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Frontiers in Research on Cystic Fibrosis: Understanding Its Molecular and Chemical Basis and Relationship to the Pathogenesis of the Disease

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In recent years a new family of transport proteins called ABC transporters has emerged. One member of this novel family, called CFTR (cystic fibrosis transmembrane conductance regulator), has received special attention because of its association with the disease cystic fibrosis (CF). This is an inherited disorder affecting about 1 in 2000 Caucasians by impairing epithelial ion transport, particularly that of chloride. Death may occur in severe cases because of chronic lung infections, especially by *Pseudomonas aeruginosa,* which cause a slow decline in pulmonary function. The prospects of ameliorating the symptoms of CF and even curing the disease were greatly heightened in 1989 following the cloning of the CFTR gene and the discovery that the mutation (ΔF508), which causes most cases of CF, is localized within a putative ATP binding/ATP hydrolysis domain. The purpose of this introductory review in this minireview series is to summarize what we and others have learned during the past eight years about the structure and function of the first nucleotide binding domain (NBF1 or NBD1) of the CFTR protein and the effect thereon of disease-causing mutations. The relationship of these new findings to the pathogenesis of CF is also discussed.

KEY WORDS: Cystic fibrosis; lung infections; *Pseudomonas aeruginosa;* pathogenesis; CFTR; nucleotide binding domain; ATPase; genetic disease.

INTRODUCTION

The disease cystic fibrosis (CF) is an inherited disorder that affects approximately 1 in 2000 people in the United States and Canada.[1,2] Individuals with severe cases frequently die before the age of 30. The disease is characterized by lung infections, pancreatic insufficiency, and increased sweat Cl^- concentration. In particular, the pathogenesis of lung infections in CF and the clinical manifestations and microbiology thereof have been extensively studied and reviewed, and are generally well known among researchers in the field.[3] Briefly, the airways of many people with CF are colonized early in life with bacteria (Fig. 1) and once infection is established it is difficult, if not impossible, to eradicate. Among those bacteria involved are *Staphylococcus aureus, Hemophilus influenzae,* and *Pseudomonas aeruginosa.* As the disease progresses, *P. aeruginosa* is frequently the major organism detected in the sputum, and despite the antibiotic regimen used for treatment, this organism persists and gains multidrug resistance. Complications resulting from such chronic infections are coupled to a slow decline in pulmonary function, often with fatal consequences.

Although mutations in the protein CFTR (cystic fibrosis transmembrane conductance regulator) are known to cause CF, the relationship between these mutations and the pathogenesis of the disease (e.g., lung infections) remains unclear. Nevertheless, tremendous advances have been made in understanding structure–function relationships in the CFTR protein

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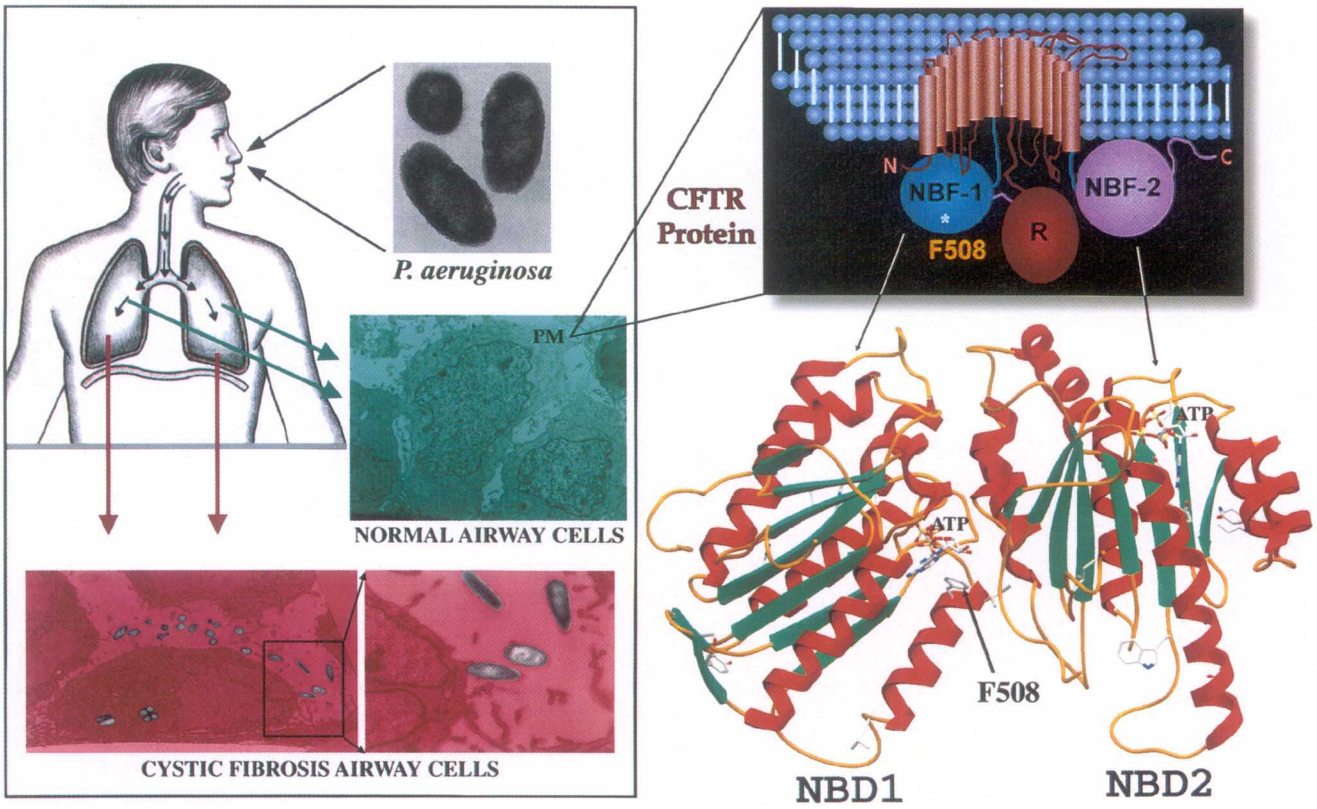

