Cystic Fibrosis: Molecular Biology and Therapeutic Implications

Francis S. Collins

Cystic fibrosis is the most common potentially lethal autosomal recessive disease of Caucasians, affecting 1 in 2500 newborns. Since the recent identification of the gene that is defective in patients with cystic fibrosis, a wealth of information about gene structure, the mutational basis of disease, and the function of the protein product has been derived. The product of the gene is a chloride channel that is regulated by adenosine 3′,5′-monophosphate (cyclic AMP)–dependent protein kinase phosphorylation and that requires binding of adenosine triphosphate (ATP) for channel opening. Several new approaches to drug therapy for cystic fibrosis are now emerging, and the possibility of successful gene therapy by transfer of the normal gene to airway epithelial cells is being vigorously pursued.

Cystic fibrosis (CF) is a complex inherited disorder that affects children and young adults (*1*). It is inherited in an autosomal recessive fashion; heterozygotes who carry one normal CF allele and one mutant allele are entirely asymptomatic and are denoted carriers, whereas the child of two carriers has a one in four chance of inheriting a mutation from each parent and being affected with CF. The frequency of the disease varies among ethnic groups and is highest in individuals of Northern European extraction, of which about 1 in 2500 newborns is affected. Similarly, in this population the heterozygote frequency reaches the rather remarkable value of about 1 in 25 individuals. CF is less common in other ethnic groups, but significant numbers of affected individuals are found in Southern Europe, in the Ashkenazi Jewish population, and in American blacks (*2*).

The first comprehensive description of CF was provided by Anderson in 1938, who introduced the term "cystic fibrosis of the pancreas" to point out the destruction of pancreatic exocrine function as a result of the disease (*3*). In 1953, DiSant'Agnese demonstrated that excessive salt loss occurs in the sweat of children with CF (*4*), a finding that led soon after to the measurement of sodium and chloride in sweat as the diagnostic standard for the disease (*5*). About 10 years ago, the salt transport abnormality was further defined by Quinton, who demonstrated attenuated chloride transport in sweat ducts (6), and by Knowles, Boucher, and co-workers, who demonstrated a similar abnormality in respiratory epithelium (*7*).

The clinical features of CF (*1*) are dominated by involvement of the respiratory tract, where obstruction of the airways by thick, sticky mucus and subsequent infection, especially with *Pseudomonas* species, predominate. There is also involvement of the gastrointestinal tract in most patients, with 85% showing pancreatic insufficiency as a result of obstruction of the pancreatic ducts and subsequent scarring and destruction of exocrine function. Five to 10% of newborns with CF are born with a form of intestinal obstruction called meconium ileus, and 2 to 5% develop liver disease at some time during the course of the illness. In adults with CF, infertility is almost universal in males and is frequent in females.

The management of CF (*1*) currently includes chest percussion to improve clearance of infected secretions, administration of antibiotics to treat infection, pancreatic enzyme replacement, and vigorous attention to nutritional status. Survival has progressively improved over the past 40 years, with median survival now being about 29 years. Because this calculation includes individuals from an earlier cohort, it is estimated that an individual born today with CF would be expected to survive about 40 years, even without further advances in therapy. Thus, this is no longer exclusively a disease of young children, and many individuals with CF survive productively into adulthood.

The Cystic Fibrosis Gene

Unlike many single-gene disorders for which a direct biochemical analysis has revealed the dysfunctional protein product, CF for years represented a frustrating puzzle to physiologists and biochemists. A break in this logjam came with the demonstration that the normal efflux of chloride ions across respiratory epithelial cell membranes in response to elevated adenosine 3′,5′-monophosphate (cyclic AMP) is lacking in cells derived from patients with CF (*8*). The activation of cyclic AMP–dependent protein kinase (PKA) by cyclic AMP occurred normally in CF cells, but PKA failed to activate a chloride conductance (*9*). However, this information was not sufficient for direct identification of the protein product of the gene that is defective in CF patients.

An alternative strategy, now referred to as positional cloning (*10*), was successful in identifying the *CF* gene in 1989 (*11–13*). The initial step in this effort was the mapping of the *CF* gene to chromosome 7 through the use of linkage analysis of multiple affected individuals and a panel of polymorphic DNA markers. Subsequent refinement of the genetic analysis placed the *CF* gene in an ~1.5-Mb interval. Through the use of a combination of chromosome jumping and chromosome walking (*11*), an intense collaborative effort eventually yielded an attractive candidate transcript, which was expressed in sweat glands, lungs, and the pancreas (*12*). The gene itself was quite large [250,000 base pairs (bp)], and its messenger RNA (mRNA) transcript was deduced to be about 6.5 kb, encoding a protein of 1480 amino acids (Fig. 1). As with any positional cloning effort, the final proof that the gene had been identified rested on the discovery of mutations that distinguish normal from affected individuals. A 3-bp deletion in exon 10 of this candidate gene, which results in the loss of a single amino acid (phenylalanine at codon 508, hence its designation as ΔF508) was identified in individuals with CF, in whom it accounted for approximately 70% of the mutant alleles (*13*). This ΔF508 mutation was never found on chromosomes from normal individuals. The protein product of this gene was named CFTR, the cystic fibrosis transmembrane conductance regulator, a designation that reflected the initial ambiguity about the exact function of the protein.

