Cystic fibrosis transmembrane conductance regulator and the etiology and pathogenesis of cystic fibrosis

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ABSTRACT **Cystic fibrosis (CF) is an inherited disorder causing pancreatic, pulmonary, and sinus disease in children and young adults. Abnormal viscosity of mucous secretions is a hallmark of the disease, and is believed to be the result of altered electrolyte transport across epithelial cell membranes. The monogenic etiology of this disease has been apparent for more than 40 years, but the defective gene has only recently been identified. This was made possible because of a revolution in genetic technology, called positional cloning, which can pinpoint disease genes without previous knowledge of the abnormal protein product. The protein encoded by the gene defective in CF has been termed the CF transmembrane conductance regulator (CFTR) because of its postulated role in electrolyte transport. Studies investigating the normal function of CFTR and how mutations affect that function, thereby causing CF, have required the combined skills of clinicians, geneticists, molecular biologists, and physiologists. From this collaborative effort a greater understanding of the pathogenesis of this disorder is now emerging. It may soon be possible to introduce novel therapies derived from this new knowledge that will be aimed directly at the basic defect. An ever-increasing number of genes of unknown function will be identified by continuing advances in molecular genetic technology and the advent of the genome sequencing project. The experience in cystic fibrosis research may prove to be a paradigm for investigation of the function of genes isolated by positional cloning methods. — McIntosh, I.; Cutting, G. R. CFTR and the etiology and pathogenesis of cystic fibrosis. *FASEB J.* 6: 2775-2782; 1992.**

Key Words: chloride channel • mutations • structure/function relationships • phosphorylation • genetic disease, human • positional cloning

CYSTIC FIBROSIS (CF)[2] AFFECTS approximately 1 in every 2500 newborn Caucasians; the disorder is infrequent in African-Americans (1:17,000) and rare in native African and Asian populations (<1:100,000) (1). The disease is characterized by thick mucous secretions that plug smaller airways of the respiratory tree and ducts of the pancreas, causing inflammation, infection, and progressive destruction of both organs (1). Lung disease limits longevity; however, recent improvements in therapy have increased the median age of survival to 29 years (CF Foundation patient registry, 1991). Measurement of sodium and chloride ion concentrations, which are elevated in the sweat of CF patients, has been used as a diagnostic test for the disorder since 1953.

Reports of children suffering with symptoms suggestive of CF have been described since the Middle Ages, but the first complete description of the disease was not published until 1936 (1). Although the etiology of the disease was unclear, it was recognized that the disorder occasionally affected several offspring of the same parents. Extensive pedigree analysis of a series of patients demonstrated that the disorder was inherited as an autosomal recessive mendelian trait. These findings demonstrated that CF is a genetic disease caused by defects at a single locus.

Significant advances in our understanding of the pathophysiology of the disorder have occurred in the past 10 years. Studies of epithelial cells from the exocrine tissues of affected individuals revealed abnormal fluid and electrolyte transport. This abnormality affects absorption in the sweat duct and secretory processes in the airways and pancreatic ducts (2). The failure of sweat glands from CF patients to respond to β-adrenergic agents localized the problem to improperly functioning cyclic AMP (cAMP)-regulated chloride channels residing in the cell membrane (3). Despite these discoveries, the defective protein in this disorder remained unknown until 1989, when the gene responsible for the disease was identified.

CFTR AND THE ETIOLOGY OF CYSTIC FIBROSIS

The CFTR gene

The first breakthrough in the search for the defective gene in CF occurred when anonymous DNA markers segregating with the disease phenotype mapped the gene to the long arm of human chromosome 7 (4). The gene responsible for CF was isolated from this region by positional cloning methods 4 years later (5, 6). The gene spans 250 kilobases containing 27 exons, which are spliced to produce a messenger RNA of approximately 6500 nucleotides (6, 7). The nucleotide sequence encodes a polypeptide of 1480 amino acids, termed the CF transmembrane conductance regulator (CFTR) (6). The protein is predicted to contain two hydrophobic regions, each composed of six transmembrane segments and two regions presumed to interact with ATP, termed nucleotide binding folds (NBF) (**Fig. 1**). The overall structure of CFTR is most similar to the multiple drug resistance (MDR) proteins and to STE6, a yeast protein (6). These proteins belong to a superfamily of ATP-dependent transport proteins that

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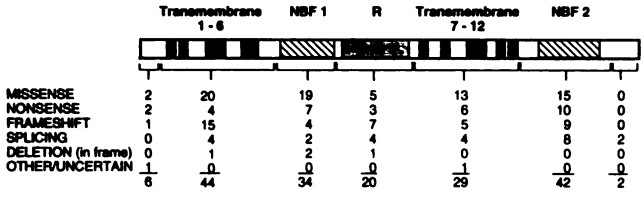
[2]Abbreviations: ABC, ATP binding cassette; ATP, adenosine triphosphate; UTP, uridine triphosphate; cAMP, cyclic 3'-5' adenosine monophosphate (AMP); CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; NBF, nucleotide binding fold; PCR, polymerase chain reaction; PKA, cAMP-dependent protein kinase; PKC, protein kinase C; MDR, multiple drug resistance.



								Total
MISSENSE	2	20	19	5	13	15	0	74
NONSENSE	2	4	7	3	6	10	0	32
FRAMESHIFT	1	15	4	7	5	9	0	41
SPLICING	0	4	2	4	4	8	2	24
DELETION (in frame)	0	1	2	1	0	0	0	4
OTHER/UNCERTAIN	1	0	0	0	1	0	0	2
	6	44	34	20	29	42	2	177

Figure 1. Diagram showing the presumed functional domains of CFTR and associated mutations (reported by members of the CF Genetic Analysis Consortium).

import or export molecules (drugs, proteins, sugars, and ions) across cell membranes in a process that appears to be coupled to ATP hydrolysis (8). Members of this group of proteins show the greatest degree of sequence similarity in the putative NBF regions, particularly in areas that contain consensus sequences for ATP binding (motifs A and B shown in **Fig. 2**). However, CFTR also has a unique region, with many charged residues and multiple potential sites for phosphorylation by cAMP-dependent kinase (PKA) and protein kinase C (PKC), situated in the middle of the protein (Fig. 1). This region is believed to be involved in regulation of protein function and has been named the R domain (6).

Sequence analysis of the 5' flanking region of the CFTR gene reveals structures similar to those found in "housekeeping" genes. It has a high guanine and cytosine nucleotide content, does not contain a TATA box, and transcription is initiated from more than one position (9, 10). These findings are rather surprising, as housekeeping genes are not expressed in a tissue-specific manner as would have been expected for CFTR based on the organ and tissue-specific manifestations of CF. Furthermore, although chronic pulmonary disease is a hallmark of CF, low levels of transcription are detected in bronchial cells by nuclear run-on assays and by quantitative PCR analyses (9, 11). It has been estimated that CFTR transcripts are present at an average of one copy per cell in bronchial epithelial cells (11). There is some evidence that CFTR transcript levels may be modulated by elements in the putative promoter region and during differentiation of certain intestinal cell lines (10, 12, 13).