Fig. 1. Relationship of the pathogenesis of cystic fibrosis (CF) to its molecular basis. The left panel compares airway cells from a normal healthy individual with those derived from a cystic fibrosis patient following infection with *Pseudomonas aeruginosa*. After 10 h the medium surrounding the normal lung cells is completely free of bacteria whereas the medium surrounding CF cells contains numerous bacteria. Some have bound to the cells whereas others have entered. Mutations in the protein called CFTR (upper right panel) cause cystic fibrosis, with the deletion of phenylalanine residue F508 within the first nucleotide binding fold/domain (called NBF1 or NBD1) causing most cases (~70%). The ΔF508 mutation prevents NBF1 from folding properly, resulting in its retention in the endoplasmic reticulum. Failure of CFTR to traffic to the cell membrane and function as a Cl^- channel is believed to either prevent the release of bacterial factors including antimicrobial peptides contained within airway cells, or to prevent bacterial killing even when antimicrobial factors/peptides have been released. In the latter case, it is suggested that the bactericidal action of antimicrobial peptides is prevented due to the higher salt content in the airways of CF patients. The lower panel shows a 3D-model of the two nucleotide binding domains of the CFTR protein, which are believed to interact, and also the position of F508. (Adapted from figures in Refs. 4, 31, and 100). In addition, a few biochemical data (68–71) show that the nucleotide binding domain 1 (NBF1) interacts with the cell membrane, implying a dynamic and mobile nature of this domain inside the cell.

during this last decade of the 20th century, and this information is being translated already into testable models for the pathogenesis of the disease.

In the first part of this brief review the authors summarize some of the advances that have been made in understanding structure–function relationships in the CFTR protein, and the effects of disease-causing mutations thereon. In the second part, we review some of the recent models that have been proposed, which attempt to relate disease-causing mutations in the CFTR protein to the pathogenesis of the disease.

THE CFTR PROTEIN: DOMAINS, PROPOSED FUNCTION, AND DISTRIBUTION OF DISEASE-CAUSING MUTATIONS

CFTR is an integral membrane protein[4] comprised within a single polypeptide chain of 1480 amino

acids (Fig. 1). The five major domains include two nucleotide binding folds (NBF1 and NBF2), a regulatory domain (R), and two transmembrane spanning regions (TMSs). The latter, at least in part, form an anion channel that is believed to require for optimal function both ATP binding and ATP hydrolysis mediated by one or both nucleotide binding folds,[5–8] and phosphorylation of the R domain mediated by protein kinase A and/or other cellular kinases.[9,10]

It seems clear from work in many laboratories (reviewed in Refs. 1 and 2, and in the more recent minireviews in this volume) that one function of CFTR is as a Cl^- channel to help regulate Cl^- and Na^+ conductive pathways in epithelial cells. However, more recent work indicates that CFTR may also be involved directly or indirectly in the secretion of ATP,[11,12] which, upon exiting the cell, may stimulate other Cl^- channels via a signal transduction pathway mediated by purinergic receptors. Thus, CFTR may function to regulate other Cl^- secretory pathways in addition to itself conducting Cl^-.

Several hundred putative disease-associated mutations have been reported in the CFTR gene by the CF Genetic Analysis Consortium.[13] Most cause mild forms of the disease, whereas others like ΔF508 can result in a severe form of the disease. Unfortunately, about 90% of all cystic fibrosis patients have been reported to have at least one ΔF508 CFTR allele.[14] Although disease-causing mutations are distributed throughout the CFTR protein,[13] i.e., within all five domains, their frequency is high within or near NBF1 and NBF2, implicating important functional roles of these domains.