The expression of the *CF* gene is largely restricted to epithelial cells, where it is transcribed at relatively low concentrations. The highest RNA levels are found in the pancreas, the salivary glands, the sweat glands, the intestine, and the reproductive tract (*12, 14*). Several investigators have raised antisera that detect the CFTR pro-

The author is in the Departments of Internal Medicine and Human Genetics and the Howard Hughes Medical Institute, University of Michigan, Ann Arbor, MI 48109–0650.

tein from a variety of cell lines and tissues (*15–20*). The mature protein migrates as an ~170-kD *N*-glycosylated species. Morphological immunofluorescent localization with an antibody to the COOH-terminal portion of CFTR has demonstrated expression in the pancreas and the sweat glands (*17*), where the protein seems to be localized to the apical membrane surfaces of specialized epithelial cells. Studies of CFTR expression in human lungs have indicated very low expression in the respiratory epithelium and considerably higher expression in the submucosal glands (*21*). Although it is risky to draw conclusions about the importance of CFTR function in a tissue solely on the basis of protein abundance, these observations suggest that the submucosal glands could be an important site for normal CFTR function.

Mutational Basis of the Disease

The finding that one particular mutation (ΔF508) is responsible for such a high percentage of all CF mutations (70%) suggests that there may have been some heterozygote selection or a very strong founder effect for this particular mutation in the Northern European population (*2*). With a single mutation accounting for 70% of disease alleles, the original expectation was that only a few mutations might account for the remainder (*13*). This has not turned out to be the case. At present more than 170 mutations have been described in the CFTR gene from individuals with CF (Fig. 2). About 20 of these commonly occur among Caucasians, while the remainder are quite rare, many having been identified in only a single individual (*2, 22*). The frequency of the more common mutations varies considerably in geographic and ethnic subgroups (*2*). This mutational information has been accumulated rapidly by the Cystic Fibrosis Genetic Analysis Consortium, a network of 88 laboratories around the world that has pursued this problem with a variety of detection techniques.

Considerable effort has been expended to correlate specific mutations with the phenotype of disease produced. It has been predicted that such correlations would not be precise, because siblings affected with CF often have quite different clinical courses. Furthermore, many of the mutations shown in Fig. 2 do not include sufficient numbers of affected homozygotes to define precisely the phenotype of that particular mutation. The ΔF508 mutation is clearly associated with classic, severe CF, with nearly universal pancreatic insufficiency and a high risk of meconium ileus (*23, 24*). On the other hand, a few mutations are associated with very mild disease (*25*), including a few that result in normal sweat chloride values. Mildest of all are a few mutations in infertile males who have little if any pulmonary or gastrointestinal symptoms (*26*). Genetically, mild mutations appear to be dominant over severe ones in defining the phenotype of a compound heterozygote (*13, 24*).

The ability to detect the common CFTR mutations now makes it possible to detect approximately 85 to 90% of CF carriers. This has raised the possibility of general population screening for CF in order to identify couples at risk and to provide them with genetic counseling before the initiation of childbearing. Pilot projects are under way in the United States, Canada, and Europe to study the appropriateness of such screening. The inability to detect all CF mutations and the difficulty in counseling large numbers of at risk individuals about a disease with such a variable course has led many to recommend caution in moving forward with a population screening program (*27*). Advances in therapy, which have been occurring at a steady pace over the past several decades and which will probably continue at an accelerated rate, further complicate the approach to realistic nondirective counseling.

Function of the CFTR Protein

The predicted amino acid sequence of CFTR reveals a striking homology to a large family of proteins involved in active trans-

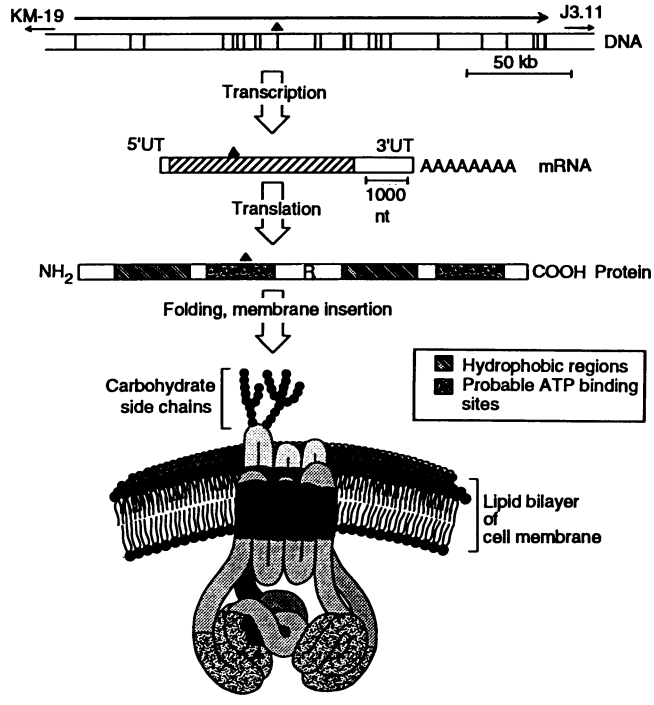

Fig. 1. Gene, transcript, and predicted structure for the CFTR protein. The gene contains 27 exons and is flanked by the genetic markers KM-19 and J3.11. The mRNA is 6129 bp long, including 5′ and 3′ untranslated (UT) regions. The protein includes two hydrophobic transmembrane domains, two nucleotide-binding sites, and a highly charged R (regulatory) domain. The diagram of the folding of the protein is hypothetical. The triangle (▲) shows the location of the 3-bp deletion in exon 10 that generates ΔF508, the most common mutation in CF patients.