Mutations in the CFTR gene cause CF

During the course of cloning the CFTR gene, a common mutation was discovered in alleles from CF patients. This mutation, a deletion of three nucleotides causing the omission of a phenylalanine residue at codon 508 (ΔF508), is found at a worldwide frequency of 68%, but varies between 30 and 88% (14). In Europe, the frequency of this mutation increases along a southeast to northwest gradient (15). The high frequency of this mutation in Caucasians is thought to be the predominant cause of the high incidence of CF in this population (16). Theories explaining why this particular mutation is so common range from random chance to selective advantage conferred to the heterozygote (reviewed in ref 4).

Three-base pair deletions have been observed as deleterious mutations in several disorders. In many cases, these mutations occurred within a region of direct nucleotide repeats. Strand slippage and mispairing of these repeats during DNA replication are believed to be the most likely events causing these deletions (17). This mutagenic process does not, however, appear to explain the generation of the common CF mutation, ΔF508, as short direct repeats are not found near the end points of this deletion. The mechanism of mutation may be related to its proximity to the repeated isoleucine codons at positions 506 and 507. Mispairing during meiosis followed by nonhomologous crossing over (18), which is believed to be extremely rare, could explain the origin of the ΔF508 mutation. The presumed rarity of this event suggests that this event occurred only once during evolution, which is consistent with theories based on haplotype analysis (19, 20).

To coordinate efficient analysis of the remaining CF-producing mutations, Lap-Chee Tsui, one of the investigators involved in cloning the CFTR gene, founded the CF Genetic Analysis Consortium. Approximately 90 laboratories from all over the world submit new findings to the Consortium for rapid dissemination of this information to participating laboratories. More than 170 mutations occurring throughout the gene have been reported to the Consortium thus far (Fig. 1). Apart from the ΔF508 mutation, five other mutations (G542X,[3] G551D, R553X, W1282X, and N1303K) occur at frequencies greater than 1% in the majority of populations (4, 16). An additional 10 mutations are found in common among Caucasians but at very low frequency (0.25–0.5%) (16). The remainder of the reported mutations have been discovered on only one, or at most a few, chromosomes and therefore appear to be rare. Several mutations have been found at frequencies greater than 5% in discrete populations, which may be the result of founder effect or genetic drift (see **Table 1**).

Knowledge of the ethnic distribution of CF mutations is a prerequisite for prenatal diagnosis and carrier screening. Establishment of patient genotype is important in understanding the relationship between mutations and disease severity. Novel therapies may require knowledge of genotype as a predictor of the CFTR abnormality. For example, different therapies may be used for patients who do not produce CFTR (see below) than those for patients producing normal amounts of dysfunctional CFTR.

THE CELLULAR ROLE OF CFTR

Expression of normal and mutant CFTR protein in primary and cultured airway, intestinal, pancreatic, and testicular tissues

Northern blot analysis has identified CFTR mRNA in epithelial tissues affected in CF (6). These observations have been extended by using RNA-PCR (PCR amplification of cDNA reverse-transcribed from RNA), and CFTR mRNA is found in a variety of epithelial and nonepithelial cell lines including fibroblasts, macrophages, and neutrophils (21). Other investigators have found some nonepithelial cells, including fibroblasts, to be negative in similar experiments (22). This controversy is probably due to the exquisite sensitivity of RNA-PCR in detecting tissue-specific transcripts in almost all cell types (23). However, because the gene promoter lacks the characteristics of a tissue-specific promoter, nonepithelial expression cannot be ruled out.

The epithelial pattern of expression is confirmed by in situ hybridization in rat tissues by using an RNA probe gener-

[3]Mutations are named by using the single letter amino acid code and the amino acid number, e.g. G551D is the substitution of glycine at codon 551 by aspartic acid. Mutations producing a termination (or stop) signal are indicated by an X. Frameshift and splice-site mutations are indicated by their location relative to the numbered nucleotide sequence of the CFTR coding sequence, e.g., 621+1G->T is a substitution of thymidine for guanine immediately following nucleotide 621, the last base in exon 4.

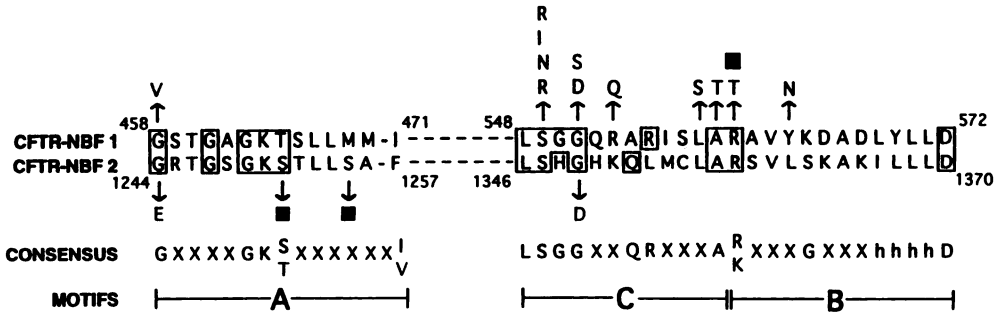


Figure 2. Missense CF mutations occurring in regions of NBF1 and NBF2 of CFTR that are conserved among members of the ATP-dependent transporter superfamily. Predicted amino acid sequence is in a single letter code and numbered according to the published sequence (6); residues that are highly conserved with the transporter proteins are boxed (6). The sites of missense mutations reported to the CF Genetic Analysis Consortium are shown by arrows; the substituted residue is indicated in single letter code for published mutations (4) or by a solid box for unpublished mutations. Consensus sequences for motifs A and B described by Walker et al. (71) and the proposed consensus for motif C are shown below the CFTR sequences; X indicates any residue and h indicates hydrophobic residue.

ated from rat CFTR cDNA. These studies demonstrate CFTR mRNA expression in pancreas, salivary gland, lung, gastrointestinal tract, uterus, and testes (24). Specifically, expression of CFTR is localized to the ductal cells of the pancreas and salivary glands, and is greater in proximal than in distal intestine and greater in intestinal crypts than in villi. Low-level expression was detected in the surface epithelia and lamina propria of bronchi and bronchioles, consistent with RNA quantification experiments (11). CFTR expression in the testes is regulated during the course of spermatogenesis, which suggests a possible role for it in this process and may explain some aspects of male infertility in CF (24).

