' *Science* 252:1643-1651, 1991.
43. Feigner, P. L., and Rhodes, G., "Gene Therapeutics," *Nature* 349:351-352, *1991.*
44. Ferrari, M., Colombo, C., Sebastio, G., et al., "Cystic Fibrosis Patients With Liver Disease Are Not Genetically Distinct," *American Journal of Human Genetics* 48:815-816, 1991.
45. Friedman, K. J., Highsmith, W. E., and Silverman, L. M., "Detecting Multiple Cystic Fibrosis Mutations by Polymerase Chain Reaction-Mediated Site-Directed Mutagenesis,' *Clinical Chemistry 37:753-755, 1991.*
46. Frizzell, R. A., "Cystic Fibrosis: A Disease of Ion Channels?," *Trends in Neuroscience* 10:190-193, *1987.*
47. Frizzell, R. A., and Cliff, W. H., "Back To the chloride Channel," *Nature* 350:277-278, 1991.
48. Gershon, D., "NIH Merger to Shorten Review," *Nature* 355:664, *1992.*
49. Gibson, A. L., Wagner, L. M., Collins, F, C., et al., "A Bacterial System for Investigating Transport Effects of Cystic Fibrosis-Associated Mutations," *Science* 254:109-111, 1991.
50. Grannell, R., Solera, J., Carrasco, S., et al., "Identification of a Nonframeshift 84-bp Deletion in Exon 13 of the Cystic Fibrosis Gene,' *American Journal of Human Genetics* 50:1022-1026, *1992.*
51. Gray, J., "Instrumentation Is the Key to Mapping, Sequencing,' *Human Genome News* 3:1-4, September 1991,
52. Gregory, R. J., Cheng, S.H., Rich, D. P., et al., ''Expression and Characterization of the Cystic Fibrosis Transmembrane Conductance Regulator,' *Nature* 347:382-386, *1990.*
53. *Griffin,* R. D., "Gene Therapy, " *Congressional Quarterly Researcher* 1:779-797,1991.
54. Haliassos, A., Chomel, F. C., Tesson, L., et al., "Modification of Enzymatically Amplified DNA for the Detection of Point Mutations, " *Nucleic Acids Research* 17:3606, *1989.*
55. Hall, J. G., "Genomic Imprinting and Its Clinical Implications, ' New *England Journal of Medicine* 326:827-829, *1992.*
56. Hammond, K. B., Abman, S. H., Sokol, R. J., et al., ' 'Efficacy of Statewide Neonatal Screening for Cystic Fibrosis by Assay of Trypsinogen Concentrations, *New England Journal of Medicine 325:769-774, 1991.*
57. Herman, R., "Gene Therapy Is No Longer a Rarity, ' *Washington Post,* Jan. 21, 1992, Health 7.
58. Higgins, C. F., "Protein Joins Transport Family," *Nature 341* :103, *1989.*
59. Higgins, C.F., and Hyde, S. C., "Channeling Our Thoughts, " *Nature* 352:194-195, 1991.
60. Higuchi, R., Dollinger, G., Walsh, P. S., et al., "Simultaneous Amplification and Detection of Specific DNA Sequences, '' *Biotechnology 10:413-417, 1992.*
61. Holden, C. (cd.), 'Aerosol Gene Therapy, ' *Science* 253:964-965, *1991.*
62. Holden, C. (cd.), "Gene Therapy Trials on the Move," *Science* 254:372, *1991.*
63. Holden, C. (cd.), "Genetics 'Force de Frappe'," *Science 251* :623, *1991.*
64. Hunkapiller, T., Kaiser, R.J., Koop, B. F., et al., "Large-Scale and Automated DNA Sequence Determination," *Science* 254:59-67, 1991.
65. Hyde, S. C., Emsley, P., Hartshorn, M. J., et al., ''Structural Model of ATP-Binding Proteins Associated With Cystic Fibrosis, Multidrug Resistance and Bacterial Transport, ' *Nature* 346:362-365, *1990.*
66. Integrated Genetics Laboratones, Inc., Framingham, MA, ''CF/12 Mutation Test for Cystic Fibrosis," brochure, fall 1991.
67. Jett, J. H., Los Alamos National Laboratory, Los Alamos, NM, personal communication, September 1991.
68. Jett, J. H., Keller, R. A., Martin, J. C., et al., "High-Speed DNA Sequencing: An Approach Based Upon Fluorescence Detection of Single Molecules, ' *Journal of Bimolecular Structure and Dynamics7:301* - 309, 1989.
69. Jilling, T., Cunningham, S., Barker, P. B., et al., ''Genetic Complementation in Cystic Fibrosis Pancreatic Cell Lines by Somatic Cell Fusion, ' *American Journal of Physiology 259:* C1010-C1015,*1990.*
70. Johnson, J. A., "Human Gene Therapy," *Congressional Research Service Issue Brief, IB901O8,* Apr. 1, 1991.
71. Jones, C.T., McIntosh, I., Keston, M., et al., "Three Novel Mutations in the Cystic Fibrosis Gene Detected by Chemical Mismatch Cleavage: Analysis of Variant Splicing and a Nonsense Mutation, ' *Human Molecular Genetics* 1:11-17, 1992.
72. Kartner, N., Hanrahan, J. W., Jensen, T. J., et al., ' 'Expression of the Cystic Fibrosis Gene in Non-Epithelial Cells Produces a Regulated Anion Conductance, " *Cell* 64:681-691,1991.
73. Kazazian, H. H., Center for Medical Genetics, Johns Hopkins Hospital, Baltimore, MD, personal communication, December 1991.
74. Kerem, B.-S., Rommens, J. M., Buchanan, J. A., et al., "Identification of the Cystic Fibrosis Gene: Genetic Analysis,' *Science* 245:1073-1080, *1989.*