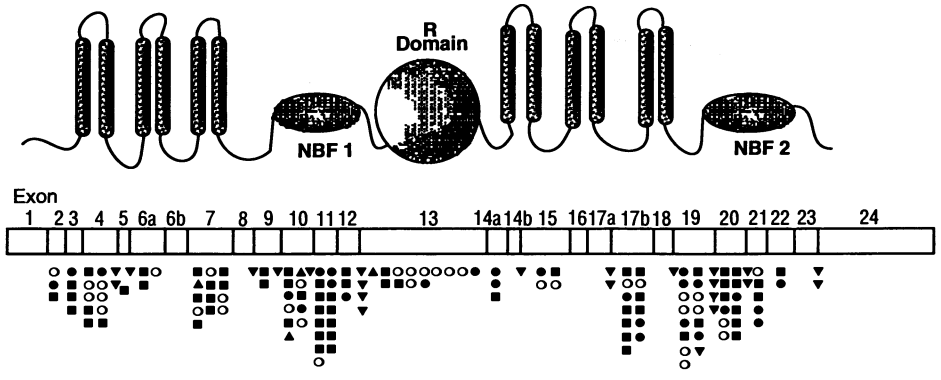

Fig. 2. Spectrum of mutations responsible for CF. The location and nature of CF mutations identified by the Cystic Fibrosis Genetic Analysis Consortium are indicated below a schematic of the CFTR protein. (▲) In-frame deletion; (■) missense mutation; (●) nonsense mutation; (○) frame-shift mutation; and (▼) splicing mutation.

port across cell membranes (*12*). These proteins are found in species as diverse as bacteria, *Drosophila*, and mammals. They thus belong to a superfamily, variably referred to as the traffic adenosine trisphosphatases (ATPases) (*28*), the ABC (ATP-binding cassette) family (*29*), or the TM6-NBF family (*30*). They have in common the presence of one or two hydrophobic transmembrane domains, each usually involving six loops that span the membrane, and the occurrence of one or two nucleotide-binding folds (NBFs) that bind and cleave ATP, providing the energy source for transport. CFTR contains two transmembrane domains and two NBFs (Fig. 1). In addition, it has a highly charged central domain, which contains a number of serine residues predicted to be targets of PKA-mediated phosphorylation. This region was therefore denoted the R (regulatory) domain (*12*).

In the 2½ years since the cloning of the gene, we have learned much about the function of the CFTR protein. These efforts began with gene transfer of the full-length, wild-type CFTR coding region into CF cells, demonstrating this to be sufficient for correction of the chloride channel defect (*31*). Several groups went on to transfect CFTR into a variety of cell types that do not normally express it (*32–37*). In every situation where wild-type CFTR protein was expressed, a chloride conductance that could be activated by cyclic AMP was observed. This occurred in diverse cell types, including mouse fibroblasts [3T3 cells (*32*) or L cells (*33*)], human HeLa cells (*32*), Chinese hamster ovary (CHO) cells (*34*), and even *Xenopus* oocytes (*35, 36*) and army worm ovary (sf9) cells (*37*). Although the simplest conclusion to be drawn from these experiments is that CFTR is itself a chloride channel, this was initially met with some skepticism because the protein amino acid sequence was far more reminiscent of that of an active transporter than of that of an ion channel.

Subsequent experiments, however, leave little doubt that CFTR can function as an ion channel. Mutagenesis of specific charged residues in the transmembrane domains resulted in an alteration in the relative permeability of CFTR to iodide, chloride, fluoride, and bromide (*38*). This effect on ion selectivity is difficult to explain if CFTR functions as a regulator of some other endogenous channel and fits much better with the notion that CFTR is itself a chloride channel, with residues in its transmembrane domains potentially lining the anion-selective pore. Perhaps most compelling in this regard is the recent reconstitution of CFTR in a planar bilayer. One of these experiments (*39*) involved the use of cellular material that might have contained other components, but a recent cell-free experiment with highly purified CFTR protein demonstrated a density of PKA-activatable channels in the resulting bilayer that could not be explained by contaminants (*40*). Thus, there is now little room for further debate about whether CFTR is a channel; although it may have other functions as well, CFTR certainly has an endogenous chloride channel function and is directly activatable by PKA.

Although much of the current experimental work on CFTR has focused on its role as a chloride channel in the apical cell membrane, the CFTR protein may carry out other important functions. For example, intracellular organelles may be defectively acidified in patients with CF, perhaps because this same chloride channel is needed for HCl accumulation (*41*). This acidification defect could potentially account for some of the abnormalities in sialylation and sulfation previously noted in CF proteins. Furthermore, observations on cultured pancreatic CF cells demonstrate a defect in cyclic AMP–mediated endocytosis and exocytosis, which is corrected by transfection of wild-type CFTR (*42*).