Immunohistochemical studies show that CFTR protein is abundant in many epithelia (including the kidney) and is concentrated in the apical but not the basolateral membrane, consistent with a role in lumenal chloride secretion (2, 25, 26). The techniques used did not detect CFTR in primary epithelia from lung tissue, probably because of low levels of CFTR protein, which is consistent with transcript studies (11, 24). CFTR has been localized to the plasma membrane in cultured airway cells from normal individuals and patients homozygous for ΔF508 (27).

The varied approaches taken to analyzing the endogenous expression of CFTR have helped to elucidate its organ-, tissue-, and cell-specific expression and its cellular localization. CFTR transcript and protein are expressed in organs classically affected in CF, and have uncovered significant levels of expression in tissues previously not suspected to be involved in the pathogenesis of this disorder. It is particularly interesting to note the high level of expression in the kidney, an organ that is not generally affected by CF (1).

Heterologous expression systems indicates that CFTR is a chloride channel

Transfection of cells from CF patients with the CFTR cDNA corrects the chloride conduction abnormality, confirming that CFTR is the defective gene in CF. Using a retroviral vector, Drumm et al. (28) restored cAMP-regulated chloride conductance to PANC-1 cells (a pancreatic adenocarcinoma cell line exhibiting the CF phenotype). Rich et al. (29) similarly corrected the defect in CF airway cells and Gregory et al. (30) showed that CFTR is phosphorylated in vitro. These experiments did not address the normal function of CFTR, i.e., Does it regulate the opening of a chloride channel encoded by another gene or does it form the channel itself? This question has been examined by introducing CFTR into nonepithelial cells (fibroblasts and insect cells). In each case, novel cAMP-activated chloride currents were observed by using a number of physiological techniques (22, 31). The channel shows a linear current-voltage relationship and other characteristics of a chloride channel present in epithelial cells that express CFTR (31, 32). Both groups conclude that the simplest explanation of the results is that CFTR forms a regulated ion channel. However, it has become apparent that ubiquitous chloride channels are present in cell types that ex-

TABLE 1. *CF mutations that occur at a frequency of 5% or greater in certain population groups*

Region	Mutation	Population group	Frequency	Reference
Transmembrane 1-6	621 + 1G→T	French Canadian; Saguenay-Lac St. Jean	0.23	72
	711 + 1G→T	French Canadian; Urban Quebec	0.09	72
NBF 1	A455E	French Canadian; Saguenay-Lac St. Jean	0.08	72
	ΔF508	Worldwide	0.30-0.88	14
	G542X	Ashkenazi Jewish	0.12	73
		Spanish	0.05	74
	G551D	Scottish	0.05	75
Transmembrane 7-12	R1162X	N.E. Italian	0.05	74
NBF 2	W1282X	Ashkenazi Jewish	0.48	56, 73

press CFTR and in those used for transient expression studies (33). This raised the possibility that exogenously expressed CFTR may regulate endogenous chloride channels.

Two lines of evidence support the proposal that CFTR by itself can function as a chloride channel. First, the anion selectivity of the channel can be altered by mutations in the transmembrane regions of CFTR (34). Second, and most convincingly, CFTR purified from over-expressing cell lines has been reconstituted into lipid bilayers (35). Patch-clamp analyses of these vesicles identify cAMP-regulated currents comparable to those observed in cells expressing CFTR. As no other proteins were present, it is most likely that CFTR is a cAMP-activated chloride channel (35).

The finding that CFTR functions as a chloride channel is somewhat surprising given its sequence and structural similarity to the superfamily of ATP-dependent unidirectional transporters (8). However, it is interesting that the human MDR 1 gene, a member of the transporter superfamily closely related to CFTR, can function as a volume-regulated chloride channel and as a unidirectional transporter (36). This raises the possibility that CFTR may have more than one function.

Activation of the CFTR-encoded chloride channel appears to require two distinct steps—phosphorylation by protein kinases and hydrolysis of ATP. These are probably properties of separate domains of CFTR—the R domain and NBFs, respectively. This is supported by a growing body of experimental evidence. Transient expression of CFTR in fibroblasts demonstrates that the chloride channel activity is regulated via phosphorylation by PKA in response to cAMP stimulation (32, 37). The R domain contains a number of consensus sequences for phosphorylation by PKA and PKC. Four serine residues in this domain have been identified as in vivo PKA phosphorylation sites but no single residue is essential for cAMP activation of the channel (38). In fact, mutation of all four sites is required to abolish cAMP activation.

The assertion that the NBF regions bind ATP is supported by the observation that a synthetic peptide corresponding to 67 amino acids in the first NBF binds ATP (39). It has been proposed that hydrolysis of ATP causes a conformational change in CFTR that allows passage of chloride ions down a concentration gradient (31). ATP analogs resistant to hydrolysis can substitute for ATP in phosphorylation but not in channel opening, demonstrating that ATP hydrolysis is required for channel opening. CFTR mutated at a critical residue in NBF2 can still hydrolyze ATP, and so it is suggested that NBF1 is the primary site of ATP hydrolysis (40).

THE EFFECT OF CF MUTATION ON CFTR PROTEIN

Functional implications of CF-producing mutations

Analysis of the location and nature of disease-producing mutations can identify functionally important regions of a protein. The common CF mutation (ΔF508) is a deletion of three nucleotides that does not alter the reading frame of the mRNA. A second three-base pair deletion, ΔI507, has been discovered in the same region of the CFTR gene that causes the loss of an isoleucine residue at codon 506 or codon 507 (20). The ΔI507 and ΔF508 mutations occur between motifs A and B, which are believed to interact with the nucleotide (Fig. 2) (6). The loss of an amino acid in this region could disrupt the alignment between the two regions or cause conformation changes that render the protein functionally inactive (39). The finding that substitution of Phe508 with cysteine or Ile506 with valine does not cause CF (41) supports the conclusion that proper spacing in this region is more important than the type of amino acid at certain locations (20).

Missense mutations are particularly informative because they change only a single amino acid in the entire protein. Regardless of its subtlety, this type of mutation can alter protein conformation and lead to improper folding and instability (42, 43). In other cases, the effects of a single amino acid alteration remain local, and overall protein structure and stability are conserved. The amount of function retained by the protein then depends on the location and nature of the amino acid substitution. Almost half of the mutations reported to the Consortium are of the missense type (Fig. 1). All but three occur at a residue conserved among CFTR of human, mouse, and cow (44), supporting the assertion that missense mutations associated with disease occur in functionally important areas. In particular, there is a concentration of missense mutations in one region immediately preceding motif B in the first NBF (4, 20, 45) (Fig. 2). For convenience, the consensus sequence from this region has been termed motif C. Although motifs A and B occur in a variety of proteins that interact with nucleotides, motif C appears to a unique feature of the ATP-dependent transporter superfamily. The lack of a similar cluster of mutations in motif C of the second NBF is not explained by ascertainment bias and suggests a difference in the functional importance of each region. Indeed, expression of CFTR mutants in COS cells indicates that disease-producing missense mutations in NBF1 affect function more profoundly than analogous mutations in NBF2 (46).