75. Kerem, B.-S., Zielenski, J., Markiewicz, D., et al., ‘ ‘Identification of Mutations in Regions Corresponding to the Two Putative Nucleotide (ATP)-Binding Folds of the Cystic Fibrosis Gene, ” *Proceedings of the National Academy of Sciences, USA* 87:8447-8451, *1990.*

76. Kerem, E., Corey, M., Kerem, B.-S., et al., ‘‘The Relation Between Genotype and Phenotype in Cystic Fibrosis-Analysis of the Most Common Mutation, ’ New *England Journal of Medicine* 323:1517-1522, *1990.*

77. Knowlton, R. G., Cohen-Haguenauer, O., Van Cong, N., et al., “A Polymorphic DNA Marker Linked to Cystic Fibrosis Is Located on Chromosome 7,” *Nature* 318:380-382, *1985.*

78. Kristidis, P., Bozon, D., Corey, M., et al., “Genetic Determination of Exocrine Pancreatic Function in Cystic Fibrosis,” *American Journal of Human Genetics* 50:1 *178-1184, 1992.*

79. Landegren, U., Kaiser, R., Caskey, C. T., et al., “DNA Diagnostics: Molecular Techniques and Automation,” *Science* 242:229-237, *1988.*

80. Langfelder, E. J., National Center for Human Genome Research, National Institutes of Health, Bethesda, MD, personal communication, February 1992.

81. Lemna, W. K., Feldman, G. L., Kerem, B.-S., et al., ‘ ‘Mutation Analysis for Heterozygote Detection and the Prenatal Diagnosis of Cystic Fibrosis, *New England Journal of Medicine* 322:291-296, 1990.

82. Lewis, R., “Innovative Alternatives to PCR Technology Are Proliferating, ’ *The Scientist* Jan. **21, 1991, p. 23.**

83. Marrone, B. L., Los Alamos National Laboratory, Los Alamos, NM, personal communication, September 1991.

84. MarWick, C., “Gene Replacement Therapy Enters Second Year,” *Journal of the American Medical Association* 266:2193, 1991.

