Journal of Bioenergetics and Biomembranes, Vol. 29, No. 5, 1997

Coupling of ATP Hydrolysis with Channel Gating by Purified, Reconstituted CFTR

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Received April 4, 1997; accepted July 1, 1997

The cystic fibrosis transmembrane conductance regulator (CFTR) is a chloride channel situated on the apical membrane of epithelial cells. Our recent studies of purified, reconstituted CFTR revealed that it also functions as an ATPase and that there may be coupling between ATP hydrolysis and channel gating. Both the ATP turnover rate and channel gating are slow, in the range of 0.2 to 1 s^{-1}, and both activities are suppressed in a disease-causing mutation situated in a putative nucleotide binding motif. Our future studies using purified protein will be directed toward understanding the structural basis and mechanism for coupling between hydrolysis and channel function.

KEY WORDS: Cystic fibrosis; transmembrane conductance regulator (CFTR); chloride channel activity; ATPase activity; purified protein.

MODEL SYSTEMS FOR STUDY OF CFTR

Cystic fibrosis is a lethal disease which is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene (Welsh *et al.*, 1995). Individuals affected with cystic fibrosis present with a diverse set of symptoms localized to the epithelial cells of the respiratory tract, sweat ducts, gastrointestinal tract, and the reproductive organs. Generally, these symptoms are thought to arise from changes in the properties of the fluid bathing the mucosa of these tissues. The high viscosity and stickiness of the mucosal fluid is thought to lead to obstruction of ductular systems and poor clearance of pathogens from the respiratory tract. Substantial evidence now supports the suggestion that CFTR functions as a chloride channel (Anderson *et al.*, 1991; Bear *et al.*, 1992), and it is reasonable to speculate that the absence of this function may form the basis for CF disease, as chloride conductance has been implicated as a driving force for fluid secretion across the mucosa (Frizzell *et al.*, 1979).

Many experimental tools have been generated for the study of normal and mutant versions of CFTR. *In vivo* assays have been developed for the study of ion transport by the epithelial cells of affected individuals (Knowles *et al.*, 1991). The generation of CFTR-knockout mice have permitted the definition of the tissue-specific sequelae of loss of CFTR function (Snouwaert *et al.*, 1992; Rozmahel *et al.*, 1996). Epithelial cell cultures have permitted evaluation of the role of CFTR in cell biology (Haws *et al.*, 1992). While these experimental systems have supported the putative chloride channel function of CFTR, they have also led to the generation of novel models in which CFTR acts to regulate several other cellular functions. Experimental data exists which suggests that CFTR may modify the activity of neighboring membrane transport molecules through various mechanisms (Gabriel *et al.*, 1993; Reisin *et al.*, 1994; Schweibert *et al.*, 1995; Stutts *et al.*, 1995). It has been suggested that CFTR can permit the electrodiffusion of ATP which in turn acts through purinergic receptors to cause a change in function of other channels (Reisin *et al.*,

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0145-479X/97/1000-0465$12.50/0

1994; Schweibert *et al.*, 1995). Alternatively, it has been proposed that CFTR could modify the activity of other membrane proteins through direct protein–protein interactions (Stutts *et al.*, 1995) or by modulating membrane traffick (Prince *et al.*, 1994).

In this review, we have discussed our studies of purified CFTR reconstituted in model membranes (Bear *et al.*, 1992; Li *et al.*, 1996a,b). This experimental system has allowed us to define some of the properties of the protein which are intrinsic to CFTR by direct biochemical and biophysical assay. We will review our studies designed to test some of the predictions relating to the structure and function of CFTR and relate our findings to some of those obtained using alternate experimental approaches. Our discussion will focus on our most recent studies of the catalytic and channel functions of CFTR as these experiments best illustrate the utility of our reconstitution system. Ultimately, we hope that we will be able to use purified CFTR to reconstitute its activity and functional interactions with other proteins in cells in order to assess its role(s) in cellular and organ physiology.

EVIDENCE SUPPORTING THE CHANNEL FUNCTION OF CFTR

Initial modeling of the peptide sequence of CFTR suggested that it may have a transport function due to structural similarities with certain members of the ATP binding cassette (ABC) superfamily whose members include P-glycoprotein (Riordan *et al.*, 1989) and the sulfonylurea receptor protein (SUR) (Inagaki *et al.*, 1995). Further, it was suggested that this transport function may be regulated by interaction with nucleotides and phosphorylation due to the identification of Walker A and Walker B consensus sites for nucleotide binding and consensus sites for phosphorylation by protein kinase A and protein kinase C.

The putative substrates for transport by several members of the ABC superfamily exhibit considerable structural heterogeneity. For example, it has been shown that P-glycoprotein can transport hydrophilic, lipophilic, or amphiphilic substrates (Sharom *et al.*, 1995; Shapiro *et al.*, 1995). Hence, there was animated debate concerning the nature of the substrate transported by CFTR when the gene was initially cloned (Hyde *et al.*, 1990; Kartner *et al.*, 1991). The evidence accrued over the past eight years has demonstrated that CFTR acts as a chloride channel. Its channel function was shown in mutagenesis studies in which substitution of certain charged residues within the putative membrane-spanning domains of CFTR caused changes in single-channel conductance or anion selectivity (Anderson *et al.*, 1991; Tabcharani *et al.*, 1993). Further, we confirmed that the chloride channel function is intrinsic to CFTR in our electrophysiological studies of the purified, reconstituted CFTR (Bear *et al.*, 1992). Fusion of liposomes containing purified CFTR with planar lipid bilayers resulted in the appearance of step-like changes in current consistent with the open and closing of single-ion channels. The channel properties of the reconstituted purified CFTR protein, namely the unitary conductance of 10 pS, selection for anions over cations (10:1), linear current–voltage relationship in the presence of symmetrical chloride concentrations, and regulation by PKA-mediated phosphorylation were identical to those features associated with CFTR expression in native cell membranes (Bear and Reyes, 1992; Tabcharani *et al.*, 1990).