The mechanism of activation of CFTR by cyclic AMP has been further investigated, and the original postulation that the R domain mediates this effect still seems valid. Cheng *et al.* (*43*) identified four serine residues (660, 737, 795, and 813) in the R domain that are phosphorylated in vivo after activation of PKA. Mutation of as many as three of these does not abolish CFTR function, but loss of all four results in the loss of activatable conductance. Further experiments are necessary to determine if the serines are completely redundant or if there is cooperativity among them. Furthermore, partial deletion of the R domain results in a channel that is constitutively active without the requirement for PKA activity (*44*). This observation suggests that the R domain is normally inhibitory to channel function in its unphosphorylated state.

Recent studies of CFTR in cell-free membrane patches have shed further light on the mechanism of channel activation. Anderson *et al.* (*45*) have shown that PKA phosphorylation of the R domain and ATP binding to the NBFs are apparently separable steps. PKA-mediated phosphorylation of CFTR in the membrane patch alone is not sufficient to produce chloride currents, but a channel that has been phosphorylated can be activated by the addition of ATP. The activation is reversible if ATP is removed, and it can be reinduced by resupplying with nucleotide. This observation, as well as other data (see below), has suggested the model for CFTR function in Fig. 3 (*36*). In this view, phosphorylation of the R domain is accomplished by PKA after cyclic AMP stimulation but is reversible by the action of one or more as yet undefined phosphatases. If the phosphorylated form of CFTR binds ATP, a conformational change is induced that allows the passive flow of chloride ions. Note that this does not mean CFTR is a chloride pump; chloride moves according to its electrochemical gradient, and there is no stoichiometric relation between molecules of ATP cleaved and numbers of chloride ions that pass through the membrane. The physiologic significance of this dual control of

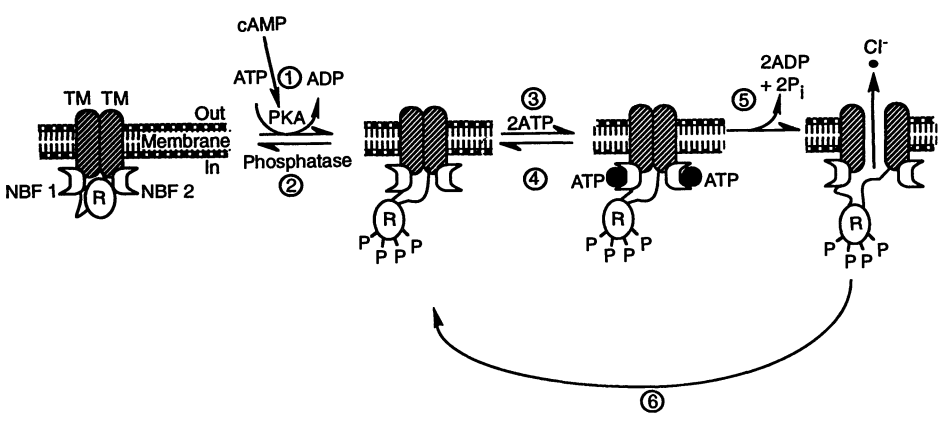

Fig. 3. Hypothesis for the dual control of CFTR by PKA and ATP. In the absence of R-domain phosphorylation, the channel is closed. After cyclic AMP stimulates PKA to phosphorylate one or more serine residues in the R domain (step 1), the CFTR channel is poised to bind ATP (step 3), which is cleaved to induce a conformational charge (step 5), opening the chloride channel. This is envisioned to decay back (step 6) to a closed state. The separation of ATP binding, hydrolysis, and channel opening (steps 3 and 5) is speculative. P_i, inorganic phosphate; cAMP, cyclic AMP; and TM, transmembrane domain.

CFTR, involving both PKA and ATP, is not entirely clear, although it may prevent overactivity of this channel in cells that are depleted of nucleotide (*46, 47*).

This model also explains observations about various CFTR missense mutations. Drumm *et al.* (*36*) studied several naturally occurring CF mutations that cause mild to severe disease, all of which result from missense mutations in the first NBF. Injection of in vitro–transcribed wild-type CFTR RNA into *Xenopus* oocytes led to a large cyclic AMP–inducible chloride current (*35, 36*). The mutant ΔF508 and the other NBF 1 mutants could also unexpectedly be induced to generate approximately comparable currents, but only if high doses of drugs that stimulate cyclic AMP, such as forskolin (which stimulates adenylate cyclase) and isobutylmethylxanthine (IBMX, which blocks phosphodiesterases) were used (*36*). This result initially seems paradoxical, because the mutations are in the NBF and not the R domain. However, consideration of Fig. 3 suggests the possibility that high concentrations of cyclic AMP are able to overcome the NBF 1 mutations by driving reaction 1 strongly to the right, providing a larger pool of substrate for reaction 3. This reaction can then proceed even in the presence of reduced ability of NBF 1 to bind or hydrolyze ATP (or both). The model also predicts that phosphatase inhibitors might be useful in activating mutant CFTRs.