85. McBride, G., ‘‘Nontraditional Inheritance+-11: The Clinical Implications,” *Mosaic* 22:12-25, 1991.

86. McCabe, E. R. B., “Genetic Screening for the Next Decade: Application of Present and New Technologies, ” *Yale Journal of Biology and Medicine* 64:9-14, *1991.*

87. McCabe, E. R. B., “Implementation of DNA Technology” *Yale Journal of Biology and Medicine 64: 19-20, 1991.*

88. McKusick, V. A., *Mendelian Inheritance in Man: Catalogs of Autosomal Dominant, Autosomal Recessive, and X-Linked Phenotypes, 9th ed.* (Baltimore, MD: The Johns Hopkins University Press, 1990).

89. Ng, I. S. L., Pace, R., Richard, M. V., et al., ‘‘Methods for Analysis of Multiple Cystic Fibrosis Mutations, ’ *Human Genetics* 87:613-617, 1991.

90. Nickerson, D. A., Kaiser, R., Lappin, S., et al., “Automated DNA Diagnostics Using an ELISA-Based Oligonucleotide Ligation Assay, ’ *Proceedings of the National Academy of Sciences, USA* 87:8923-8927, *1990.*

91. *Nunes,* V., Bonizzato, A., Gaona, A., et al., “A Frameshift Mutation (2869insG) in the Second Transmembrane Domain of the CFTR Gene: Identification, Regional Distribution, and Clinical Presentation, ’ *Amen”can Journal of Human Genetics* 50:1 140-1142, 1992.

92. Osborne, L., Knight, R., Santis, G., et al., “A Mutation in the Second Nucleotide Binding Fold of the Cystic Fibrosis Gene, ’ *American Journal of Human Genetics* 48:608-612, 1991.

93. Parham, P., “Transporters of Delight,” *Nature* 348:674-675, *1990.*

94. Pollard, M., Lawrence Berkeley Laboratory, Berkeley, CA, personal communication, September 1991.

95. Quinton, P. M., “Cystic Fibrosis: A Disease in Electrolyte Transport,” *FASEB Journal 4:2709-2717,* 1990.

96. Quinton, P. M., “Righting the Wrong Protein, ” *Nature* 347:226, *1990.*

97. Rich, D. P., Anderson, M. P., Gregory, R.J., et al., “Expression of Cystic Fibrosis Transmembrane Conductance Regulator Corrects Defective Chloride Channel Regulation in Cystic Fibrosis Airway Epithelial Cells, ” *Nature* 347:358-363, 1990.

98. Rich, D. P., Gregory, R.J., Anderson, M.P., et al., “Effect of Deleting the R Domain on CFTR-Generated Chloride Channels,” *Science 253:205-207, 1991.*

99. Rigot, J.-M., Lafitte, J.-J., Dumur, V., et al., ‘Cystic Fibrosis and Congenital Absence of the Vas Deferens,’ New *England Journal of Medicine* 325:64-65, *1991.*

100. Ringe, D., and Petsko, G. A., “A Transport Problem’?,” *Nature* 346:312-313, *1990.*

101. Riordan, J. R., Rommens, J. M., Kerem, B.-S., et al., “Identification of the Cystic Fibrosis Gene: Cloning and Characterization of Complementary DNA, ’ *Science* 245:1066-1072, *1989.*

102. Roberts, L., “Cystic Fibrosis Corrected in Lab,” *Science* 249:1503, *1990.*

103. Roberts, S. S., “Push Buttons and the Genome: The Role of Automation in DNA Sequencing, ’ *Journal of NIH Research* 3:89-92, 1991.

104. Rogers, J., “Nontraditional Inheritance-I: Mechanisms Mendel Never Knew, ’ *Mosaic* 22:3-1 1, 1991.

105. Romeo, G., and Devoto, M. (eds.), “Population Analysis of the Major Mutation in Cystic Fibrosis, ’ *Human Genetics* 85:391-445, *1990.*

