Journal of Bioenergetics and Biomembranes, Vol. 33, No. 6, December 2001 (© 2001)

Cystic Fibrosis: A Brief Look at Some Highlights of a Decade of Research Focused on Elucidating and Correcting the Molecular Basis of the Disease

Young Hee Ko[1,2] and Peter L. Pedersen[1,2]

The disease Cystic Fibrosis (CF) is caused by mutations in the protein called CFTR, cystic fibrosis transmembrane conductance regulator, an ABC-transporter–like protein found in the plasma membrane of animal cells. CFTR is believed to function primarily as a Cl^- channel, but evidence is mounting that this protein has other roles as well. Structurally, CFTR consists of a single polypeptide chain (1480 amino acids) that folds into 5 distinct domains. These include 2 transmembrane domains that are involved in channel formation; 2 nucleotide-binding domains (NBF1 and NBF2), the first of which clearly binds and hydrolyzes ATP; and 1 regulatory domain (R) that is phosphorylated in a cAMP-dependent process. Currently, the 3D structure of neither CFTR nor its domains has been elucidated, although both nucleotide domains have been modeled in 3D, and solution structures in 3D have been obtained for peptide segments of NBF1. The most common mutation causing CF is the deletion (Δ) of a single phenylalanine (F) in position 508 within a putative helix located in NBF1. CF patients bearing this ΔF508 mutation frequently experience chronic lung infections, particularly by *Pseudomonas aeruginosa*, and have a life span that rarely exceeds the age of 30. Since the CFTR gene was cloned and sequenced in 1989, there has been over a decade of research focused on understanding the molecular basis of CF caused by the ΔF508 mutation, with the ultimate objective of using the knowledge gained to carry out additional research designed to correct the underlying defect. In general, this pioneering or "ground roots" research has succeeded according to plan. This brief review summarizes some of the highlights with a focus on those studies conducted in the authors' laboratory. For us, this research has been both exciting and rewarding mainly because the results obtained, despite very limited funding, have provided considerable insight, not only into the chemical, molecular, and pathogenic basis of CF, but have made it possible for us and others to now develop novel, chemically rational, and "cost effective" strategies to identify agents that correct the structural defect in the ΔF508 CFTR protein causing most cases of CF.

KEY WORDS: Cystic fibrosis; CFTR; ABC transporter; ion channel; nucleotide domain; protein folding; lung infections; CF drugs; CF drug screening; rational drug design.