In addition to these qualitative effects on protein function, there is evidence that some missense mutations of CFTR also alter protein processing, at least in some cells. Expression of a wide variety of CFTR missense mutations in COS (monkey kidney) cells reveals that the majority produce a protein that is incompletely glycosylated, indicating that the protein product is held up in the endoplasmic reticulum and apparently does not reach the plasma membrane (*47*). These observations suggest that the primary defect in many CF mutations could be in protein processing. However, there are exceptions to this observation, even in COS cells; certain mutations located only a few amino acid residues apart sometimes led to faulty processing and sometimes did not. Furthermore, analysis of the in vivo concentrations of ΔF508 protein in respiratory epithelium has revealed nearly wild-type levels of an apparently normally glycosylated form (*19, 20*). This contrasts with recent data on the sweat duct, where ΔF508 protein did not appear to be localized properly (*48*). Recently, Dalemans *et al.* (*49*) have compared the expression of wild-type and ΔF508 CFTR in Vero cells. Although there was a marked decrease in the amount of ΔF508 CFTR that reached the plasma membrane compared to wild-type, a cyclic AMP–activatable chloride current was clearly demonstrable in the ΔF508-transfected cells, and patch-clamping suggested that the primary difference between wild-type and ΔF508 CFTR was that the ΔF508 channels remained closed a greater proportion of the time. This would fit with the model in Fig. 3 if the change from the closed to the open state is represented by steps 3 and 5.

The most likely explanation for these discrepancies is that the ability to process mutant forms of CFTR depends on the cell type being studied and perhaps also on the amounts of protein being expressed. It is crucial to resolve the question of in vivo localization of mutant CFTR in a variety of tissues, because the presence of at least some mutant protein on the cell surface has important consequences for both drug therapy and gene therapy of CF: a modest amount of defective protein might still be potentially activatable by appropriate drug manipulations, and the presence of such protein reduces the likelihood of an immune response against wild-type CFTR in a gene therapy or protein therapy approach.

Prospects for Improved Drug Therapy

The management of CF currently is a multifaceted approach, simultaneously aimed at several steps in the pathophysiologic process. Table 1 outlines our understanding of the basis of the respiratory complications of CF. Although one must not neglect the extrapulmonary manifestations of CF, 95% of the morbidity and mortality of the disease arises from the pulmonary complications (*1*), so most approaches to therapy will be focused on these.

Three new pharmacological approaches are currently in clinical trials and show considerable promise. Amiloride, a potassium-sparing diuretic, blocks the uptake of sodium ions by respiratory epithelium. Analysis of the ion flux abnormalities in CF has revealed an increased uptake of sodium ions, as well as the better-characterized block in chloride efflux (*50*). This sodium transport abnormality apparently contributes to the general dehydration of airway mucus by enhancing salt and water absorption. Accordingly, blocking this sodium uptake with amiloride might be expected to lead to improved mucus hydration and better clearance of secretions. A study (*51*) of moderately affected CF patients has documented a slowing of pulmonary deterioration with aerosolized amiloride, and a larger clinical trial is now under way.

A second potential ion-channel intervention has emerged with the observation that the application of ATP or uridine triphosphate (UTP) to the apical surface of respiratory epithelial cells results in chloride efflux. This efflux occurs equally well in CF cells and normal cells, apparently operating through a different pathway than that mediated by CFTR (*52*). Circumstantial evidence indicates that the action of ATP and UTP is mediated through the P_2 nucleotide receptor (*52*). These observations suggest that the delivery of aerosolized ATP or UTP might be beneficial to patients with CF, and clinical trials are being designed to test this hypothesis.

In a rather different approach, the use of recombinant human deoxyribonuclease (DNase) is also being tested for the treatment of CF (*53*). One of the major clinical problems in CF patients is the high viscosity of the large volumes of mucus produced by CF patients. This mucus is heavily in-

Table 1. Approaches to CF lung disease.

Abnormality	Solution	Approach
Abnormal CF gene	Provide normal gene	Gene therapy
Abnormal CFTR protein	Provide normal protein Activate mutant form	Protein therapy ? Phosphodiesterase inhibitors ? Phosphatase inhibitors ? Others
Abnormal salt transport	Block Na^+ uptake Increase Cl^- efflux	Amiloride ATP/UTP
? Abnormal mucus	Decrease viscosity	DNase
Impaired clearance	Augment ciliary action	Chest percussion
? *Pseudomonas* infection	Reduce bacterial count	Antibiotics
Inflammatory response	Decrease host reaction	Antiproteases Anti-inflammatory drugs (steroids, ibuprofen)
Bronchiectasis	Replace irreversibly damaged areas	Lung transplantation

Table 2. Gene transfer approaches to the lung CF; + indicates a particular feature is present, − indicates that it is absent, and ± indicates that it is weak. NA indicates that it is not applicable and ? indicates not known.

Feature	Retrovirus	Adenovirus	AAV	Liposomes	DNA-protein complexes
Tropism for airway	−	±	−	Potential	Potential
Titer (per milliliter)	10^6	$>10^{10}$	10^8	NA	NA
Need for dividing cells	+	−	?	−	−
Integration	+	−?	+	±	±
Repeated dosing needed	?	+	?	?	?
Anticipated immune response	−	+	±	?	?

fected with bacterial organisms and contains numerous white blood cells, many of which die and release their DNA, further contributing to the high viscosity (Table 1). It was therefore hypothesized that digestion of the DNA component of the mucus might reduce the viscosity and lead to better clearance. In order to avoid an allergic response to a nonhuman protein, the human DNase I gene was cloned and expressed, allowing purification of large amounts of protein. A small clinical trial of aerosolized recombinant DNase has resulted in better clearance of secretions and an apparent improvement in clinical status (*53*).