Study of the expression of CFTR in cells from CF patients

The expression of mutant CFTR mRNA and protein in epithelial cells has been studied in a small number of patients. The common mutation, ΔF508, is associated with normal levels of mRNA and protein in airway epithelia, suggesting that the defect is at the level of protein function and not due to mRNA or protein instability (11, 26, 27). Messenger RNA levels from CFTR genes bearing nonsense mutations are greatly reduced, and compound heterozygotes for two nonsense alleles have no detectable mRNA or protein (47–49); thus, the approximately 3% of CF patients with nonsense mutations in both CF alleles will probably have no CFTR.

The effect of specific nucleotide changes on mRNA splicing has been investigated in some instances. Variation in the length of a polypyrimidine tract in the splice acceptor site in intron 8 has been associated with variable deletion of exon 9 from CFTR transcripts (50). Surprisingly, four individuals without features of CF have been discovered in whom 73–92% of the CFTR transcripts in bronchial epithelial cells were missing exon 9 (51). This suggests either that exon 9 is not necessary for CFTR function or that as little as 8% of normal CFTR mRNA is sufficient to avoid a CF phenotype. Three putative splice mutations (621+1G→T, 711+1G→T, 1717−1G→A) account for approximately 1% of CF alleles; however, their effects on splicing have yet to be examined. One rare mutation, Q1291H, the result of a G→C change at the last nucleotide in exon 20, results in the use of a cryptic splice site in intron 20 that disrupts the reading frame. Most transcripts from this allele appear to be spliced normally, suggesting that the change from glutamine to histidine affects CFTR function (48).

Possible associations between genotype and phenotype

The degree to which the nature of a mutation (genotype) influences the form of the disease (phenotype) is under inves-

tigation (see ref 52 for review). This type of analysis can provide prognostic information for the patient and insight into the functional role of a protein at the cellular, tissue, organ, and organism level. Before the CFTR gene was cloned, there was evidence that the severity of pancreatic disease is determined by genetic factors (4). Analysis of patients with the ΔF508 mutation in one or both CFTR genes revealed a strong correlation with pancreatic status and degree of sweat chloride abnormality, but not with the severity of pulmonary disease (19, 53). A multicenter collaborative study comparing patients heterozygous for ΔF508 and the missense mutation G551D with age- and sex-matched ΔF508 homozygotes found no clinical differences except a lower risk of meconium ileus in the compound heterozygotes (54). Several reports have been published describing patients bearing nonsense mutations in each CFTR gene. Pancreatic insufficiency is a consistent feature; however, pulmonary disease ranges from mild to severe, again demonstrating that genotype is a better predictor of pancreatic than of pulmonary phenotype (55, 56).

Functional analysis of CFTR mutants using heterologous expression systems

To investigate the effect of disease-causing mutations on CFTR function, a number of investigators have expressed mutant CFTR by using various transient expression systems. In COS cell experiments, CFTR bearing the ΔF508 mutation is incompletely glycosylated and has a subcellular distribution markedly different from that of the wild-type CFTR (57). Glycosylation, however, does not appear to be necessary for chloride channel function (31, 46). These observations suggest that the ΔF508 mutation alters the structure of CFTR, leading to abnormalities in posttranslational modification and intracellular transport (46, 57). Some other CFTR mutants expressed in COS cells were also incompletely glycosylated, raising the possibility that defects in CFTR synthesis account for the majority of CF. This hypothesis is quite plausible considering that defects in protein synthesis and transport are the major consequences of disease-producing mutations in a number of other disorders, e.g., α_1-antitrypsin (43). In this case the disease could be caused by misplaced mutant CFTR protein interfering with cellular metabolism, as suggested by abnormalities recorded in the pH of intracellular organelles in CF cells (58).

Studies of mutant CFTR in other cell types, however, indicate that the situation is not straightforward. Transient expression of CFTR in monkey kidney fibroblasts (Vero cells) reveals that the ΔF508 mutation affects intracellular transport of CFTR, but some mutant CFTR exhibiting abnormal chloride channel kinetics is present in the cell membrane (59). The finding of mutant CFTR in the cell membrane is consistent with studies of CFTR in primary and cultured epithelial cells from CF patients (26, 27). Furthermore, CFTR mutants (ΔF508, G551D, and G551S) expressed in Xenopus oocytes demonstrate partial function after stimulation of cAMP levels with high concentrations of the phosphodiesterase inhibitor, IBMX (60).

Identification of the CFTR gene and the demonstration that CF patients carry mutations in each CFTR gene together establish the etiology of this disorder. Understanding the basis for aberrations in CFTR processing, function, and ion transport is a current goal of CF research. Functional analysis of CFTR containing naturally occurring mutations has provided some insight into the possible relationship between chloride transport and disease severity. One conclusion of the aforementioned study by Drumm and colleagues (60) is that mutations in the first NBF region reduce the ability of CFTR to be activated by cAMP. Variations in the sensitivity of the various CFTR mutants to cAMP stimulation of chloride conductance are correlated with the severity of the disease observed in patients carrying the respective mutations. It was unclear from Drumm et al. (60), however, whether the mutant CFTR proteins differ with respect to stability.

Under standard conditions, Xenopus oocytes expressing mutant CFTR-containing NBF1 mutations ΔF508 and G551D do not exhibit any appreciable chloride currents. However, a mutation in the first transmembrane domain (R117H) is partially functional (61). Statistical analysis of a group of patients bearing the latter mutation reveals that it is clearly associated with a milder phenotype. Indeed, the chloride conductances measured in Xenopus oocytes expressing these mutant CFTR molecules correlate with the sweat chloride levels of patients carrying these mutations (61). These studies demonstrate that the basic genetic defect (mutation), primary cellular defect (chloride conductance), and abnormality at the organ and organism level (pancreatic function, disease severity) are interrelated.

NEW THERAPIES BASED ON INCREASED UNDERSTANDING OF THE PATHOGENESIS OF CF

Recent advances in our understanding of the etiology and pathogenesis of cystic fibrosis have opened a whole new array of therapeutic possibilities for this disorder. The discovery that CFTR containing the common mutation F508 may be partially functional suggests that CF might be treated by pharmacologically increasing the function of mutant CFTR. Application of high levels of the phosphodiesterase inhibitor IBMX appears to stimulate the chloride currents exhibited by mutant CFTR expressed in Xenopus oocytes (60). Although these observations are very recent and need to be confirmed in other expression systems and cells from CF patients, they have suggested a new avenue of possibilities for treatment.