Finally, it should be noted that CFTR is a member of a superfamily of proteins referred to as ABC transporters,[15] traffic ATPases,[16] or M-type ATPases.[17] This family includes the multidrug resistance conferring protein, MDR1 or P-glycoprotein, which is involved in drug resistance, the STE-6 protein involved in yeast mating, the TAP proteins involved in antigen processing and presentation, and a number of bacterial permeases.[15,16] Common features of these proteins include much sequence homology particularly within their nucleotide binding domains, and all predicted domain types shown in Fig. 1 except the R domain, which is unique to the CFTR protein. Among those well-characterized ABC transporter family members, CFTR is also considered to be unique in functioning as a gated anion channel rather than as a transporter *per se*.[1,2,4] However, the possible role as a transporter cannot be excluded and other functions of the CFTR protein may yet to be discovered.

Nucleotide Binding Domain 1: Structure, Function, Disease-Causing Mutations, and Subcellular Location

To understand in molecular and chemical terms the underlying cause of most cases of cystic fibrosis, we must first understand the relationship between the structure of NBF1 (also called NBD1) and its functions, and define its subcellular location. Information obtained to date is briefly summarized below.

1. Structure. NBF1, defined on the basis of homology with other nucleotide binding proteins,[4] spans an ~157 amino acid region of CFTR from F433 through S589. It includes a Walker *A* (GX_4GKT/S), a Walker *B* ($RX_{6-8}h_4D$), and a so-called "linker" or *C* consensus (LSXGXR/K). The Walker *A* and *B* consensus are found in many nucleotide binding proteins[18] including adenylate kinases, ATP synthases, the Rec A protein, and all members of the ABC transporter superfamily.[15] The *C* consensus, however, appears to be a rather unique feature of the ABC transporter superfamily.[15]

As no 3-dimensional structural data is available for CFTR, or for any other member of the ABC transporter superfamily, NBF1 has been assumed to fold in a manner similar to adenylate kinase,[20–22] the crystal and partial NMR structure of which are known.[23–26] Recently, however, because of progress made on the crystal structures of two ATPases, the Rec A protein[27] and the mitochondrial F_1-ATPase,[28–30] an improved model for NBF1 (Fig. 2) has been obtained in a collaborative study between the authors' laboratory and the laboratory of Dr. Mario Amzel and Mario Bianchet of this institution.[31] The improvement over earlier models derives from the fact that significant amino acid sequence homology/similarity exists between NBF1 and the mitochondrial F_1-ATPase β-subunit.[31] Moreover, the F_1-β subunit, unlike adenylate kinase, spans the entire NBF1 sequence

The resultant model, presented in simplified form in Fig. 2A and in greater detail in Ref. 31, includes CFTR amino acid residues from L441 through K684, thus comprising >94% of the NBF1 core region (F433 through S589) with an additional 95 amino acids at the C-terminus. (The modeling studies predict that NBF1 is really a domain of 240-250 amino acids rather