Other as yet theoretical approaches to pharmacological treatment of CF are suggested by the model in Fig. 3. The observation that many mutant forms of CFTR, including the common ΔF508, can be activated by high concentrations of cytosolic cyclic AMP suggests that such an approach might be feasible in vivo as long as sufficient levels of mutant protein are present in the proper cellular location. An alternative would be the development of specific phosphatase inhibitors. Obviously, a great deal must be learned about protein localization and the specific phosphodiesterases and phosphatases in the respiratory tract in order for this idea to be exploitable. The recent development of a cell-free system for studying the function of CFTR in a lipid bilayer (*40*) raises the possibility that a screening system could be developed to look for other drugs that could activate mutant forms of CFTR.

An animal model of the disease would be of enormous assistance in evaluating new forms of therapy; several laboratories have been vigorously attempting to mutate the mouse homolog of CFTR by homologous recombination. Although successful targeting of the locus in embryonic stem cells has been accomplished (*54*), a stable line has been very difficult to generate. Differences between mouse and human airway anatomy, especially the reduced number of submucosal glands in the mouse, make it impossible to predict whether the mouse model will closely mimic the human disease.

Prospects for Gene Therapy

Because CF is a recessive disease and because heterozygotes who produce 50% of wild-type protein are normal, one would predict that the transfer and expression of a normal version of the CFTR gene into a sufficient number of the correct cells in the respiratory tract would result in correction of the disease. The cloning of the *CFTR* gene (*11–13*) and the successful demonstration of complementation of the chloride channel defect by gene transfer in tissue culture (*31*) have raised hopes that a gene therapy approach to this disease could be developed. The unique accessibility of the airway and the potential use of aerosol delivery systems have further increased the attractiveness of CF as one of the first major targets for human gene therapy. A number of important issues and questions need to be addressed, however:

1) What are the relevant cells to treat? As noted above, the amount of expression of CFTR in airway lining cells is quite low, and higher expression is found in the submucosal glands (*21*). This suggests that the abnormal CF mucus may have its origins in these glands, which are potentially much less accessible to an aerosolized vector. However, the chloride channel defect, which is the hallmark of CF, is demonstrable in the surface airway lining cells (*9*), and correcting these cells, even without altering the submucosal glands, could conceivably still be quite beneficial.

2) What fraction of the responsible cell types must be corrected to achieve clinical benefit? It is unlikely that gene transfer approaches will be 100% efficient. Mixing of CF cells with genetically modified CF cells that express wild-type CFTR, followed by measurement of the ion transport properties of the resulting monolayer, has indicated that correcting as little as 6% of the cells can produce a monolayer with essentially normal physiologic function (*55*). This felicitous outcome may potentially be the result of the gap junctions between cells, which allow the passage of chloride ions, so one normal cell can control the chloride flow from several of its CF neighbors. It will be important to determine whether this same phenomenon occurs in vivo.

3) Is overexpression of CFTR toxic? Given the low endogenous concentrations of RNA and protein and the fact that a chloride channel might have the potential to seriously alter cellular homeostasis, it is reassuring that overexpression of CFTR in transgenic mice has produced no apparent toxicity (*56*). This experiment included animals in which CFTR expression was controlled by a strong bronchioalveolar promoter (SP-C). Overexpression of CFTR may thus be less dangerous than might have been predicted.

4) How long will expression persist? Permanent expression will probably require the integration of the CFTR gene into the host chromosome of airway "stem cells," which are not yet well defined. Vectors that efficiently integrate (such as retroviruses) have a much higher likelihood of long-term persistence but also carry with them the potential for oncogene activation by random promoter insertion. Vectors such as adenovirus that do not integrate can potentially achieve efficient expression in nondividing cells (approximately 98% of the normal airway) but would not be expected to persist permanently.

5) Will the immune system intervene? The possibility of an immune response to the vector, or to wild-type CFTR, must be given serious consideration in any gene transfer approach. As noted above, the recent observations that detectable levels of CFTR are likely to be present in all but a few patients (those who carry knock out mutations of both alleles) reduce the likelihood that immune response against CFTR itself will occur. However, many of the vectors contemplated, especially adenovirus, are known to induce a host immune response. This may limit the repeated dosing that is likely to be necessary with nonintegrating vectors.

6) Can safety be ensured? Although largely theoretical now, the possibility of inadvertent gene transfer into the germ line or of contamination of the environment with aerosol vectors has to be considered for each new approach.

Table 2 shows a summary of five of the gene transfer approaches that are being considered for CF. The adenovirus vectors are the best developed, and they are attractive because they are a naturally occurring pathogen for the human airway and their molecular biology is well understood. Foreign genes can be readily inserted into the adenovirus genome and produce very high

titers of recombinant virus. With this system, highly efficient transfer of a wild-type human CFTR gene to the airway of the Cotton rat has been accomplished (*57*). A significant proportion of the rat airway epithelium then appeared to express human CFTR RNA for up to 6 weeks, and the protein was detectable in cells obtained from bronchial washings. The physiologic effects of this treatment are as yet unknown. The effects of adenovirus treatment might be expected to be transitory, because the viral genome is not integrated. It will be important to determine whether the need to treat repetitively will be thwarted by the appearance of a specific immune response to the adenoviral vector.