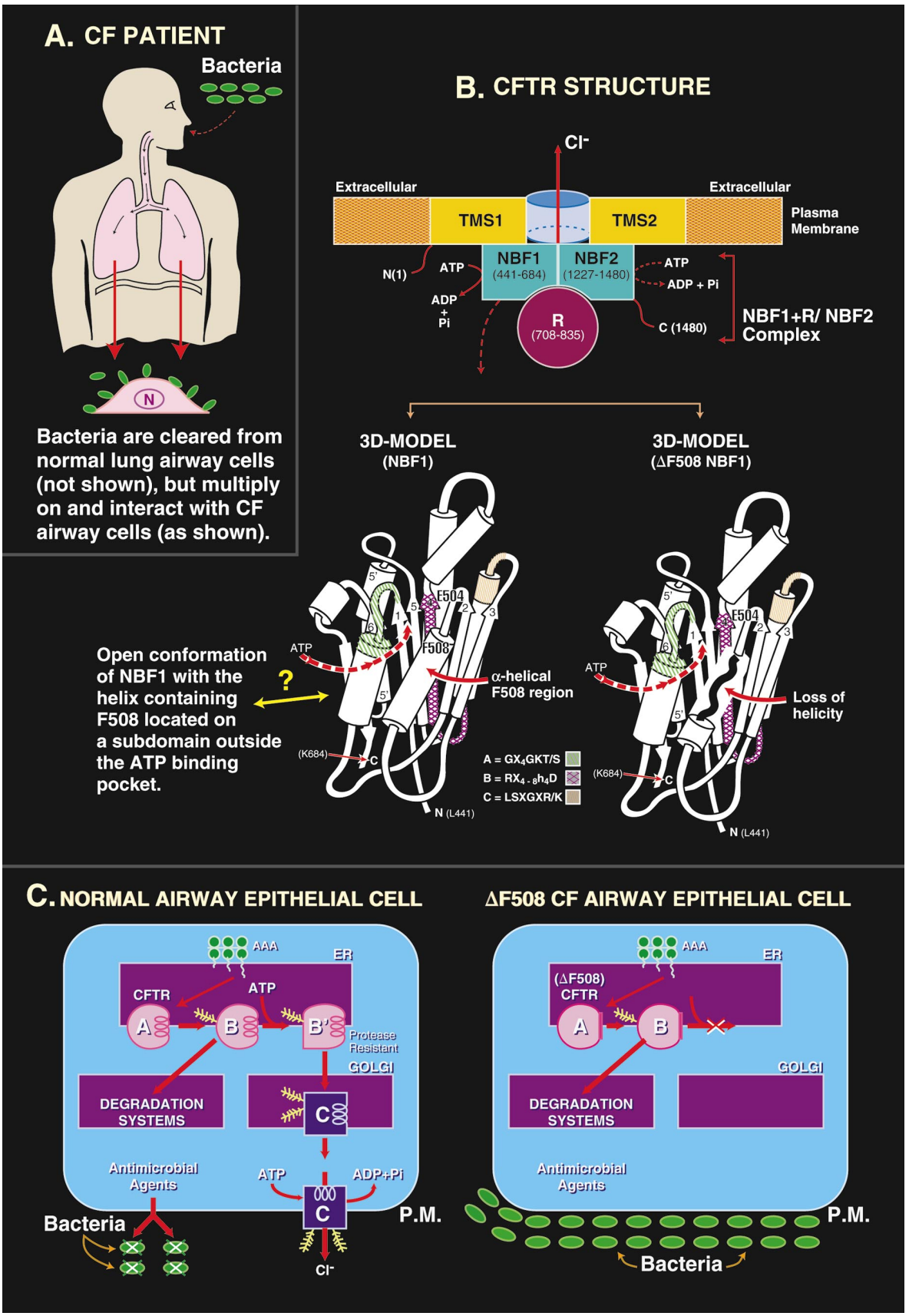
INTRODUCTION

CF remains a life-threatening disease, and to date we do not fully understand its underlying basis, as it relates to the changes induced by disease-causing mutations in the CFTR protein. CF is an autosomal recessive disease of epithelial cell origin, affecting nearly 30,000 people in the United States (Welsh *et al.*, 1995; Welsh and Ramsey, 1998). It is one of the most common lethal genetic diseases known, with a mean patient survival time of 31 years. The disease is characterized by a number of different clinical manifestations, which include an increased sweat Cl^-, the inability to secrete sufficient pancreatic enzymes for digestive purposes, and the inability to combat lung infections (Fig. 1(A)). The increased sweat Cl^- has been a cornerstone to the diagnosis of CF

[1] Department of Biological Chemistry, Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, Maryland 21205–2185.

[2] To whom correspondence should be addressed; e-mail: yko@jhmi.edu or ppederse@jhmi.edu.

0145-479X/01/1200-0513$19.50/0 © 2001 Plenum Publishing Corporation


A. CF PATIENT
Bacteria
N
Bacteria are cleared from normal lung airway cells (not shown), but multiply on and interact with CF airway cells (as shown).
B. CFTR STRUCTURE
Cl-
Extracellular
Extracellular
TMS1
TMS2
Plasma Membrane
NBF1 (441-684)
NBF2 (1227-1480)
R (708-835)
N(1)
ATP
ADP + Pi
C (1480)
NBF1+R/ NBF2 Complex
3D-MODEL (NBF1)
3D-MODEL (ΔF508 NBF1)
Open conformation of NBF1 with the helix containing F508 located on a subdomain outside the ATP binding pocket.
?
α-helical F508 region
Loss of helicity
E504
F508
(K684)
N (L441)
A = GX4GKT/S
B = RX4 - 8h4D
C = LSXGXR/K
C. NORMAL AIRWAY EPITHELIAL CELL
ΔF508 CF AIRWAY EPITHELIAL CELL
AAA
ER
CFTR
(ΔF508) CFTR
Protease Resistant
GOLGI
DEGRADATION SYSTEMS
Antimicrobial Agents
ADP+Pi
P.M.
Bacteria
Cl-

for over 40 years, but unlike pancreatic insufficiency and chronic lung infections, it does not contribute to the mortality caused by the disease. Significantly, pancreatic insufficiency can be treated by supplementing the CF patient's diet with pancreatic enzymes, but to date there is no "magic bullet" for effectively treating lung infections for prolonged time periods. Chronic lung infections (Fig. 1(A)), particularly by *Pseudomonas aeruginosa*, ultimately lead to 90% of the mortality in CF (Welsh *et al.*, 1995; Welsh and Ramsey, 1998). Therefore, CF remains a common life-threatening and lethal disease for many young Americans, and for many other young people throughout the world. It will continue to be life-threatening for future generations until either a cure is found, or until novel therapeutic approaches that consistently ameliorate the symptoms of the disease are devised.

Following the discovery in 1989 (Riordan *et al.*, 1989) of the CFTR gene and the major disease-causing mutation (ΔF508) within this gene, hope soared so high that ΔF508 CF would be cured soon by gene replacement therapy (Korst *et al.*, 1995; Rosenfeld *et al.*, 1993; Wilson, 1993; Zaber *et al.*, 1993) that only a handful of investigators attempted to do *in vitro* work on the CFTR protein or domains thereof. However, in recent years, the hopes for a quick gene therapy cure have been severely dampered by numerous difficult and unforeseen experimental and safety problems (Welsh and Ramsey, 1998; Zahllen, 2000). Consequently, more interest, consideration, and patience has been given to the more conservative approach, first initiated in 1991 in the authors' laboratory (Thomas *et al.*, 1991), of learning more about the CFTR protein *in vitro*, particularly the first nucleotide-binding domain (NBF1) where the major disease-causing mutation (ΔF508) occurs. Significantly, this approach has already provided important new insights into CFTR function and dysfunction, and has led to more rational approaches to "fixing what is broken" by the ΔF508 mutation.

DISCUSSION

The CFTR Protein

This protein, in which mutations causing CF are found, normally traffics, following its synthesis in the E.R., to its site of action in the plasma membrane. Here, acting as a chloride channel, and perhaps as a transporter of a still undefined substrate, or substrates, CFTR aids in combating bacterial infections in the lung. In other tissues where CFTR is also present it may have other roles that remain to be clearly defined.

Domains: Number, Size, and Roles

The CFTR protein (Riordan *et al.*, 1989) is a single polypeptide chain 1480 amino acids in length that is predicted to fold into five domains: two transmembrane-spanning domains (TMS1 and TMS2), two nucleotide-binding domains (NBF1 and NBF2), and a regulatory domain (R) (Fig. 1(B)). Notably, CFTR is a unique member of a large family of ATP-dependent proteins known either as "ABC transporters" or "Traffic ATPases" that are involved in transporting various metabolites, peptides, or ions across biological membranes (Doige and Ames, 1993; Higgins, 1992). CFTR's uniqueness derives from the findings that it is the only known member of the ABC transporter superfamily that has a distinct R domain and the only member believed by many to function predominantly as a channel rather than as a transporter, although a transport function has certainly not been ruled out. TMS1 and TMS2 are each predicted to form six transmembrane-spanning segments, of which the M1 and M6 segments have been implicated in forming the Cl^- channel (Akabas *et al.*, 1997, 1994). The focus of this brief review is on the soluble domains of CFTR, in particular NBF1 and NBF2. Here, the amino acid residues that we predict to constitute NBF1 and NBF2 are, respectively, the 244 residues