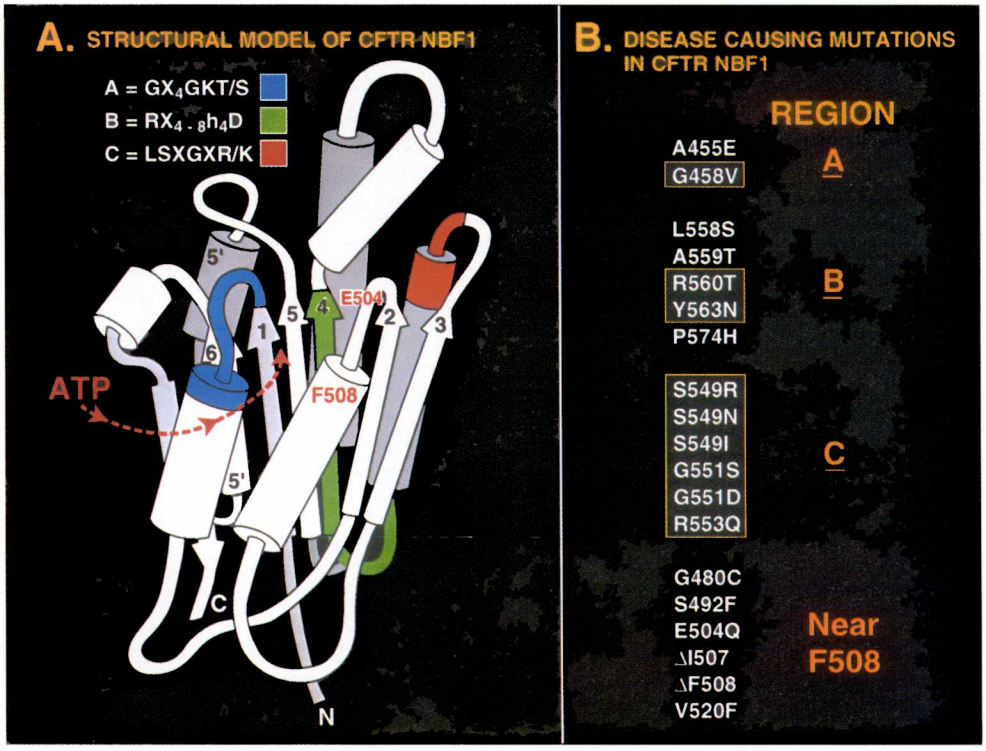

Fig. 2. Diagrammatic representation of a three-dimensional model of the first nucleotide binding domain (NBF1 or NBD1) of the CFTR protein. A. the model is based on the known X-ray structures of the F_1 moiety of mitochondrial ATP synthase,[29,30] and is described in great detail in an article in this issue.[31] The Walker *A* and Walker *B* motifs are indicated in *blue* and *green* respectively, and the *C* or linker region, a signature of ABC transporters, is indicated in *orange*. The position of phenylalanine 508 (F508), which is predicted to lie near the ATP binding pocket, is shown in *red*, at the end of a predicted α-helical region. Also shown in *red* is glutamic acid 504 (E504), a predicted catalytic base, i.e., a residue that facilitates removal of a proton from a water molecule, leaving a hydroxyl ion which attacks the terminal phosphorus atom of ATP, resulting in its release from ADP. Significantly, NBF1 (NBD1) has been shown to function as an ATPase (see Ref. 42) B. A list of mutations in NBF1 (NBD1) that cause cystic fibrosis. With the exception of the *C* region and the predicted catalytic base (E504) it will be noted that mutations that cause CF lie within or near the nucleotide binding pocket. Mutations within the *C* region may cause CF either by inducing a structural change that is transmitted to the catalytic base E504, or by altering interactions of NBF1 with other domains within CFTR.

than the 157 amino acids originally predicted.[4]) The model shows as expected that the Walker *A* and *B* consensus regions reside near ATP and contribute to the nucleotide binding. Significantly, amino acid residue F508 is predicted to lie within bonding distance of the purine ring of ATP, and the nearby E504 is predicted to lie in the same position as the so-called "catalytic base" in the F_1-β subunit.[29] In F_1-β, the catalytic base is believed also to be a glutamate, which promotes ATP hydrolysis by removing a proton from a water molecule allowing the resultant hydroxide ion to facilitate the displacement of the γ-phosphate of ATP.[29] In brief, the catalytic base is one of the most important functional residues within an ATPase, and its replacement by mutagenesis with a more neutral residue is predicted to reduce ATPase activity to very low levels, as in the case of F_1-ATPase.[32,33] Finally, it is important to note that the *C* or "linker region" is predicted to lie nearer the catalytic base than to the nucleotide binding pocket *per se*.

2. *Function.* Consistent with the structural model depicted in Fig. 2, it has been demonstrated in the authors' laboratory,[35,36] and more recently in other laboratories,[22,37] that NBF1, or peptide segments thereof containing the Walker *A* consensus, bind ATP. Moreover, mutations within the Walker *A* consensus

region of NBF1 have been shown to impair CFTR function *in situ*.[38,39] The structural model and *in situ* studies with the nonhydrolyzable ATP analog AMP-PNP[5–7,40,41] predict also that NBF1 may not only bind ATP, but similar to the F_1-ATPase β subunit, may be an ATP hydrolyzing domain. Recently, we have demonstrated this directly with an overexpressed, purified preparation of NBF1 in fusion with the maltose binding protein, MBP.[36] Using three different assays for ATP hydrolysis, we have shown that MBP-NBF1 does catalyze the hydrolysis of ATP by a reaction pathway inhibited both by AMP-PNP and by mutations of the lysine residing in the Walker *A* consensus (GX_4GKT).[42] [It is important to note that neither the F_1-ATPase β-subunit alone[43,44] nor the NBF1 of CFTR alone[22,35] have been shown to catalyze the hydrolysis of ATP. Although F_1-β contains an ATP binding site and a catalytic base, some interaction with the α-subunit,[29] also a nucleotide binding subunit and analogous to the NBF2 of the CFTR protein, is essential to effect catalysis. We know that, in the MBP-NBF1 fusion protein, MBP is undergoing some interaction with NBF1 (36), a stabilizing interaction that in intact CFTR may be provided by another domain, e.g., NBF2. See model in Fig. 1 and in Bianchet *et al.*[31]

In summary, *in situ*, *in vitro*, and modeling studies carried out to date indicate that the functions of NBF1 are to both bind and hydrolyze ATP in order to promote normal CFTR channel function. Significantly, Bear and coworkers[45] have demonstrated recently that the purified CFTR protein reconstituted in liposomes couples ATP hydrolysis to channel gating. Other work[8,46,47] indicates that *in situ* ATP binding/hydrolysis by NBF1 opens the CFTR chloride channel while those events by NBF2 result in its closure.