Several groups have demonstrated that epithelial cells contain chloride channels that are distinct from the channel encoded by CFTR (33, 62, 63). These channels can be activated by mechanisms different from those used by CFTR. Therefore it may be possible to exploit other pathways of epithelial chloride secretion and compensate for electrolyte abnormalities caused by mutations in CFTR. The observation that ATP and UTP applied to nasal epithelium elicits chloride secretion in normal and CF patients demonstrates the feasibility of this approach (64). Alternatively, it may be possible to increase the hydration of epithelial secretions by affecting the movement of ions other than chloride. For example, blockage of sodium absorption in airway epithelia of CF patients by aerosolized amiloride resulted in reduced sputum viscosity and elasticity and in subtle signs of improved pulmonary status (65). A clearer understanding of the role of CFTR and other ion channels in epithelial electrolyte and fluid transport will aid in the design of such therapies.

The cloning and expression of CFTR have raised the possibility of treating CF by replacement therapy. The presence of CFTR in the milk of mice transgenic for the human CFTR gene suggests that large quantities of this protein could be produced inexpensively (66). The biological activity of this form of CFTR and a method of administration need to be determined. However, it is reasonable to expect that CF could be treated with recombinant CFTR protein if it were

functional and could be delivered in sufficient quantities to the affected tissues. New therapies aimed at improving clearance of viscous secretions from the lung, such as aerosolized recombinant DNase I (67), may aid in the delivery of CFTR to the airway epithelia.

Of all the treatment regimens being considered for CF, gene therapy has received the greatest attention. The possibility of providing affected epithelial tissues with a long-lasting source of normally functioning CFTR is an attractive and desirable goal. Using a replication-deficient adenovirus vector, Rosenfeld et al. (68) have observed expression of CFTR in rat airway cells for as long as 6 wk after exposure to virus instilled directly into the trachea. While this is encouraging, the safety of virally based systems needs to be verified. Furthermore, in situ hybridization has shown that CFTR expression in the airways is not restricted to the surface epithelia (24). Therefore, it may be difficult to treat the defect in cells inaccessible to virus.

Major improvements in understanding CF pathogenesis and in treatment may both be possible once an animal model has been generated. To this end, a number of groups have interrupted the murine CFTR gene in embryonic stem cells and are attempting to generate chimeric mice that can be bred to homozygosity (69, 70).

IMPLICATIONS FOR OTHER GENETIC DISORDERS

Identification of the gene responsible for CF provided a new direction for research in this field. As our understanding of the etiology of CF has grown, the number of questions regarding the role that mutant forms of this protein play in disease has increased. It can be safely predicted that this second phase of research will take more time and effort than did the cloning of the gene. This also appears to be the case for other disorders where the gene in question has been identified by positional cloning techniques (e.g., neurofibromatosis type 1, Duchenne muscular dystrophy), and even when the responsible gene encodes a previously characterized protein (e.g., Marfan syndrome). The Human Genome Project will provide the sequences of many genes of unknown function. Improved methods of linkage analysis should enable researchers to associate some of those genes with disease conditions. Identification of the defective gene will establish the etiology of a number of inherited disorders. However, this is only the first step toward a complete understanding of the pathogenesis of the disease. FJ

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REFERENCES

1. Boat, T. F., Welsh, M. J., and Beaudet, A. L. (1989) Cystic Fibrosis. In *The Metabolic Basis of Inherited Disease* (Scriver, C. L., Beaudet, A. L., Sly, W. S., and Valle, D., eds) pp. 2649–2680, McGraw-Hill, New York
2. Quinton, P. M. (1990) Cystic fibrosis: a disease in electrolyte transport. *FASEB J.* **4**, 2709–2717
3. Welsh, M. J. (1990) Abnormal regulation of ion channels in cystic fibrosis epithelia. *FASEB J.* **4**, 2718–2725
4. Tsui, L-C., and Buchwald, M. (1991) Biochemical and molecular genetics of cystic fibrosis. *Adv. Hum. Genet.* **20**, 153–266
5. Rommens, J. M., Iannuzzi, M. C., Kerem, B., Drumm, M. L., Melmer, G., Dean, M., Rozmahel, R., Cole, J. L., Kennedy, D., Hidaka, N., Zsiga, M., Buchwald, M., Riordan, J. R., Tsui, L-C., and Collins, F. S. (1989) Identification of the cystic fibrosis gene: chromosome walking and jumping. *Science* **245**, 1059–1065
6. Riordan, J. R., Rommens, J. M., Kerem, B., Alon, N., Rozmahel, R., Grzelczak, Z., Zielenski, J., Lok, S., Plavsic, N., Chou, J. L., Drumm, M. L., Iannuzzi, M. C., Collins, F. S., and Tsui, L-C. (1989) Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* **245**, 1066–1073
7. Zielinski, J., Rozmahel, R., Bozon, D., Kerem, B-S., Grzelczak, Z., Riordan, J. R., Rommens, J., and Tsui, L-C. (1991) Genomic DNA sequence of the cystic fibrosis transmembrane conductance regulator (CFTR) gene. *Genomics* **10**, 214–228
8. Hyde, S. C., Emsley, P., Hartshorn, M. J., Mimmack, M. L., Gileadi, U., Pearce, S. R., Gallagher, M. P., Gill, D. R., Hubbard, R. E., and Higgins, C. F. (1990) Structural and functional relationships of ATP-binding proteins associated with cystic fibrosis, multidrug resistance and bacterial transport. *Nature (London)* **346**, 362–366
9. Yoshimura, K., Nakamura, H., Trapnell, B. C., Dalemans, W., Pavirani, A., Lecocq, J-P., and Crystal, R. G. (1991) The cystic fibrosis gene has a "housekeeping"-type promoter and is expressed at low levels in cells of epithelial origin. *J. Biol. Chem.* **266**, 9140–9144
10. Chou, J.-L., Rozmahel, R., and Tsui, L.-C. (1991) Characterization of the promoter region of the cystic fibrosis transmembrane conductance regulator gene. *J. Biol. Chem.* **266**, 24471–24476
11. Trapnell, B. C., Chu, C-S., Paakko, P. K., Banks, T. C., Yoshimura, K., Ferrans, V. J., Chernick, M. S., and Crystal, R. G. (1991) Expression of the cystic fibrosis transmembrane conductance regulator gene in the respiratory tract of normal individuals and individuals with cystic fibrosis. *Proc. Natl. Acad. Sci. USA* **88**, 6565–6569
12. Montrose-Rafizadeh, C., Guggino, W. B., and Montrose, M. H. (1991) Cellular differentiation regulates expression of Cl- transport and cystic fibrosis transmembrane conductance regulator mRNA in human intestinal cells. *J. Biol. Chem.* **266**, 4495–4499
13. Trapnell, B. C., Zeitlin, P. L., Chu, C-S., Yoshimura, K., Nakamura, H., Guggino, W. B., Bargon, J., Banks, T. C., Dalemans, W., Pavirani, A., Lecocq, J.-P., and Crystal, R. G. (1991) Down-regulation of cystic fibrosis gene mRNA transcript levels and induction of the cystic fibrosis chloride secretory phenotype in epithelial cells by phorbol ester. *J. Biol. Chem.* **266**, 10319–10323
14. Cystic Fibrosis Genetic Analysis Consortium. (1990) Worldwide survey of the deltaF508 mutation-Report from the Cystic Fibrosis Genetic Analysis Consortium (CFGAC). *Am. J. Hum. Genet.* **47**, 354–359
15. European Working Group on CF Genetics (EWGCFG) (1990) Gradient of distribution in Europe of the major CF mutation and of its associated haplotype. *Hum. Genet.* **85**, 436–441
16. Cutting, G. R., Curristin, S. M., Nash, E., Rosenstein, B. J., Lerer, I., Abeliovich, D., Hill, A., and Graham, C. (1992) Analysis of four diverse population groups indicates that a subset of cystic fibrosis mutations occur in common among Caucasians. *Am. J. Hum. Genet.* **50**, 1185–1194
17. Efstratiadis, A., Posakony, J. W., Maniatis, T., Lawn, R. M., O'Connell, C., Spritz, R. A., DeRiel, J. K., Forget, B. G., Weissman, S. M., Slightom, J. L., Blechl, A. E., Smithies, O., Baralle, F. E., Shoulders, C. C., and Proudfoot, N. J. (1980) The structure and evolution of the human B-globin gene family. *Cell* **21**, 653–668
18. Marotta, C. A., Wilson, J. T., Forget, B. G., and Weissman, S. M. (1977) Human β-globin messenger RNA. *J. Biol. Chem.* **252**, 5040–5053
19. Kerem, B., Rommens, J. M., Buchanan, J. A., Markiewicz, D., Cox, T. K., Chakravarti, A., Buchwald, M., and Tsui, L. C. (1989) Identification of the cystic fibrosis gene: genetic analysis. *Science* **245**, 1073–1080
20. Kerem, B., Zielenski, J., Markiewicz, D., Bozon, D., Gazit, E.,