←

Fig. 1. Brief overview of cystic fibrosis (CF), the CFTR protein, and the structural, cellular, and pathogenic consequences of the ΔF508 mutation that causes most cases of CF. (A) CF Phenotype. Chronic bacterial lung infections, especially by *Pseudomonas aeruginosa*, represent one of the most common phenotypes of CF patients. (B) The CFTR protein. The protein, a Cl^- channel, which also may be a transporter, is composed of five domains, as indicated. The single phenylalanine (F508), which when deleted causes most cases of CF, is predicted to lie in a flexible α-helical region of NBF1. This helical region is predicted in one conformation of NBF1 to form part of the ATP-binding pocket (Bianchet *et al.*, 1997) but in a second conformation to lie outside this binding pocket as shown in the recent X-ray–derived structure of the His-P protein (Hung *et al.*, 1998). The structural consequences of deleting F508 are believed to be local, causing loss of helicity only in the ΔF508 region (Massiah *et al.*, 1999). This is predicted to impair catalytic function but not to prevent it. (C) Cellular and pathogenic consequences of the ΔF508 mutation. The critical step in the trafficking pathway of CFTR that ΔF508 CFTR is believed not to undergo is the ATP-dependent step (B to B′) in the E.R. Thus, in contrast to normal cells where CFTR traffics to the plasma membrane and operates as a Cl^- channel, ΔF508 CFTR is retained in the E.R. and degraded. As the presence of CFTR in the plasma membrane is necessary to facilitate the natural killing of invading bacteria, its absence in the mutant CF cells results in the multiplication of these infectious microorganisms.

L441–K684 and the 254 residues L1227–L1480 (Bianchet *et al.*, 1997). Earlier predictions (Riordan *et al.*, 1989) for NBF1 (433–589) and NBF2 (1218–1386) fall 50–100 amino acids short of the 200–250 now known to constitute many nucleotide domains based on X-ray structural analysis (Abrahams *et al.*, 1994; Bianchet *et al.*, 1998; Schulz *et al.*, 1974; Story and Steitz, 1992). Therefore, the 3D models for NBF1 and NBF2 described below and in Fig. 1(B) are based respectively on the 244 residues L441–K684 and the 254 residues L1227–L1480.

The NBF1 domain of CFTR clearly binds and hydrolyzes ATP (Howell *et al.*, 2000; Ko *et al.*, 1993; Thomas *et al.*, 1991). This accounts, at least in part, for the recent findings that purified CFTR reconstituted with phospholipid vesicles also catalyzes ATP hydrolysis (Bear *et al.*, 1997; Li *et al.*, 1996; Ramjeesingh *et al.*, 1999). Evidence is less clear that NBF2 catalyzes ATP hydrolysis, as the only direct demonstration for this was obtained using an apparently impure fraction that catalyzed other enzyme activities as well, e.g., adenylate kinase (Randak *et al.*, 1997). As NBF1 and NBF2 exhibit more motif and sequence similarity to the nucleotide domains of the F_1-ATPase (catalytic unit of ATP synthase) (Bianchet *et al.*, 1997) and myosin (Maita *et al.*, 1987) than to those of P-type ATPases (e.g., Na^+/K^+, Ca^{++}, and H^+) (Gunteski-Hamblin *et al.*, 1988; Hager *et al.*, 1996; Kano *et al.*, 1989; Mercer *et al.*, 1993; Takeyasu *et al.*, 1987), it seems likely that the reaction pathway for ATP hydrolysis catalyzed by CFTR does not involve a covalent phosphorylated intermediate. However, covalent phosphorylation of the R domain of CFTR does occur in response to one or more signal transduction pathways (Cheng *et al.*, 1991; Tabcharani *et al.*, 1991), an event that is apparently not required for the Cl^-channel functions (i.e., opening and closing) but rather for optimizing these functions when necessary.

One attractive functional view (Gadsby and Nairn, 1994) based on data, at least in part, obtained in several laboratories (Baukrowitz *et al.*, 1994; Carson *et al.*, 1995; Gadsby and Nairn, 1994; Gunderson and Kopito, 1994; Hwang *et al.*, 1994; Lu and Pedersen, 2000; Ma *et al.*, 1997; Wilkinson *et al.*, 1996) is that the soluble domains form an NBF1+R/NBF2 complex [now demonstrated (Lu and Pedersen, 2000)], and catalyze ATP hydrolysis at one of the two nucleotide domains to drive the opening of the Cl^- channel, and later, ATP hydrolysis at the other nucleotide domain to drive its closing. This would suggest that, within the NBF1+R/NBF2 complex, the catalytic sites alternate in hydrolyzing ATP. Despite its initial attractiveness, this alternating catalytic site hypothesis as it relates to the Cl^- channel function of CFTR is currently facing some very serious challenges. First, there is no direct evidence that NBF2 catalyzes the hydrolysis of ATP, where "direct" refers to the measured formation of ADP and P_i *in vitro* catalyzed by the purified domain free of contaminants. Second, omitting Mg^{++}, which is required for ATPase activity, did not prevent CFTR channels from opening and closing (Schultz *et al.*, 1996). Third, the ATP hydrolytic activity of isolated reconstituted CFTR (in phospholipid vesicles) is not tightly coupled to channel function (Ramjeesingh *et al.*, 1999). Finally, the earlier assumed action of AMP-PNP *in vivo*, i.e., as an inhibitor of NBF2, which had been taken in earlier studies as evidence for the alternating catalytic site hypothesis for the function of CFTR's nucleotide domains, has now been shown to stimulate rather than inhibit NBF2 (Aleksandrov *et al.*, 2001).