3. Disease-Causing Mutations. Of the 19 major mutations within NBF1 that cause cystic fibrosis,[13] it is interesting to note that 13 lie within or near the nucleotide binding consensus regions (Fig. 2B). The remaining 6 mutations reside in a centrally located region consisting of an α-helix that connects to a sharp loop. F508, the residue which when deleted causes most cases of cystic fibrosis, lies near the end of the helix and E504, the predicted catalytic base, lies at the top of the connecting loop (Fig. 2A). Consistent with the predicted functional importance of E504 as a catalytic base (see Discussion above), it will be noted that the inherited mutation E504Q also causes cystic fibrosis.[13]

The prediction that F508 resides within an α-helix is based on the secondary structure observed in this region in the crystal structures of known ATPases[28–30] used in our recent modeling studies.[31] Interestingly, all predictive programs for secondary structure indicate that F508 resides within a β-strand region,[35] predictions that are supported by circular dichroism spectroscopy on a 67 amino acid peptide fragment (P67) previously synthesized and studied in the authors' laboratory.[35,48] This suggests, therefore, that in the folding pathway β-strand/β-sheet formation may precede the formation of the α-helix in the region of F508 in the finally folded NBF1 domain. In support of this view, we have now shown that the β-strand/β-sheet structure characteristic of the P-67 peptide studied earlier[35] can be converted to an α-helical structure (unpublished data).

The consequences of the ΔF508 mutation have been studied extensively both at the protein level[22,32,36,48,49] and at the cellular level[50–56] and are generally consistent with the folding hypothesis first proposed by Thomas and the authors in 1992 (Fig. 3).[57] Studies in the authors' laboratory on wild type and ΔF508 peptide segments of CFTR[35,48,49] revealed that ΔF508 induces a marked instability in the peptide, which is accompanied by a significant structural change in a localized region. Studies conducted in other laboratories on ΔF508 CFTR expressed in intact cells demonstrated that the protein is retained within the endoplasmic reticulum at 37°C[50–53] and unlike wild type CFTR fails to become fully glycosylated and traffic to the plasma membrane.[53] However, upon lowering the temperature ΔF508 CFTR was shown to also traffic to the plasma membrane and function as a Cl^- channel.[54–56] More recent studies indicate that ΔF508 CFTR when retained in the ER is functional[58] but more readily targeted for degradation,[59,60] most likely by one or more chaperones[61,62] that recognize the localized structural alteration.

Following up on the suggestion by Thomas and the authors[48] that chemical agents that stabilize protein structure may restore wild type function to ΔF508 CFTR, two different laboratories[63,64] demonstrated that glycerol, when added to cultured cells bearing the ΔF508 mutation in CFTR, resulted in normal trafficking behavior of the mutant protein. Glycerol referred to in these studies by Welch and colleagues[63] as a "chemical chaperone" has recently been studied in depth together with other chemical agents for their relative capacities to restore normal trafficking behavior of the ΔF508 CFTR protein in intact cells.[65]