Yahaf, J., Kennedy, D., Riordan, J. R., Collins, F. S., Rommens, J. M., and Tsui, L.-C. (1990) Identification of mutations in regions corresponding to the 2 putative nucleotide (ATP)-binding folds of the cystic fibrosis gene. *Proc. Natl. Acad. Sci. USA* **87**, 8447–8451

21. Yoshimura, J., Nakamura, H., Trapnell, B. C., Chu, C.-S., Dalemans, W., Pavirani, A., Lecocq, J.-P., and Crystal, R. G. (1991) Expression of the cystic fibrosis transmembrane conductance regulator gene in cells of non-epithelial origin. *Nucleic Acids Res.* **19**, 5417–5423
22. Anderson, M. P., Rich, D. P., Gregory, R. J., Smith, A. E., and Welsh, M. J. (1991) Generation of cAMP-activated chloride currents by expression of CFTR. *Science* **251**, 679–682
23. Sarkar, G., and Sommer, S. S. (1989) Access to a messenger RNA sequence or its protein product is not limited by tissue or species specificity. *Science* **244**, 331–334
24. Trezise, A. E. O., and Buchwald, M. (1991) In vivo cell-specific expression of the cystic fibrosis transmembrane conductance regulator. *Nature (London)* **353**, 434–437
25. Crawford, I., Maloney, P., Zeitlin, P. L., Guggino, W. B., Hyde, S. C., Turley, H., Gatter, K. C., Harris, A., and Higgins, C. F. (1991) Immunocytochemical localization of the cystic fibrosis gene product CFTR. *Proc. Natl. Acad. Sci. USA* **88**, 9262–9266
26. Zeitlin, P. L., Crawford, I., Lu, L., Woek, S., Cohen, M. E., Donowitz, M., Montrose, M. H., Hamosh, A., Cutting, G. R., Gruenert, D., Huganir, R., Maloney, P., and Guggino, W. B. (1992) CFTR protein expression in primary and cultured epithelia. *Proc. Natl. Acad. Sci. USA* **89**, 344–347
27. Sarkadi, B., Bauzon, D., Huckle, W. R., Earp, H. S., Berry, A., Suchindran, H., Price, E. M., Olsen, J. C., Boucher, R. C., and Scarborough, G. A. (1992) Biochemical characterization of the cystic fibrosis transmembrane conductance regulator in normal and cystic fibrosis epithelial cells. *J. Biol. Chem.* **267**, 2087–2095
28. Drumm, M. L., Pope, H. A., Cliff, W. H., Rommens, J. M., Marvin, S. A., Tsui, L.-C., Collins, F. S., Frizzell, R. A., and Wilson, J. M. (1990) Correction of the cystic fibrosis defect in vitro by retrovirus-mediated gene transfer. *Cell* **62**, 1227–1233
29. Rich, D. P., Anderson, M. P., Gregory, R. J., Cheng, S. H., Paul, S., Jefferson, D. M., McCann, J. D., Klinger, K. W., Smith, A. E., and Welsh, M. J. (1990) Expression of cystic fibrosis transmembrane conductance regulator corrects defective chloride channel regulation in cystic fibrosis airway epithelial cells. *Nature (London)* **347**, 358–363
30. Gregory, R. J., Cheng, S. H., Rich, D. P., Marshall, J., Paul, S., Hehir, K., Ostedgaard, L., Klinger, K. W., Welsh, M. J., and Smith, A. E. (1990) Expression and characterization of the cystic fibrosis transmembrane conductance regulator. *Nature (London)* **347**, 382–386
31. Kartner, N., Hanrahan, J. W., Jensen, T. J., Naismith, A. L., Sun, S., Ackerley, C. A., Reyes, E. F., Tsui, L.-C., Rommens, J. M., Bear, C. E., and Riordan, J. R. (1991) Expression of the cystic fibrosis gene in non-epithelial invertebrate cells produces a regulated anion conductance. *Cell* **64**, 681–691
32. Berger, H. A., Anderson, M. P., Gregory, R. J., Thompson, S., Howard, P. W., Maurer, R. A., Mulligan, R., Smith, A. E., and Welsh, M. J. (1991) Identification and regulation of the cystic fibrosis transmembrane conductance regulator-generated chloride channel. *J. Clin. Invest.* **88**, 1422–1431
33. Thiemann, A., Grunder, S., Pusch, M., and Jentsch, T. J. (1992) A chloride channel widely expressed in epithelial and non-epithelial cells. *Nature (London)* **356**, 57–60
34. Anderson, M. P., Gregory, R. J., Thompson, S., Souza, D. W., Paul, S., Mulligan, R. C., Smith, A. E., and Welsh, M. J. (1991) Demonstration that CFTR is a chloride channel by alteration of its anion selectivity. *Science* **253**, 202–205
35. Bear, C. E., Li, C., Kartner, N., Bridges, R. J., Jensen, T. J., Ramjeesingh, M., and Riordan, J. R. (1992) Purification and functional reconstitution of the cystic fibrosis transmembrane conductance regulator (CFTR). *Cell* **68**, 809–818
36. Valverde, M. A., Diaz, M., Sepulveda, F. V., Gill, D. R., Hyde, S. C., and Higgins, C. F. (1992) Volume-regulated chloride channels associated with the human multidrug-resistance P-glycoprotein. *Nature (London)* **355**, 830–833