When taken together, these two sets of apparently conflicting data may be telling us something very important about CFTR function. Perhaps the simplest interpretation is that CFTR can function both as a Cl^- channel and as a transporter. Chloride channel function may require ATP binding but not ATP hydrolysis, whereas a yet unidentified natural substrate (or family of substrates) may require both ATP binding and ATP hydrolysis to be transported across the plasma membrane. Should this suggestion turn out to be true, it might reconcile the confusion as to why CFTR appears to wear a coat of different color from all other members of the ABC transporter superfamily. Perhaps in its real world CFTR wears two coats, one of a different color from other members of the ABC tranporter superfamily when it acts as a Cl^-channel, and the other of the same color when it acts as a transporter. We the scientists may have become color-blind because our glasses were designed immediately following the discovery of CFTR to focus primarily on a single function, i.e., chloride conductance (Riordan *et al.*, 1989). However, it may now be time for us to purchase new glasses, and take a closer look.

NBF1 and NBF2 Structure

To date, a 3D structure has not been determined for CFTR as the protein has proven very difficult to express in amounts necessary for crystallization trials. However, 3D models based on F_1 ATPase and the Rec A protein have been obtained by the authors and their colleagues (Bianchet *et al.*, 1997) for both of CFTR's nucleotide domains, NBF1 and NBF2. Such modeling was possible because NBF1 and NBF2 of CFTR show significant homology in their sequences to the β and α subunits, respectively, of F_1. The 3D models obtained for NBF1 (Fig. 1(B)) and NBF2 of CFTR are very similar, showing direct contribution of the Walker A and Walker B motifs to the nucleotide binding pocket with a C motif,

a signature for ABC transport proteins (Higgins, 1992), lying near but outside the binding pocket. Significantly, F508, which when deleted causes most cases of CF, is found to reside within an α helix and to contribute to the nucleotide-binding pocket in NBF1. Near the end of this helix is a glutamic acid residue (E504), which, in sequence alignment with the β subunit of F_1, corresponds to a catalytic base believed to be involved in ATP hydrolysis (Abrahams *et al.*, 1994; Bianchet *et al.*, 1998). Predictions of the 3D model for NBF1 gain support from three different experiments. First, consistent with the prediction that this domain should have the capacity to hydrolyze ATP, we have demonstrated this directly in studies with the MBP-NBF1 fusion protein (Ko and Pedersen, 1995), where MBP is the maltose-binding protein. Second, consistent with the prediction that F508 lies within an α helix, we have also demonstrated this directly in a collaborative study (Massiah *et al.*, 1999) by performing NMR studies of a peptide segment representative of this region. Third, consistent with the prediction that F508 contributes in part to the nucleotide-binding pocket and interacts with the purine ring of ATP, we have shown in unpublished work that the same peptide segment of NBF1, on which we completed the NMR work, binds the ATP analog trinitrophenyl-ATP (TNP-ATP).

Subsequent to the publication of our modeling studies on NBF1 and NBF2 (Bianchet *et al.*, 1997), the 3D structure of the nucleotide domain (called His-P) of a distantly related bacterial permease (histidine transporter) was reported (Hung *et al.*, 1998). This His-P structure, determined by X-ray analysis at 1.5 Å in the presence of ATP, shows that the γ phosphate interacts with the Walker A motif, and that the region homologous with the F508 region of CFTR is an α helix, both findings of which are in agreement with our 3D model of CFTR's NBF1 (Bianchet *et al.*, 1997). Interestingly, however, and in contrast to our 3D model of NBF1 (Bianchet *et al.*, 1997), the region of His-P homologous with the F508 region lies outside the ATP-binding pocket on a separate subdomain (Hung *et al.*, 1998). There are several explanations for these differences, one of which is that the His-P protein contains several extra β strands, derived from sequences not found in NBF1, that stabilize its ATP-binding pocket, and therefore there is no need for the helix homologous to the F508 region of NBF1 to serve this role. Another explanation that we find more attractive is that the F508 region of CFTR's NBF1, and the homologous region in the His-P protein, are flexible and both can either constitute part of the nucleotide-binding pocket in one conformation of the domain or lie outside the binding pocket in another conformation (Fig. 1(B)). Thus, when the nucleotide-binding domain (NBF1 of CFTR, or His-P of the histidine permease) is isolated in the absence of their natural partner(s), the F508 region may lie outside the ATP-binding pocket, but when within the intact protein (CFTR or histidine permease), it may form part of the ATP-binding pocket under some cellular conditions and lie outside this pocket under others. Specifically, as it applies to CFTR, the F508 region may have a good functional reason to form part of the ATP-binding pocket when the protein has reached its final destination, i.e., the plasma membrane, but during CFTR's trafficking to the plasma membrane, the F508 region (which is hydrophobic) may reside outside the ATP-binding pocket in order to interact with membranes. Here, it is important to note that several groups (Arispe *et al.*, 1992; Gruis and Price, 1997; Ko *et al.*, 1997a) have documented the capacity of NBF1 to interact with membranes. These include *in vitro* studies in which NBF1 is shown to interact with phospholipid vesicles (Arispe *et al.*, 1992) and *in vivo* studies where NBF1 is shown to interact both with the *E. coli* cytoplasmic membrane (Ko *et al.*, 1997a) and the plasma membrane of animal cells (Gruis and Price, 1997).

Finally, it is important to note that 3D structures of nucleotide-binding domains of two other ABC transporter superfamily members (MalK and MJ0796) have been elucidated recently (Diederichs *et al.*, 2000; Yuan *et al.*, 2001) and shown to contain the F_1-ATPase core structure (Abrahams *et al.*, 1994; Bianchet *et al.*, 1998) predicted originally from modeling work on the CFTR nucleotide domains carried out in our earlier collaborative work (Bianchet *et al.*, 1997).