4. Subcellular Location. There is very little published work about the subcellular location of NBF1.

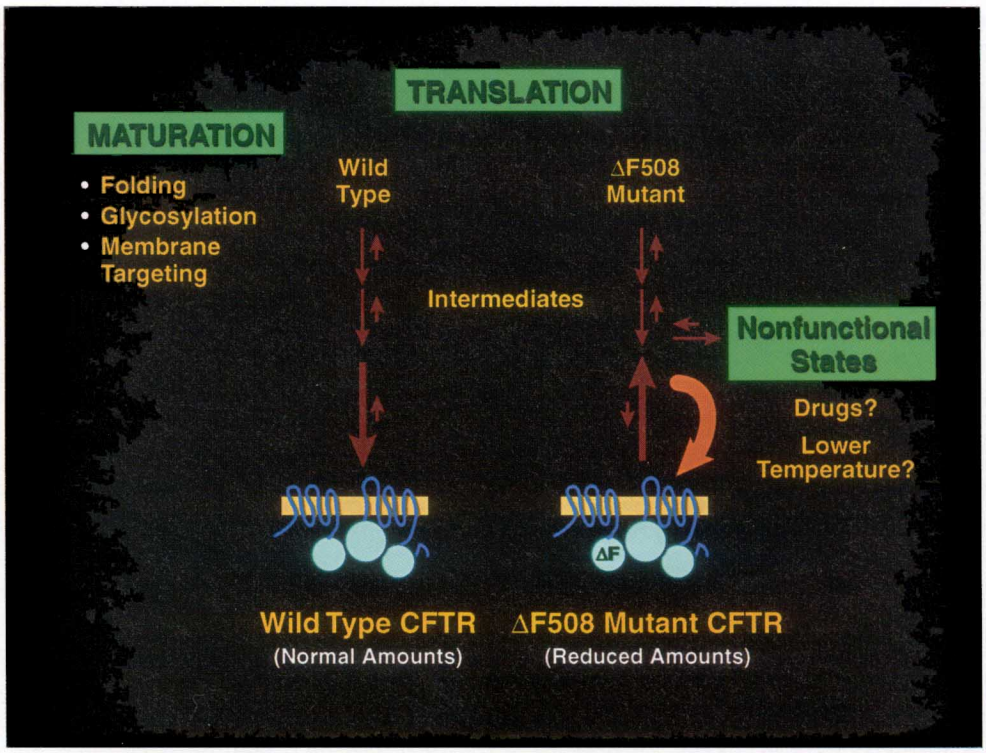

Fig. 3. Comparison of the folding pathways of wild type and ΔF508 CFTR. The figure is from Ref. 57 in which Thomas and the authors first indicated, on the basis of biochemical and biophysical studies on CFTR peptide models,[35,48,49] that ΔF508 cystic fibrosis results from a problem in protein folding. More details about this aberrant folding pathway and chemical chaperones that correct it are found in back-to-back articles in the minireviews by Thomas and colleagues[101] and Welch and colleagues.[65]

The original model[4] and a more recent version[66] from which Fig. 1 was adapted places both NBF1 and NBF2 within the cytosol. However, other published models depict both nucleotide binding domains as being directly attached to the membrane.[12,67] The first published evidence for an interaction of either domain with the membrane was derived from a study conducted with purified NBF1 reconstituted into a planar lipid bilayer.[68] A second report[69] suggested that an α-helical region within NBF1, similar to a comparable membrane binding region in the ATP binding domain of the bacterial His P protein, may interact with the membrane. Significantly, in two other recent studies[70,71] direct evidence has been obtained that NBF1 can interact with the membrane when expressed *in vivo*. It is important to note that NBF1 is predicted to be connected to the anion channel via amino acids extending only from its N-terminus (Fig. 1). Significantly, the cluster of exons 9 through 12, within the CFTR gene originally implicated as NBF1,[4] encodes not only the core NBF1 region (F433-S589), but 29 additional amino acids (G404-N432) extending from the N-terminus of NBF1. Although to date, little is known about this "connecting" region, it seems likely that it plays a critical role in CFTR function.

Nucleotide Binding Domain 2 (Called NBF2 or NBD2) and the R Domain: Relationship to NBF1 and to CFTR Function

A significant number of mutations that cause cystic fibrosis occur also within the NBF2 domain and the R domain.[13,72] Therefore, the relationship of these two domains to NBF1 and to each other is of considerable interest and of potential importance in understanding structure–function relationship within the CFTR protein.

Significantly, NBF2 exhibits considerable amino acid sequence homology to NBF1.[39] Moreover, both domains contain the three consensus sequences *A*, *B*, and *C* mentioned above for NBF1. For these reasons,

NBF2 is predicted according to our recent three-dimensional modeling studies[31] to fold in a manner very similar to NBF1.[31] Relative to the mitochondrial F_1-ATPase, NBF1 is predicted to be more similar to the F_1-β subunit and NBF2 more similar to the F_1-α subunit.[31] Moreover, just as in the F_1-ATPase where the catalytic base, a glutamate amino acid residue,[29,30] is present in the β-subunit but replaced with a glutamine residue in the α-subunit, the same applies to CFTR where E504 is predicted to be the catalytic base in NBF1 but is replaced in NBF2 with glutamine, Q1291.[31] This suggests that NBF2 may hydrolyze ATP at a much lower rate than NBF1, or not hydrolyze ATP at all.

Recent work focused on the function of NBF2 is flavored both with areas of agreement and apparent discrepancies. There is good agreement with the original report from the authors' laboratory[73] demonstrating directly that a 51 amino acid segment of NBF2 containing the Walker *A* consensus can bind ATP. In fact, two different laboratories[22,74] have now shown that purified recombinant preparations of NBF2 bind ATP. To date, however, none of these NBF2 preparations have been reported to hydrolyze ATP, leading to the suggestion that NBF2 may play a regulatory role.[74] However, on the basis of patch clamp studies with CFTR mutated in its nucleotide binding domains a different conclusion was reached, i.e., ATP hydrolysis at NBF1 initiates a burst of Cl^- channel activity and hydrolysis at NBF2 terminates the burst.[8] In support of a requirement for NBF1 in channel function is the finding that the N-terminal half of CFTR alone exhibits channel activity in the presence of ATP.[75]

Recent studies of PKA-dependent Cl^- channel activity indicate that there is significant interdependency and "cross-talk" among NBF1, NBF2, and the R domain. Thus, PKA-dependent activation of Cl^- channel function mediated through the R domain was shown to be inhibited by mutations in the *C* consensus region of either NBF1 or NBF2.[39] In contrast, mutations in the *A* and *B* consensus regions of NBF1 inhibited channel function but those in the same region of NBF2 produced an activation. Finally, ADP was shown to inhibit channel function by a mechanism that could be relieved by mutations in the *A* and *B* consensus regions of NBF2.[38] Although there may be several possible interpretations of these findings, it is difficult to rationalize them without implicating direct interactions of NBF1 and NBF2 with each other and/or with the R domain. For example, phosphorylation of the R domain may induce NBF2 to release ADP causing a conformational change transmitted by direct interaction to NBF1 activating its ATP hydrolytic activity, which in turn induces a conformational change transmitted to the R domain. This final change may then remove blockage of the Cl^- channel by the R domain. Consistent with the above views, NBF1 and NBF2 are predicted to interact,[31] and a conformational change in the R domain upon phosphorylation by protein kinase A has been demonstrated directly.[76] Also, recent work in the authors' laboratory has demonstrated that the [NBF1 + R] combined domain does interact with the NBF2 domain *in vitro*.[77]