37. Tabcharani, J. A., Chang, X-B., Riordan, J. R., and Hanrahan, J. W. (1991) Phosphorylation-regulated Cl channel in CHO cells stably expressing the cystic fibrosis gene. *Nature (London)* **352**, 628–631
38. Cheng, S. H., Rich, D. P., Marshall, J., Gregory, R. J., Welsh, M. J., and Smith, A. E. (1991) Phosphorylation of the R domain by cAMP-dependent protein kinase regulates the CFTR chloride channel. *Cell* **66**, 1027–1036
39. Thomas, P. J., Shenbagamurthi, P., Ysern, X., and Pedersen, P. L. (1991) Cystic fibrosis transmembrane conductance regulator: nucleotide binding to a synthetic peptide. *Science* **251**, 555–557
40. Anderson, M. P., Berger, H. A., Rich, D. P., Gregory, R. J., Smith, A. E., and Welsh, M. J. (1991) Nucleoside triphosphates are required to open the CFTR chloride channel. *Cell* **67**, 775–784
41. Kobayashi, K., Knowles, M. R., Boucher, R. C., O'Brien, W. E., and Beaudet, A. L. (1990) Benign missense variations in the cystic fibrosis gene. *Am. J. Hum. Genet.* **47**, 611–615
42. Adams, J. G., III, and Coleman, M. B. (1990) Structural hemoglobin variants that produce the phenotype of thalassemia. *Semin. Hematol.* **27**, 229–238
43. Crystal, R. G. (1989) The α_1-antitrypsin gene and its deficiency states. *Trends Genet.* **5**, 411–417
44. Diamond, G., Scanlin, T. F., Zasloff, M. A., and Bevins, C. L. (1991) A cross-species analysis of the cystic fibrosis transmembrane conductance regulator. *J. Biol. Chem.* **266**, 22761–22769
45. Cutting, G. R., Kasch, L. M., Rosenstein, B. J., Zielenski, J., Tsui, L.-C., Antonarakis, S. E., and Kazazian, H. H., Jr. (1990) A cluster of cystic fibrosis mutations in the first nucleotide binding domain of the CFTR protein. *Nature (London)* **346**, 366–369
46. Gregory, R. J., Rich, D. P., Cheng, S. H., Souza, D. W., Paul, S., Manavalan, P., Anderson, M. P., Welsh, M. J., and Smith, A. E. (1991) Maturation and function of cystic fibrosis transmembrane conductance regulator variants bearing mutations in putative nucleotide-binding domains 1 and 2. *Mol. Cell Biol.* **11**, 3886–3893
47. Hamosh, A., Trapnell, B. C., Zeitlin, P. L., Montrose-Rafizadeh, C., Rosenstein, B. J., Crystal, R. G., and Cutting, G. R. (1991) Severe deficiency of CFTR mRNA carrying nonsense mutations R553X and W1316X in respiratory epithelial cells of patients with cystic fibrosis. *J. Clin. Invest.* **88**, 1880–1885
48. Jones, C. T., McIntosh, I., Keston, M., Ferguson, A., and Brock, D. J. H. (1992) Three novel mutations in the cystic fibrosis gene detected by chemical cleavage: analysis of variant splicing and a nonsense mutation. *Hum. Mol. Genet.* **1**, 11–17
49. Hamosh, A., Rosenstein, B. J., and Cutting, G. R. (1992) Nonsense mutations G542X and W1282X in the CFTR gene are associated with severe reduction of mRNA in respiratory epithelial cells. *Pediatr. Res.* **31**, 133A
50. Chu, C.-S., Trapnell, B. C., Murtagh, J. J., Moss, J., Dalemans, W., Jallat, S., Mercenier, A., Pavirani, A., Lecocq, J.-P., Cutting, G. R., Guggino, W. B., and Crystal, R. G. (1991) Variable deletion of exon 9 coding sequences in cystic fibrosis transmembrane conductance regulator gene mRNA transcripts in normal bronchial epithelium. *EMBO J.* **10**, 1355–1363
51. Chu, C.-S., Trapnell, B. C., Curristin, S. M., Cutting, G. R., and Crystal, R. G. (1992) Extensive post-translational deletion of the coding sequences for part of nucleotide-binding fold 1 in respiratory epithelial mRNA transcripts of the cystic fibrosis transmembrane conductance regulator gene is not associated with the clinical manifestations of cystic fibrosis. *J. Clin. Invest.* In press
52. Hamosh, A., and Cutting, G. R. (1992) Genotype/phenotype relationships in cystic fibrosis. In *Current Topics in Cystic fibrosis* (Dodge, J. A., Brock, D. J. H., and Widdicombe, J. H., eds) Wiley and Sons, Chichester. In press
53. Kerem, E., Corey, M., Kerem, B.-S., Rommens, J., Markiewicz, D., Levison, H., Tsui, L.-C., and Durie, P. (1990) The relation between genotype and phenotype in cystic fibrosis—analysis of the most common mutation (ΔF508). *New Engl. J. Med.* **323**, 1517–1522
54. Hamosh, A., King, T. M., Rosenstein, B. J., Corey, M., Levi-

son, H., Durie, P., Tsui, L.-C., McIntosh, I., Keston, M., Brock, D. J. H., Macek, M., Jr., Zemkova, D., Krasnicanova, H., et al. (1992) Cystic fibrosis patients bearing the common missense mutation Gly → Asp at codon 551 and the deltaF508 are indistinguishable from deltaF508 homozygotes except for decreased risk of meconium ileus. *Am. J. Hum. Genet.* In press