Trafficking and Role in Lung Pathogenesis

This is an area of CFTR research that has seen a great deal of activity in recent years, and sufficient information is now available to outline the rudiments of the trafficking pathway and speculate on what role the CFTR protein may play in combating bacterial infections when it reaches the plasma membrane of lung-airway epithelial cells (Fig. 1(C)). Briefly, it can be stated that, following its synthesis, the CFTR protein must advance through at least four different forms (Cheng *et al.*, 1990; Lukacs *et al.*, 1994), which will be referred to here as A, B, B′, and C. Form A is the unglycosylated CFTR protein that comes off the ribosome immediately following its synthesis; Form B results when Form A is partially glycosylated in the E.R., after which it must undergo a critical ATP-dependent step to a form designated here as B′. The latter form can exit the E.R. and move on to the Golgi where it is fully glycosylated to become what is designated here as Form C. Trafficking is completed when Form C integrates

into the plasma membrane where it functions as a chloride channel, or as a transporter of an as yet unidentified function. Significantly, past experiments (Ko *et al.*, 1997b; Smith *et al.*, 1996) show that normal-airway epithelial cells have the capacity to kill bacteria plated on their surface, a defense response that cannot be mounted by airway cells from ΔF508 CF patients, where the mutant CFTR protein becomes trapped in the E.R. This has led to the view that CFTR is critical for preventing, or helping to prevent, lung infections—a view that is supported by experiments conducted in the authors' laboratory (Ko *et al.*, 1997b). The exact role that CFTR plays in this defense process is unknown, with one view being that it maintains a salt concentration that is optimal for bacterial killing (Smith *et al.*, 1996) and another view being that CFTR participates directly or indirectly in the transport or secretion of some antibacterial factor (Ko *et al.*, 1997b). Other views have been proposed also (Imundo *et al.*, 1995; Pier *et al.*, 1996), thus emphasizing the need for additional research.

The ΔF508 CFTR Protein

Entrapment and Release From the E.R.

Recent evidence supports the view that this major disease-causing form of CFTR is unable to undergo a critical ATP-dependent step in the E.R. and, rather than trafficking normally to the plasma membrane, becomes targeted for degradation (Fig. 1(C)). Since the key discovery made in 1990 (Cheng *et al.*, 1990) that ΔF508 CFTR becomes trapped in the E.R. and does not traffic to the plasma membrane, a number of important *in vitro* and *in vivo* experiments have been conducted that shed light on the problem involved. Specifically, early *in vitro* circular dichroism studies in the authors' laboratory (Thomas *et al.*, 1991, 1992) on peptide segments representative of the F508 and ΔF508 regions of normal and mutant CFTR showed clearly that the ΔF508 peptide is less structured and less stable than the normal peptide. Subsequently, we suggested that in ΔF508 CFTR there may be a localized protein-folding problem (Thomas and Pedersen, 1993), and that most cases of CF may be due to this problem (Thomas *et al.*, 1992). This view is supported by our more recent *in vitro* NMR collaborative studies of peptide segments representative of the F508 and ΔF508 regions (Massiah *et al.*, 1999, Fig. 2), and also by four different types of experiments *in vivo* which showed that (1) lowering cell temperature from 37 to 25°C (Denning *et al.*, 1992) or addition of protein-stabilizing agents like glycerol (Brown *et al.*, 1996; Sato *et al.*, 1996) causes ΔF508 CFTR to traffic to the plasma membrane; (2) the ΔF508 protein when trapped in the E.R. can function, at least in part, as a Cl^- channel (Pasyk and Foskett, 1995); (3) the normal and ΔF508 CFTR proteins are degraded at similar rates while in the E.R. (Ward and Kopito, 1994); and (4) a significant fraction of the normal CFTR protein can escape degradation in an ATP-dependent step that the ΔF508 protein cannot undergo (Lukacs *et al.*, 1994). Collectively, these studies suggest that the change produced in CFTR's NBF1 domain by the ΔF508 mutation is subtle and most likely confined to the F508 region (Fig. 1(B)) that becomes unstable, and that this region, and F508's presence therein, is critical for CFTR to undergo the ATP-dependent B-to-B′ step (described above) necessary for its exit from the E.R. Finally, it is important to note that there remains some question as to whether ΔF508 CFTR that does traffic to the plasma membrane is fully functional as a Cl^- channel, with some investigators reporting a significant reduction in activity (Dalemans *et al.*, 1991; Denning *et al.*, 1992).

REAPING THE REWARDS OF PAST ACCOMPLISHMENTS AND PROSPECTS FOR THE FUTURE

Although it is clear from this brief review that we still have much to learn about the structure and function of CFTR, we have learned enough about the molecular and chemical basis of ΔF508-induced CF to commence screening for new drugs that may ameliorate the symptoms of most cases of the disease. Although in the best of all possible worlds it might be most effective to replace the defective gene or the defective CFTR protein in patients with CF, progress has been slow and difficult in these areas of research. Rather, from the authors' point of view the major success has come from basic research focused on the molecular and chemical bases of the disease. This has been "tracked down" to what appears to be a localized folding problem within an α-helical region of NBF1 (Fig. 1(B), Fig. 2; see Massiah *et al.*, 1999), now making it possible to screen rationally for agents that repair the ΔF508 defect. We are currently engaged in this research and in so doing are first taking an *in vitro* approach to screen for those nontoxic agents that repair the ΔF508 region in our peptide model (Fig. 2). This screening approach based on rational drug design should be able to select first for a family of compounds that can be tested later in "*in vivo*" cell systems, thus avoiding the rather blind uncertainty and extremely high cost of high-throughput screening approaches that unfortunately have not lived up to their original expectations (Ausman, 2001). Our simple approach may prove useful also in screening for agents