Finally, the possibility remains open that all PKA-dependent phosphorylation events do not target exclusively the R domain but may be directed to sites on NBF1 and/or NBF2. Such phosphorylation events might also be important in regulating the functions of these domains, their interaction with one another, and their interaction with other CFTR domains. Consistent with this view is the earlier report[78] that PKA dependent Cl^- channel activity still occurs even when the 10 PKA consensus phosphorylation sites of the R domain have been mutated.

RELATIONSHIP OF THE ABOVE STUDIES TO THE PATHOGENESIS OF CYSTIC FIBROSIS

As indicated in the introduction, the airways of many patients with CF are colonized early in life with bacteria (Fig. 1), and once established the bacterial infection is difficult or impossible to eradicate. This is particularly the case with ΔF508 CF. Special attention has been given to lung infections by *Pseudomonas aeruginosa* as this organism persists and eventually results in a slow decline in pulmonary function. Although considerable effort has been devoted to understanding how well-known components of the immune system, eg., T and B lymphocytes, neutrophils, macrophages, IgG, and complement deal with bacterial infections in CF,[3] a molecular and chemical understanding of why normal healthy individuals do not acquire chronic lung infections while many CF patients do has escaped our notice. Nevertheless, valuable insights into this intriguing mystery are gradually being synthesized, both by taking lessons from how lower life forms defend themselves against invading predators and competitors,[79–82] and how components of our own immune system, particularly phagocytic cells, kill bacteria.[83–87] The common denominator

among the chemical weapons used are that they are small molecules which are stored inside the host cell until an "inducer" from the invading predator or competitor effects their release.[79]

Of particular interest among those chemical weapons used in biological warfare are small peptides. Thus, it is now well known that "melittin," the main toxic component of honey bee venom, is a 26 amino