106. Rommens, J. M., Dho, S., Bear, C. E., et al., ''Cyclic-AMP-Inducible Chloride Conductance in Mouse Fibroblast Lines Stably Expressing Human Cystic Fibrosis Transmembrane Conductance Regulator (CFTR),'' *Proceedings of the National Academy of Sciences, USA* 88:7500-7504, *1991.*
107. Rommens, J. M., Iannuzzi, M. C., Kerem, B.-S., et al., ''IdentKlcation of the Cystic Fibrosis Gene: Chromosome Walking and Jumping,' *Science* 245:1059-1065, *1989.*
108. Rosenfeld, M. A., Siegfried, W., Yoshirnura, K., et al., ' 'Adenovirus-Mediated Transfer of a Recombinant Alpha-1 -Antitrypsin Gene to the Lung Epithelium In Vivo,'' Science 252:431-434, 1991.
109. Rosenfeld, M. A., Yoshimura, K., Trapnell, B. C., et al., ''In Vivo Transfer of the Human Cystic Fibrosis Transmembrane Conductance Regulator *Gene* to the Airway Epitheliums, " *Cell* 68:143-155, *1992.*
110. Santis, G., Osborne, L., Knight, R. A., et al., "Genetic Influences on Pulmonary Severity in Cystic Fibrosis," *Lancet* 334:294, 1990.
111. Santis, G., Osborne, L., Knight, R. A., et al., "Independent Determinants of Pancreatic and Pulmonary Status in Cystic Fibrosis, " *Lancet 336:1081-1084,* 1990.
112. Santis, G., Osborne, L., Knight, R. A., et al., ''Linked Marker Haplotypes and the ΔF508 Mutation in Adults With Mild Pulmonary Disease and Cystic Fibrosis," *Lancet* 335:1426-1429, 1990.
113. Schloesser, M., Arleth, S., Lenz, U., et al., "A Cystic Fibrosis Patient With the Nonsense Mutation G542X and the Splice Site Mutation 171-1," *Journal of Medical Genetics* 28:878-880, *1991.*
114. Shoshani, T., Augarten, A., Gazit, E., et al., "Association of a Nonsense Mutation (W1282X), the Most Common Mutation in the Ashkenazi Jewish Cystic Fibrosis Patients in Israel, With Presentation of Severe Disease, *American Journal of Human Genetics* 50:222-228, *1992.*
115. Sorscher, E. J., Kirk, K., Weaver, M. L., et al., ''Antisense Oligodeoxynucleotide to the Cystic Fibrosis Gene Inhibits Anion Transport in Normal Cultured Sweat Duct Cells,' *Proceedings of the National Academy of Sciences, USA* 88:7759-7762, *1991.*
116, Strong, TV., Smit, L. S., Turpin, S. V., et al., ' 'Cystic Fibrosis Gene Mutation in Two Sisters With Mild Disease and Normal Sweat Electrolyte Levels,'' *New' EnglandJournal of Medicine 325:* 1630-1634, 1991.
117. Swinbanks, D., "The New Automated DNA Sequencer, ' *Nature 351* :593, *1991.*
118 Tabcharani, J. A., Chang, X.-B., and Riordan, J. R., ''Phosphorylation-Regulated Cl- Channel in CHO Cells Stably Expressing the Cystic Fibrosis Gene, ' *Nature* 352:628-631, 1991.
119. Taylor, C. J., Hughes, H., Hardcastle, P. T., et al., "Genotype and Secretory Response in Cystic Fibrosis," *Lancet* 339:67-58, 1992.
120. Thiel, E., Lawrence Berkeley Laboratory, Berkeley, CA, personal communication, September 1991.
121. Thiemann, A., Grunder, S., Pusch, M., et al., "A Chloride Channel Widely Expressed in Epithelial and Non-Epithelial Cells, ' *Nature 356:57-60, 1992.*
122. Thomas, P. J., Shenbagamurthi, P., Ysern, X., et al., "Cystic Fibrosis Transmembrane Conductance Regulator: Nucleotide Binding to a Synthetic Peptide, ' *Science* 251:555-557, *1991.*
123. Trezise, A. E., and Buchwald, M., "In Vivo Cell-Specific Expression of the Cystic Fibrosis Transmembrane Conductance Regulator, ' *Nature 353:434-427, 1991.*
124. Tsui, L.-C., and Buchwald, M., "Biochemical and Molecular Genetics of Cystic Fibrosis," *Advances in Human Genetics,* vol. 20, H. Harris and K. Hirschom (eds.) (New York, NY: Plenum Press, 1991).
125. Tsui, L.-C., Buchwald, M., Barker, D., et al., "Cystic Fibrosis Locus Defined by a Genetically Linked Polymorphic DNA Marker, " *Science 230: 1054-1057, 1985.*
126. Tsui, L.-C., Zengerling, S., Willard, H. F., et al., ''Mapping of the Cystic Fibrosis Locus on Chromosome 7,' *Cold Spring Harbor Symposia on Quantitative Biology 51* :325-335, *1986.*
127. Uber, D., Lawrence Berkeley Laboratory, Berkeley, CA, personal communication, September 1991.
128. U.S. Congress, Office of Technology Assessment, *Human Gene Therapy, OTA-BP-BA-32* (Washington, DC: U.S. Government Printing Office, December 1984).
129. U.S. Congress, Office of Technology Assessment, *Techniques for Detecting Heritable Mutations in Human Beings, OTA-H-298* (Washington, DC: U.S. Government Printing Office, September 1986).
130. U.S. Congress, Office of Technology Assessment, New *Developments in Biotechnology: Ownership of Human Tissues and Cells,* OTA-BA-337 (Washington, DC: U.S. Government Printing Office, March 1987).
131, U.S. Congress, Office of Technology Assessment, *New Developments in Biotechnology>: Public Perceptions of Biotechnology>, OTA-B P-* BA-45 (Washington, DC: U.S. Government Printing Office, May 1987).
132, U.S. Congress, Office of Technology Assessment, *Mapping Our Gene.~-The Genome Projects: How Big, How Fast?,* OTA-BA-373 (Washington, DC: U.S. Government Printing Office, April 1988).