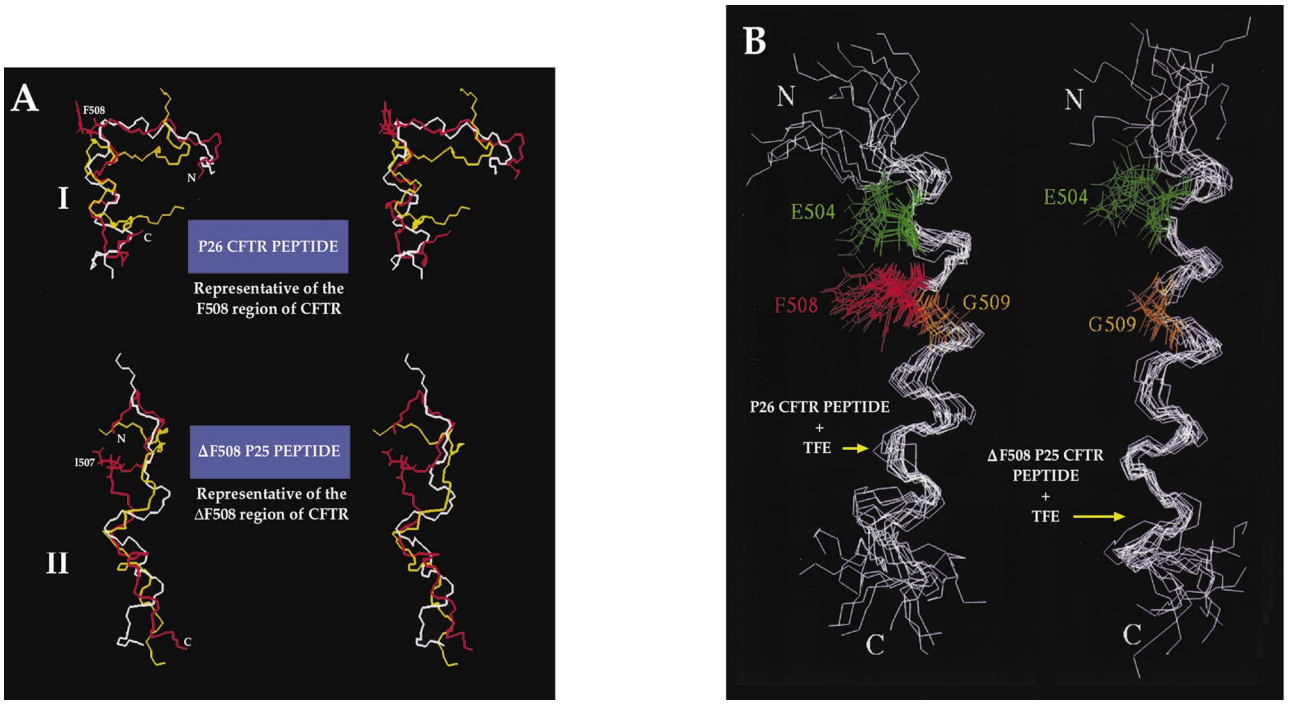


Fig. 2. (A) Solution structures derived by NMR of 26 (I) and 25 (II) amino acid segments representative respectively of the helical region of NBF1 containing F508 and of the same when F508 is deleted (also see Fig. 1(B)). Several structures are shown in each case. The main points are that the NMR data both confirm the modeling predictions that F508 lies in a helical region of NBF1 of the CFTR protein (Bianchet *et al.*, 1997), and demonstrate directly that deletion of F508 causes a loss of this helical structure, thus providing a likely explanation for the molecular and chemical bases of most cases of CF. Significantly, the recent X-ray–derived structure of the His-P protein, a nucleotide-binding domain related to that of the NBF1 of CFTR, also predicts that F508 lies within a helical region (Hung *et al.*, 1998). (B) Solution structures derived by NMR in the solvent trifluoroethanol (TFE) of the 26 and 25 (ΔF508) amino acid NBF1 peptides shown in (A). The main point here is that the solvent TFE is capable of normalizing the structures of both peptides: converting the "diseased" peptide back to its normal predicted structure, i.e., an α helix. The importance of the experiment is it shows that the structural damage to CFTR caused by the ΔF508 mutation can be readily corrected in the "test tube." These findings form the basis of a simple screening assay to identify those agents that may correct the ΔF508 mutation in CF patients. (Both Fig. (A) and (B) were reproduced with permission of the American Chemical Society from Massiah *et al.*, 1999).

that ameliorate the symptoms of other diseases in which the underlying cause appears to be a defect in protein folding (Thomas *et al.*, 1995).

In addition to the above, there is a real need for more investigators to study the CFTR protein in pure form in reconstituted membrane systems (Bear *et al.*, 1997; Li *et al.*, 1996; Ramjeesingh *et al.*, 1999). This approach has provided invaluable information about numerous membrane proteins in the past, and for some it has led to their crystallization as well as the elucidation of their 3D structures at atomic resolution. Amazing examples of the latter are the mitochondrial proteins cytochrome oxidase (Tsukihara *et al.*, 1995) and the b–c_1 complex (Xia *et al.*, 1997) that are composed, respectively, of 13 and 11 different subunit types. Although such accomplishments may be realized by commercial investment, it is likely that the breakthroughs will come from those individuals in a university setting who are both very committed to CFTR research and also very familiar with the difficulties of working with and overexpressing membrane proteins. Currently, what is lacking are new ideas that may lead to new approaches for making large scale preparations of CFTR that can be made available to all interested investigators. Currently, the few methods available for making CFTR (Peng *et al.*, 1993; Ramjeesingh *et al.*, 1997), although representing major accomplishments, do not provide the large amounts of pure CFTR protein necessary for the extensive "*in vitro*" work that needs to be conducted in many different laboratories.

ACKNOWLEDGMENTS

The authors are most grateful for consistent support from the NIH (Grant DK 43962) to PLP that has supported much of our work and that of previous colleagues. The authors are grateful also for earlier support from the CF

foundation (PLP and YHK) and from the American Lung Association, Maryland Division (YHK).

REFERENCES

Abrahams, J. P., Leslie, A. G., Lutter, R., and Walker, J. E. (1994). *Nature* **370**, 621–628.

Akabas, M. H., Cheung, M., and Guinamard, R. (1997). *J. Bioenerg. Biomembr.* **29**, 453–463.