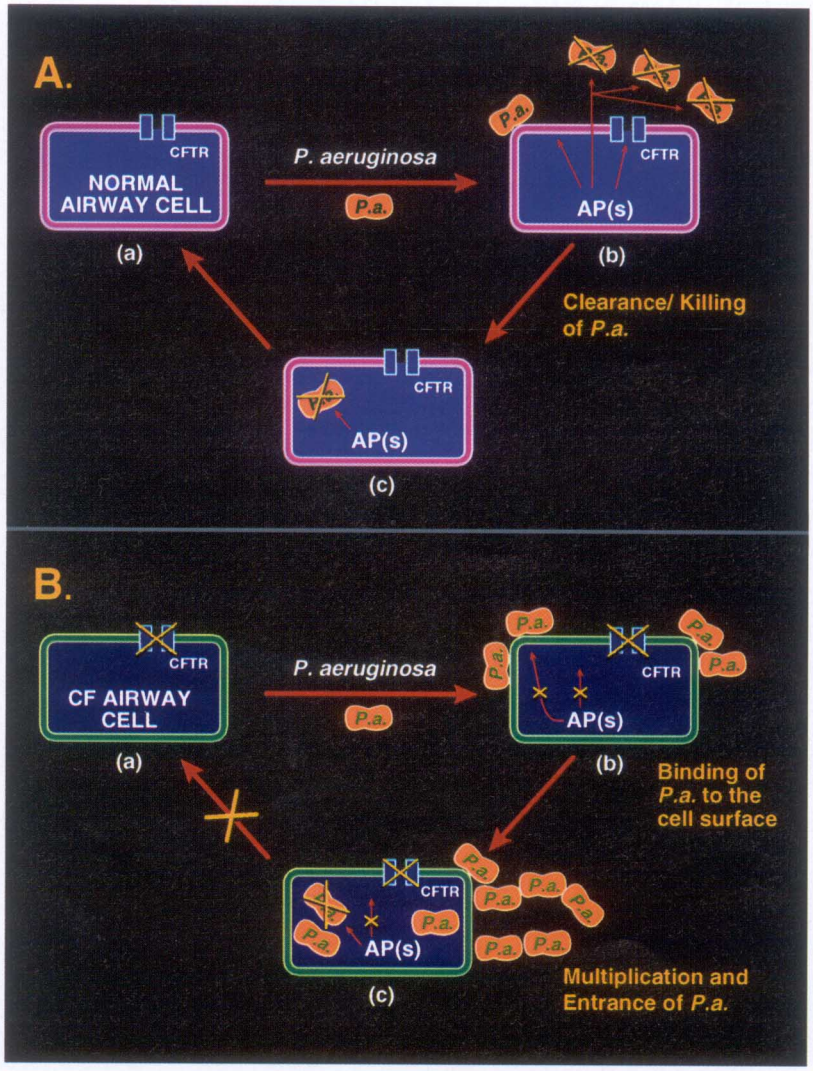

Fig. 4. A working model depicting the possible relationship between the pathogenesis of ΔF508 cystic fibrosis and the CFTR protein. (Adapted from Ref. 100.) *P. aeruginosa* that invade the human lungs of normal healthy people are depicted as being readily killed by antimicrobial peptides (APs) secreted from normal airway cells via a CFTR-dependent mechanism. B, In contrast to the scenario in A, *P. aeruginosa* that invade the lungs of CF patients bearing the ΔF508 mutation in the CFTR protein readily multiply, surround, and bind to the airway cells, with some even entering. One view[100] in the field of CF research is that, in the ΔF508 cells, antimicrobial peptides cannot be secreted because secretion is dependent on a functional CFTR on the plasma membrane surface. The alternative view[97,98] is that antimicriobial peptides are secreted in ΔF508 cells, but because CFTR is defective, the external salt concentration is high, and this high salt prevents the antimicrobial peptide from interacting effectively with the invading bacteria.

acid hemolytic peptide,[84,88] that the "magainins" isolated from the skin of the African toad *Xenopus laevis,* are antimicrobial peptides of only 23 amino acids,[90,91] and that the "defensins" of phagocytic cells of animals and humans are a family of small peptides (MW = 3500–4000).[83,87] Although the detailed mechanism by which these peptides exert their effect is still being resolved, it seems clear that they all have the capacity to "punch" holes in the membranes of their enemies by forming channels.[84,92,93] Consequently, membrane potentials cannot be established, energy (ATP) cannot be generated, nor can ions be pumped, the net result being either a quick death for the invader, or a severe crippling effect.

Recently, several laboratories have taken on the challenge of better understanding how normal and CF cells respond to bacterial infection. Prince[94] and Al-Awquati and colleagues[95] provide evidence that *P. aeruginosa* interact directly with normal and CF cells *via* asialoganglioside 1, the postulated receptor, and that increased amounts of this receptor in CF cells may help explain the pathogenesis. Studies by Pier *et al.*[96] led to the suggestion that normal epithelial cells combat bacterial infections by a phagocytic mechanism involving engulfment of bacteria via CFTR as the receptor, a mechanism proposed to be impaired in CF epithelial cells. Finally, studies by Welsh and colleagues[97] and Wilson and colleagues[98] implicate bactericidal factors, presumably released by normal epithelial cells, as responsible for killing *P. aeruginosa,* and suggest that the release or potency of such factors by CF cells may be impaired because of a high salt concentration. The bactericidal factor identified by Wilson and colleagues[98] is called human β-defensin-1 and shows homology to the bovine protein TAP, previously shown to be located in bovine lungs and to kill *Pseudomonas aeroginosa.*[99]

The authors of this minireview have also examined in great detail the CF pathogenesis problem as it relates to lung infections.[100] Using an *in vitro* assay to examine the capacity of normal and CF tracheal epithelial cells to kill *P. aeruginosa,* the following three key observations were made. First, *P. aeruginosa* do not multiply when planted onto tracheal epithelial cells from healthy humans but do so profusely on cells from ΔF508 CF patients. Second, some bacteria bind, and gain entrance into CF cells, even at a physiological salt concentration (104 mM). Third, human tracheal epithelial cells express an ~4kDa peptide (hTAP), which is known in its bovine form to exhibit bactericidal action against *P. aeruginosa.*[99] To account for these results a plausible working model is depicted in Fig. 4. Thus, one or more antimicrobial peptides (APs) may play a role as part of a first line defense mechanism in a CFTR-dependent manner, functioning outside of, or if necessary inside of, tracheal epithelial cells. This role may be dramatically compromised in diseased (ΔF508) cells.

Finally as it concerns the relationship between structure–function based studies on the CFTR protein and the pathogenesis of lung infections, the following scenario appears to occur. First, the ΔF508 mutation prevents the NBF1 from folding properly within the F508 region. Consequently, ΔF508 is retained in the endoplasmic reticulum by the quality control machinery and targeted for degradation. Second, failure of ΔF508 CFTR to traffic to the plasma membrane either prevents release of antimicrobial peptides from the lung cells as implicated in Fig. 4, or results in an abnormal salt concentration within the airway fluid which interferes with bacterial killing. Further work will be necessary to distinguish between these two possibilities.

In summary, it seems clear that much progress has been made in the past 7 years resulting in a much better understanding of the molecular and chemical basis of CF and its relationship to the pathogenesis of the disease. Testable models are now available, and it seems likely that results obtained therefrom will soon lead to new improved therapies for treating lung infections in CF patients.

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