The use of recombinant retroviruses for transfer of CFTR has been achieved in cell culture (*31, 38, 58*), and in vivo experiments are under way with a number of species. Although the retrovirus approach is attractive because of efficient host integration, it may be necessary to stimulate airway cells to enter the cell cycle (perhaps by oxidant injury) in order to achieve a high enough efficiency of gene transfer to be beneficial. Paradoxically, the intense inflammation in the CF lung may increase the proportion of cells that are actively dividing.

Another vector capable of integration is the adeno-associated virus (AAV), which can be produced at high titers and integrates at a particular site on chromosome 19 (*59*). A potential drawback to the use of this vector is the small packaging capability of AAV, which only barely accommodates the coding sequence of CFTR.

Vector-free systems that make use of liposomes or DNA-protein complexes could also deliver the wild-type CFTR gene. Encapsulation of DNA in various types of liposomes, followed by delivery to the airway of rodents, has resulted in some gene transfer, although the efficiency and persistence have not yet been fully explored (*60*). The possibility of complexing CFTR DNA with a protein normally taken up by airway cells, such as transferrin (*61*), or with an antibody against the polymeric immunoglobulin A receptor (*62*) also represents an attractive way to harness the normal properties of the respiratory epithelium, although the facts that most of the DNA taken up through these pathways is normally degraded in lysosomes and only a small fraction is expressed will reduce the efficiency of the procedure. This degradation could possibly be blocked by cotransfecting with adenovirus to disrupt endosomes (*63*).

Summary

A little more than 10 years ago most scientists surveying research in CF were pessimistic about the possibility of major advances, given decades of frustration and the inability to understand the basic disease defect. Beginning with advances in defining the CF phenotype on the basis of ion channel abnormalities in the early 1980s, and continuing with the identification of the CFTR gene in 1989, the CF field has now moved into a satisfying phase, in which observations of significance are accumulating at a dizzying pace. This advance in research potential as the result of the availability of the cloned CF gene is likely to presage events that will occur for other diseases successfully targeted by positional cloning. Current research in CF represents a blend of contributions from diverse disciplines, including molecular biology, cell biology, biochemistry, ion channel physiology, and pharmacology. Researchers in CF, aided by the foresight of the Cystic Fibrosis Foundation, have largely abandoned the notion that CF can be approached from a narrow view. An array of disciplines and researchers with vastly different techniques and languages that might normally represent "worlds in collision" has been forged into a happy mixture, more reminiscent of "worlds in collusion." It is to be hoped, and even expected, that the consequences of this intensely exciting scientific activity will be the development of better treatments, and perhaps even a cure, for this devastating disease that has ravaged so many young lives.