Akabas, M. H., Kaufmann, C., Cook, T. A., and Archdeacon, P. (1994). *J. Biol. Chem.* **269**, 14865–14868.

Aleksandrov, L., Mengos, A., Chang, X., Aleksandrov, A., and Riordan, J. R. (2001). *J. Biol. Chem.* **276**, 12918–12923.

Arispe, N., Rojas, E., Hartman, J., Sorscher, E. J., and Pollard, H. B. (1992). *Proc. Natl. Acad. Sci. U.S.A.* **89**, 1539–1543.

Ausman, D. J. (2001). *Modern Drug Discovery*, American Chemical Society, Washington, DC, pp. 33–39.

Baukrowitz, T., Hwang, T. C., Nairn, A. C., and Gadsby, D. C. (1994). *Neuron* **12**, 473–482.

Bear, C. E., Li, C., Galley, K., Wang, Y., Garami, E., and Ramjeesingh, M. (1997). *J. Bioenerg. Biomembr.* **29**, 465–473.

Bianchet, M. A., Hullihen, J., Pedersen, P. L., and Amzel, L. M. (1998). *Proc. Natl. Acad. Sci. U.S.A.* **95**, 11065–11070.

Bianchet, M. A., Ko, Y. H., Amzel, L. M., and Pedersen, P. L. (1997). *J. Bioenerg. Biomembr.* **29**, 503–524.

Brown, C. R., Hong-Brown, L. Q., Biwersi, J., Verkman, A. S., and Welch, W. J. (1996). *Cell Stress*, Chapter 1, 117–125.

Carson, M. R., Travis, S. M., and Welsh, M. J. (1995). *J. Biol. Chem.* **270**, 1711–1717.

Cheng, S. H., Gregory, R. J., Marshall, J., Paul, S., Souza, D. W., White, G. A., O'Riordan, C. R., and Smith, A.(1990). *Cell* **63**, 827–834.

Cheng, S. H., Rich, D. P., Marshall, J., Gregory, R. J., Welsh, M. J., and Smith, A. E. (1991). *Cell* **66**, 1027–1036.

Dalemans, W., Barbry, P., Champigny, G., Jallat, S., Dott, K., Dryer, D., Crystal, R. G., Pavirani, A., Lecocq, J. P., and Lazdunski, M. (1991). *Nature* **354**, 526–528.

Denning, G. M., Anderson, M. P., Amara, J. F., Marshall, J., Smith, A. E., and Welsh, M. J. (1992). *Nature* **358**, 761–764.

Denning, G. M., Ostedgaard, L. S., and Welsh, M. J. (1992). *J. Cell. Biol.* **118**, 551–559.

Diederichs, K., Diez, J., Greller, G., Muller, C., Breed, J., Schnell, C., Vonrhein, C., Boos, W., and Welte, W. (2000). *EMBO J.* **19**, 5951–5961.

Doige, C. A., and Ames, G. F. (1993). *Annu. Rev. Microbiol.* **47**, 291–319.

Gadsby, D. C., and Nairn, A. C. (1994). *Trends Biochem. Sci.* **19**, 513–518.

Gruis, D. B., and Price, E. M. (1997). *Biochemistry* **36**, 7739–7745.

Gunderson, K. L., and Kopito, R. (1994). *J. Biol. Chem.* **269**, 19349–19353.

Gunteski-Hamblin, A. M., Greeb, J., and Shull, G. E. (1988). *J. Biol. Chem.* **263**, 15032–15040.

Hager, K. M., Mandala, S. M., Davenport, J. W., Speicher, D. W., Benz, E. J., Jr., and Slayman, C. W. (1996). *Proc. Natl. Acad. Sci. U.S.A.* **83**, 7693–7697.

Higgins, C. F. (1992). *Annu. Rev. Cell. Biol.* **8**, 67–113.

Howell, L. D., Borchardt, R., and Cohen, J. A. (2000). *Biochem. Biophys. Res. Commun.* **271**, 518–525.

Hung, L. W., Wang, I. X., Nikaido, K., Liu, P. Q., Ames, G. F., and Kim, S. H. (1998). *Nature* **396**, 703–707.

Hwang, T. C., Nagel, G., Nairn, A. C., and Gadsby, D. C. (1994). *Proc. Natl. Acad. Sci. U.S.A.* **91**, 4698–4702.

Imundo, L., Barasch, J., Prince, A., and Al-Awqati, Q. (1995). *Proc. Natl. Acad. Sci. U.S.A.* **92**, 3019–3023.

Kano, I., Nagai, F., Satoh, K., Ushiyama, K., Nakao, T., and Kano, K. (1989). *FEBS Lett.* **250**, 91–98.

Karpowich, N., Martsinkevich, O., Miller, L., Yuan, Y.-R., Dai, P. L., MacVey, K., Thomas, P. J., and Hunt, J. F. (2001). *Structure* **9**, 571–586.

Ko, Y. H., Delannoy, M., and Pedersen, P. L. (1997a). *Biochemistry* **36**, 5053–5064.

Ko, Y. H., Delannoy, M., and Pedersen, P. L. (1997b). *FEBS Lett.* **405**, 200–208.

Ko, Y. H., and Pedersen, P. L. (1995). *J. Biol. Chem.* **270**, 22093–22096.

Ko, Y. H., Thomas, P. J., Delannoy, M. R., and Pedersen, P. L. (1993). *J. Biol. Chem.* **268**, 24330–24338.

Korst, R. J., McElvaney, N. G., Chu, C. S., Rosenfeld, M. A., Mastrangeli, A., Hay, J., Brody, S. L., Eissa, N. T., Danel, C., Jaffe, H. A., and Crystal, R. G. (1995). *Am. J. Crit. Care Med.* **151**, S75–S87.