55. Cutting, G. R., Kasch, L. M., Rosenstein, B. J., Tsui, L.-C., Kazazian, H. H., Jr., and Antonarakis, S. E. (1990) Two patients with cystic fibrosis, nonsense mutations in each cystic fibrosis gene, and mild pulmonary disease. *New Engl. J. Med.* **323,** 1685–1689
56. Shoshani, T., Augarten, A., Gazit, E., Bashan, N., Yahav, Y., Rivlin, Y., Tal, A., Seret, H., Yaar, L., Kerem, E., and Kerem, B.-S. (1992) Association of a nonsense mutation (W1282X), the most common mutation in the Ashkenazi Jewish cystic fibrosis patients in Israel, with severe disease presentation. *Am. J. Hum. Genet.* **50,** 222–228
57. Cheng, S. H., Gregory, R. J., Marshall, J., Paul, S., Souzo, D. W., White, G. A., O'Riordan, C. R., and Smith, A. E. (1990) Defective intracellular transport and processing of CFTR is the molecular basis of most cystic fibrosis. *Cell* **63,** 827–834
58. Barasch, J., Kiss, B., Prince, A., Salman, L., Gruenert, D., and Al-Awqati, Q. (1991) Defective acidification of intracellular organelles in cystic fibrosis. *Nature (London)* **352,** 70–73
59. Dalemans, W., Barbry, P., Champigny, G., Jallat, S., Dott, K., Dreyer, D., Crystal, R. G., Pavirani, A., Lecocq, J.-P., and Lazdunski, M. (1991) Altered chloride ion channel kinetics associated with the deltaF508 cystic fibrosis mutation. *Nature (London)* **354,** 526–528
60. Drumm, M. L., Wilkinson, D. J., Smit, L. S., Worrell, R. T., Strong, T. V., Frizzell, R. A., Dawson, D. C., and Collins, F. S. (1991) Chloride conductance expressed by deltaF508 and other mutant CFTRs in Xenopus oocytes. *Science* **254,** 1797–1799
61. Carroll, T. P., McIntosh, I., Zeitlin, P. L., Cutting, G., and Guggino, W. B. (1991) Expression of CFTR in *Xenopus laevis* oocytes. *Pediatr. Pulmonol. Suppl.* **6,** 223 (abstr.)
62. Wagner, J. A., Cozens, A. L., Schulman, H., Gruenert, D. C., Stryer, L., and Gardner, P. (1991) Activation of chloride channels in normal and cystic fibrosis airway epithelial cells by multifunctional calcium/calmodulin-dependent protein kinase. *Nature (London)* **349,** 793–796
63. Ward, C. L., Krouse, M. E., Gruenert, D. C., Kopito, R. R., and Wine, J. J. (1991) Cystic fibrosis gene expression is not correlated with rectifying Cl- channels. *Proc. Natl. Acad. Sci. USA* **88,** 5277–5281
64. Knowles, M. R., Clarke, L. L., and Boucher, R. C. (1991) Activation by extracellular nucleotides of chloride secretion in the airway epithelia of patients with cystic fibrosis. *N. Engl. J. Med.* **325,** 533–538
65. Knowles, M. R., Church, N. L., Waltner, W. E., Yankaskas, J. R., Gilligan, P., King, M., Edwards, L. J., Helms, R. W., and Boucher, R. C. (1990) A pilot study of aerosolized amiloride for the treatment of lung disease in cystic fibrosis. *N. Engl. J. Med.* **322,** 1189–1194
66. DiTullio, P., Cheng, S. H., Marshall, J., Gregory, R. J., Ebert, K. M., Meade, H. M., and Smith, A. E. (1992) Production of cystic fibrosis transmembrane coductance regulator in the milk of transgenic mice. *Bio/Technology* **10,** 74–77
67. Hubbard, R. C., McElvaney, N. G., Birrer, P., Shak, S., Robinson, W. W., Jolley, C., Wu, M., Chernick, M. S., and Crystal, R. G. (1992) A preliminary study of aerosolized recombinant human deoxyribonuclease I in the treatment of cystic fibrosis. *N. Engl. J. Med.* **326,** 812–815
68. Rosenfeld, M. A., Yoshimura, K., Trapnell, B. C., Yoneyama, K., Rosenthal, E. R., Dalemans, W., Fukayama, M., Bargon, J., Stier, L. E., Stratford-Perricaudet, L., Perricaudet, M., Guggino, W. B., Pavirani, A., Lecocq, J.-P., and Crystal, R. G. (1992) In vivo transfer of the human cystic fibrosis transmembrane coductance regulator gene to the airway epithelium. *Cell* **68,** 143–155
69. Koller, B. H., Kim, H.-S., Latour, A. M., Brigman, K., Boucher, R. C., Jr., Scambler, P., Wainwright, B., and Smithies, O. (1991) Toward an animal model of cystic fibrosis: Targeted interruption of exon 10 of the cystic fibrosis transmembrane regulator gene in embryonic stem cells. *Proc. Natl. Acad. Sci. USA* **88,** 10730–10734
70. Dorin, J. R., Dickinson, P., Emslie, E., Clarke, A. R., Dobbie, L., Hooper, M. L., Halford, S., Wainwright, B. J., and Porteous, D. J. (1992) Successful targeting of the mouse CFTR gene in embryonal stem cells. *Transgenic Res.* **1,** 101–105
71. Walker, J. E., Saraste, M., Runswick, M. J., and Gay, N. J. (1982) Distantly related sequences in the alpha and beta subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold. *EMBO J.* **1,** 945–951
72. Rozen, R., De Braekeleer, M., Daigneault, J., Ferreira-Rajabi, L., Gerdes, M., Lamoureux, L., Aubin, G., Simard, F., Fujiwara, T. M., and Morgan, K. (1992) Cystic fibrosis mutations in French Canadians: Three CFTR mutations are relatively frequent in a Quebec population with an elevated incidence of cystic fibrosis. *Am. J. Med. Genet.* **42,** 360–364
73. Lerer, I., Sagi, M., Cutting, G. R., and Abeliovich, D. (1991) Cystic fibrosis mutations delta F508 and G542X in Jewish patients. *J. Med. Genet.* **29,** 131–133
74. Nunes, V., Gasparini, P., Novelli, G., Gaona, A., Bonizzato, A., Sangiuolo, F., Balasopoulou, A., Gimenez, F. J., Dognini, M., Ravnik-Glavac, M., Cikuli, M., Mokini, V., Komel, R., Dallapiccola, B., Pignatti, P. F., Loukopoulos, D., Casals, T., and Estivill, X. (1991) Analysis of 14 cystic fibrosis mutations in five South European populations. *Hum. Genet.* **87,** 737–738
75. Shrimpton, A. E., McIntosh, I., and Brock, D. J. (1991) The incidence of different cystic fibrosis mutations in the Scottish population: effects on prenatal diagnosis and genetic counselling. *J. Med. Genet.* **28,** 317–321