REFERENCES AND NOTES

1. T. F. Boat, M. J. Welsh, A. L. Beaudet, *The Metabolic Basis of Inherited Disease* (McGraw-Hill, New York, 1989), pp. 2649–2680.
2. L.-C. Tsui and M. Buchwald, *Adv. Hum. Genet.* **20**, 153 (1991).
3. D. H. Anderson, *Am. J. Dis. Child.* **56**, 344 (1938).
4. P. A. DiSant'Agnese, R. E. Darling, G. A. Perera, E. Shea, *Pediatrics* **12**, 549 (1953).
5. L. E. Gibson and R. E. Cooke, *ibid.* **23**, 545 (1959).
6. P. M. Quinton, *Nature* **301**, 421 (1983).
7. M. R. Knowles, J. Gatzy, R. Boucher, *N. Engl. J. Med.* **305**, 1489 (1981); M. R. Knowles *et al.*, *Science* **221**, 1067 (1983).
8. K. Sato and F. Sato, *J. Clin. Invest.* **73**, 1763 (1984).
9. Reviewed in P. M. Quinton, *FASEB J.* **4**, 2709 (1990) and M. J. Welsh, *ibid.*, p. 2718.
10. F. S. Collins, *Nature Genet.* **1**, 3 (1992).
11. J. M. Rommens *et al.*, *Science* **245**, 1059 (1989).
12. J. R. Riordan *et al.*, *ibid.*, p. 1066.
13. B. Kerem *et al.*, *ibid.*, p. 1073.
14. A. E. O. Trezise and M. Buchwald, *Nature* **353**, 434 (1991).
15. R. J. Gregory *et al.*, *ibid.* **347**, 382 (1990)
16. N. Kartner *et al.*, *Cell* **64**, 681 (1991).
17. J. A. Cohn, O. Melhus, L. J. Page, K. L. Dittrich, S. R. Vigna, *Biochem. Biophys. Res. Commun.* **181**, 36 (1991); C. R. Marino, L. M. Matovcik, F. S. Gorelick, J. A. Cohn, *J. Clin. Invest.* **88**, 712 (1991); J. A. Cohn, A. C. Nairn, C. R. Marino, O. Melhus, J. Kole, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 2340 (1992).
18. I. Crawford *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 9262 (1991).
19. P. L. Zeitlin *et al.*, *ibid.* **89**, 344 (1992).
20. B. Sarkadi *et al.*, *J. Biol. Chem.* **267**, 2087 (1992).
21. J. F. Engelhardt and J. M. Wilson, in preparation.
22. G. R. Cutting *et al.*, *Am. J. Hum. Genet.*, in press.
23. E. Kerem *et al.*, *N. Engl. J. Med.* **323**, 1517 (1990).
24. P. Kristidis *et al.*, *Am. J. Hum. Genet.*, in press.
25. T. V. Strong *et al.*, *N. Engl. J. Med.* **325**, 1630 (1991); W. E. Highsmith *et al.*, *Pediatr. Pulmonol.* **5** (suppl.), 11A (1990).
26. A. Anguiano *et al.*, *J. Am. Med. Assoc.* **267**, 1794 (1992).
27. C. T. Caskey, M. M. Kaback, A. L. Beaudet, *Am. J. Hum. Genet.* **46**, 393 (1990); A. L. Beaudet *et al.*, *N. Engl. J. Med.* **323**, 70 (1990); L. Biesecker, B. Bowles-Biesecker, F. S. Collins, M. M. Kaback, B. F. Wilfond, *Am. J. Hum. Genet.* **50**, 438 (1992).
28. G. F. Ames, C. S. Mimura, V. Shyamala, *FEMS Microbiol. Rev.* **6**, 429 (1990).
29. S. C. Hyde *et al.*, *Nature* **346**, 362 (1990).
30. J. R. Riordan, N. Alon, Z. Grzelczak, S. Dubel, S. Sun, *Adv. Exp. Med. Biol.* **290**, 19 (1991).
31. M. L. Drumm *et al.*, *Cell* **62**, 1227 (1990); D. P Rich *et al.*, *Nature* **347**, 358 (1990).
32. M. P. Anderson, D P. Rich, R. J. Gregory, A. E. Smith, M. J. Welsh, *Science* **251**, 679 (1991); H. A. Berger *et al.*, *J. Clin. Invest.* **88**, 1422 (1991).
33. J. M. Rommens *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 7500 (1991).
34. J. A. Tabcharani *et al.*, *Nature* **352**, 628 (1991).
35. C. E. Bear *et al.*, *J. Biol. Chem.* **266**, 19142 (1991).
36. M. L. Drumm *et al.*, *Science* **254**, 1797 (1991).
37. N. Kartner *et al.*, *Cell* **64**, 681 (1991).
38. M. P. Anderson *et al.*, *Science* **253**, 202 (1991).
39. B. C. Tilly *et al.*, *J. Biol. Chem.*, in press.
40. C. E. Bear *et al.*, *Cell* **68**, 809 (1992).
41. J. Barasch *et al.*, *Nature* **352**, 70 (1991).
42. N. A. Bradbury *et al.*, *Science* **256**, 530 (1992).
43. S. H. Cheng *et al.*, *Cell* **66**, 1027 (1991).
44. D. P. Rich *et al.*, *Science* **253**, 205 (1991).
45. M. P. Anderson *et al.*, *Cell* **67**, 775 (1991).
46. P. M. Quinton, *Nature* **347**, 226 (1990).
47. S. H. Cheng *et al.*, *Cell* **63**, 827 (1990); R. J. Gregory *et al.*, *Mol. Cell. Biol.* **11**, 3886 (1991).
48. N. Kartner and J. R. Riordan, *J. Cell. Biochem.* **16F** (suppl.), 12 (1992).
49. W. Dalemans *et al.*, *Nature* **354**, 526 (1991).
50. R. C. Boucher *et al.*, *J. Clin. Invest.* **78**, 1245 (1986).
51. M. R. Knowles *et al.*, *N. Engl. J. Med.* **322**, 1189 (1990).
52. M. R. Knowles *et al.*, *ibid.* **325**, 533 (1991).
53. S. Shak *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 9188 (1990); R. C. Hubbard *et al.*, *N. Engl. J. Med.* **326**, 812 (1991).
54. B. H. Koller *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 10730 (1991).
55. L. Johnson and R. Boucher, in preparation.
56. J. A. Whitsett *et al.*, *Pediatr. Pulmonol.* **6** (suppl.), 185 (1991).
57. M. A. Rosenfeld *et al.*, *Cell* **68**, 143 (1992).
58. J. C. Olsen *et al.*, *Hum. Gene Ther.*, in press.
59. R. J. Samulski *et al.*, *EMBO J.* **10**, 3941 (1991); B. Carter *et al.*, *Pediatr. Pulmonol.* **6** (suppl.), 187 (1991).
60. L. Huang, *Pediatr. Pulmonol.* **6** (suppl.), 189 (1991); T. A. Hazinski, P. A. Ladd, C. A. DeMatteo, *Am. J. Respir. Cell Mol. Biol.* **4**, 206 (1991).
61. M. Cotten *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 4033 (1990).
62. T. Ferkol, R. Hanson, C. Kaetzel, P. Davis, *Clin. Res*, in press.
63. D. T. Curiel, S. Agarwal, E. Wagner, M. Cotten, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 8850 (1991).

64. I thank M. Drumm and L. Smit for preparing figures and P. Fitzgerald for preparing the manuscript. I am also grateful to numerous members of the CF research community for providing helpful comments and preprints prior to publication, especially Q. Al-Awqati, R. Beall, R. Boucher, G. Cutting, P. Davis, D. Dawson, M. Knowles, P. Quinton, J. Riordan, L.-C. Tsui, M. Welsh, J. Wilson, and members of the Collins, Wilson, and Dawson CF research groups at the University of Michigan. Supported by a Cystic Fibrosis Foundation Research Development Program Center grant and NIH grants DK42718 and DK39690. F.S.C. is an investigator of the Howard Hughes Medical Institute.