Li, C., Ramjeesingh, M., Wang, W., Garami, E., Hewryk, M., Lee, D., Rommens, J. M., Galley, K., and Bear, C. E. (1996). *J. Biol. Chem.* **271**, 28463–28468.

Lu, N. T., and Pedersen, P. L. (2000). *Arch. Biochem. Biophys.* **375**, 7–20.

Lukacs, G. L., Mohamed, A., Kartner, N., Chang, X. B., Riordan, J. R., and Grinstein, S. (1994). *EMBO J.* **13**, 6076–6086.

Ma, J., Zhao, J. K., Drumm, M. L., Xie, J., and Davis, P. B. (1997). *J. Biol. Chem.* **272**, 28133–28141.

Maita, T., Onishi, H., Yajima, E., and Matsuda, G. (1987). *J. Biochem.* **102**, 133–145.

Massiah, M. A., Ko, Y. H., Pedersen, P. L., and Mildvan, A. S. (1999). *Biochemistry* **38**, 7453–7461.

Mercer, R. W., Biemesderfer, D., Bliss, D. P., Jr., Collins, J. H., and Forbush, B. (1993). *J. Cell. Biol.* **121**, 579–586.

Pasyk, E. A., and Foskett, J. K. (1995). *J. Biol. Chem.* **270**, 12347–12350.

Peng, S., Sommerfelt, M., Logan, J., Huang, Z., Jilling, T., Kirk, K., Hunter, E., and Sorscher, E. (1993). *Protein Expr. Purif.* **4**, 95–100.

Pier, G. B., Grout, M., Zaidi, T. S., Olsen, J. C., Johnson, L. G., Yankaskas, J. R., and Goldberg, J. B. (1996). *Science* **271**, 64–67.

Ramjeesingh, M., Li, C., Garami, E., Huan, L. J., Galley, K., Wang, Y., and Bear, C. E. (1999). *Biochemistry* **38**, 1463–1468.

Ramjeesingh, M., Li, C., Garami, E., Huan, L. J., Hewryk, M., Wang, Y., Galley, K., and Bear, C. E. (1997). *Biochem. J.* **327**, 17–21.

Randak, C., Neth, P., Auerswald, E. A., Eckerskron, C., Assfalg-Machleidt, I., and Machleidt, W. (1997). *FEBS Lett.* **410**, 180–186.

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). *Science* **245**, 1066–1073.

Rosenfeld, M. A., Ronald, G., and Crystal, R. G. (1993). *Pathol. Biol.* (Paris) **41**, 677–680.

Sato, S., Ward, C. L., Krouse, M. E., Wine, J. J., and Kopito, R. R. (1996). *J. Biol. Chem.* **271**, 635–638.

Schultz, B. D., Bridges, R. J., and Frizzel, R. A. (1996). *J. Memb. Biol.* **151**, 63–75.

Schulz, G. E., Elzinga, M., Marx, F., and Schirmer, R. H. (1974). *Nature* **250**, 120–123.

Smith, J. J., Travis, S. M., Greenberg, E. P., and Welsh, M. J. (1996). *Cell* **85**, 229–236.

Story, R. M., and Steitz, T. A. (1992). *Nature* **355**, 374–376.

Tabcharani, J. A., Chang, X. B., Riordan, J. R., and Hanrahan, J. W. (1991). *Nature* **352**, 628–631.

Takeyasu, K., Tamkun, M. M., Siegel, N. R., and Fambrough, D. M. (1987). *J. Biol. Chem.* **262**, 10733–10740.

Thomas, P. J., Ko, Y. H., and Pedersen, P. L. (1992). *FEBS Lett.* **312**, 7–9.

Thomas, P. J., and Pedersen, P. L. (1993). *J. Bioenerg. Biomembr.* **25**, 11–19.

Thomas, P. J., Qu, B. H., and Pedersen, P. L. (1995). *Trends Biochem. Sci.* **20**, 456–459.

Thomas, P. J., Shenbagamurthi, P., Sondek, J., Hullihen, J. M., and Pedersen, P. L. (1992). *J. Biol. Chem.* **267**, 5727–5730.

Thomas, P. J., Shenbagamurthi, P., Ysern, X., and Pedersen, P. L. (1991). *Science* **251**, 555–557.

Tsukihara, T., Aoyama, H., Yamashita, E., Tomizaki, T., Yamaguchi, H., Shinzawa-Itol, K., Nakashima, R., Yaono, R., and Yoshikawa, S. (1995). *Science* **269**, 1069–1074.

Ward, C. L., and Kopito, R. R. (1994). *J. Biol. Chem.* **269**, 25710–25718.

Welsh, M. J., and Ramsey, B. W. (1998). *Am. J. Respir. Crit. Care Med.* **157**, S148–S154.

Welsh, M. J., Tsui, L.-C., Boat, T. F., and Beaudet, A. L. (1995). In: *The Metabolic Basis of Inherited Disease* (Scrivers, C. R., Beaudet, A. L., Sly, W. S., and Valle, D., eds.), McGraw-Hill, New York, pp. 3799–3876.

Wilkinson, D. J., Mansoura, M. K., Watson, P. Y., Smit, L. S., Collins, F. S., and Dawson, D. C. (1996). *J. Gen. Physiol.* **107**, 103–119.

Wilson, J. M. (1993). *Nature* **365**, 691–692.

Xia, D., Yu, C.-A., Kim, H., Xia, J.-Z., Kachurin, A. M., Zhang, L., Yu, L., and Deisenhofer, J. (1997). *Science* **277**, 60–66.

Yuan, Y.-R., Blecker, S., Martsinkevich, O., Millen, L., Thomas, P. J., and Hunt, J. F. (2001). *J. Biol. Chem.* **276**, 32313–32321.

Zaber, J., Coutre, L. A., Gregory, R. J., Graham, S. M., Smith, A. E., and Welsh, M. J. (1993). *Cell* **75**, 207–216.

Zahllen, D. T. (2000). *Trends Genet.* **16**, 272–275.