133. U.S. Congress, Office of Technology Assessment, *Genetic Witness: Forensic Uses of DNA Tests,* OTA-BA-438 (Washington, DC: U.S. Government Printing Office, July 1990).
134. U.S. Congress, Office of Technology Assessment, *Genetic Monitoring and Screening in the Workplace,* OTA-BA-455 (Washington, DC: U.S. Government Printing Office, September 1990).
135. Valverde, M. A., Diaz, M., Sepulveda, F. V., et al., ''Volume-Regulated chloride Channels Associated With the Human Multidrug-Resistance P-glycoprotein,' *Nature* 355:830-833, *1992.*
136. Verma, I. M., "Gene Therapy," *Scientific American* 264:68-84, *1990.*
137. Wainwright, B.J., Scambler, P. J., Schmidtke, J., et al., ''Localization of Cystic Fibrosis Locus to Human Chromosome 7cenq22," *Nature 318:384-385, 1985.*
138. Weatherall, D.J., "Gene Therapy in Perspective," *Nature* 349:275-276, 1991.
139. Weiss, R., "Hot Prospect for New Gene Amplifier,'' *Science* 254:1292-1293, 1991.
140. Welsh, M. J., "Abnormal Regulation of Ion Channels in Cystic Fibrosis Epithelia,'' *FASEB Journal* 4:2718-2725, *1990.*
141. Welsh, M. J., 'Acidification Identification," *Nature* 352:23-24, *1991.*
142. Welsh, M. J., and Fick, R. B., "Cystic Fibrosis," *Journal of Clinical Investigation* 80:1523-1526, *1987.*
143. White, R., Woodward, S., Leppert, M., et al., "A Closely Linked Genetic Marker for Cystic Fibrosis," *Nature* 318:382-384, 1985.
144. Wine, J.J., "The Mutant Protein Responds," *Nature* 354:503-504, *1991.*
145. Wine, J.J., 'No CFTR: Are CF Symptoms Milder?," *Nature Genetics* 1:10, 1992.
146. Wivel, N., Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, MD, personal communications, March 1992, June 1992.
147. Zeitlin, P. I., Crawford, I., Lu, L., et al., ''CFTR Protein Expression in Primary and Cultured Epithelia,'' *Proceedings of the National Academy of Sciences, USA* 89:344-347, *1992.*