A selectivity sequence for anion permeation from the external to internal membrane through CFTR has been defined; $P_{Br} > P_{Cl} > P_{I}$. (Anderson *et al.*; Tabcharani *et al.*, 1992). Recent reports that the CFTR channel may also permit the permeation of ATP (Reisin *et al.*, 1994; Schweibert *et al.*, 1995) seem to challenge the consensus reached among the above biophysicists regarding the specificity of CFTR for small anions. On the other hand, the hypothesis regarding ATP permeation appears to reconcile the known channel function of CFTR with emerging data supporting a role for CFTR as a regulator for other ion channels. Hypothetically, efflux of ATP through CFTR could lead to purinergic receptor activation and stimulation of signaling mechanism which could in turn converge on neighboring ion channels (Schweibert *et al.*, 1995). Recently, we examined the ability of purified CFTR to conduct ATP directly using our reconstitution system (Li *et al.*, 1996). We found that, even in the presence of a very large favorable electrochemical driving force, no ATP conductance through purified CFTR could be detected. Therefore, while there may exist indirect mechanisms through which CFTR mediates ATP transport, CFTR itself does not provide a conductance path for this bulky anion.

EVIDENCE FOR REGULATION OF CFTR FUNCTION BY PHOSPHORYLATION

The putative secondary structure of CFTR contains a unique cytoplasmic domain, the R domain

which possesses several consensus sites for PKA and PKC phosphorylation and links the two halves of the molecule (Riordan *et al.*, 1989). Subsequent studies have provided direct biochemical evidence that the R domain is phosphorylated by PKA at multiple sites (Cheng *et al.*, 1991; Chang *et al.*, 1993). The purified, soluble R domain can be phosphorylated by PKA and analyses of circular dichroism spectra revealed that the structure of the isolated R domain is altered by this modulation (Dulhanty and Riordan, 1994).

As previously discussed, patch clamp studies of CFTR channel activity shows that phosphorylation by PKA is essential for channel gating (Tabcharani *et al.*, 1991). The structural and functional consequences of phosphorylation at multiple sites on the R domain has yet to be understood. Although four serines residues are predominantly phosphorylated *in vivo* (Cheng *et al.*, 1991), phosphorylation of the remaining sites has also been shown to modify the activation properties of the CFTR channel (Chang *et al.*, 1993). Studies wth recombinant, purified R domain suggest that unphosphorylated R domain binds to the channel pore region and phosphorylation acts to inhibit this interaction (Ma *et al.*, 1996). However, as discussed later in this review, the effect of phosphorylation on channel gating is likely to be more complex than simply removing an inhibitory effect. Recent biophysical data suggests that phosphorylation of serine residues on the R domain may have two effects, first to activate channel function and second, to modulate channel function once it has been stimulated (Hwang *et al.*, 1994). Future studies with purified, reconstituted protein will be aimed at understanding the structural basis for this differential regulation.

EVIDENCE FOR REGULATION OF CFTR FUNCTION BY INTERACTION WITH NUCLEOTIDES

Evidence for Nucleotide Binding

As predicted on the basis of sequence homology with other nucleotide-binding proteins, CFTR possesses two putative nucleotide-binding folds (Riordan *et al.*, 1989). Direct biochemical evidence exists which confirms that the recombinant CFTR protein in cell membranes binds the nucleotide analogue, 8-N_3ATP (Travis *et al.*, 1993). We have shown that purified, reconstituted CFTR binds the fluorescent ATP analog, TNP-ATP, and this interaction can be competed by the natural ligand (Ramjeesingh *et al.*, 1995). Further, both nucleotide-binding folds have the capacity to bind ATP as shown in TNP-ATP binding studies of synthetic peptides corresponding to the first and second nucleotide-binding folds (NBFs) (Hartman *et al.*, 1992; Thomas *et al.*, 1991; Ko *et al.*, 1994). However, the number of nucleotide-binding sites that need to be occupied to confer channel function has yet to be determined.

Evidence for ATP Hydrolysis

To date we, and others, have been unable to study CFTR ATPase activity in biological membranes. Due to the lack of specific agonists or antagonists of CFTR function it has been impossible to distinguish the ATPase activity due to CFTR from the activity of other ATPases, such as Na^+/K^+ ATPase and V-type ATPases which are known to reside in the biological membranes. Recently, using methods employed in the study of purified GT Passes (Gout *et al.*, 1993) we have shown that purified, reconstituted CFTR protein is capable of hydrolyzing ATP (Li *et al.*, 1996). We found that ATP hydrolysis by purified, reconstituted CFTR (V_{max} = 50 nmol/mg/min) is very slow in comparison with ion-translocating ATPases such as Na/K ATPase ($V_{max} \geq$ 800–1000 nmol/mg/min) (Racker, 1985) and the ABC superfamily member P-glycoprotein (V_{max} = 300–1000 nmol/mg/min) (Shapiro and Ling, 1994; Sharom *et al.*, 1995) and higher than the hydrolysis rate exhibited by small G proteins like Ras (V_{max} = 0.4 nmol/mg/min) (Bourne *et al.*, 1991). We confirmed that this slow rate of hydrolysis measured in CFTR proteoliposomes was, in fact, due to CFTR by showing that the activity was directly correlated to the quantity of purified protein. Further, we showed that a purified, mutant version of CFTR, CFTRG551D, known to be associated with human disease (Tsui, 1995), exhibited defective ATPase activity in our reconstitution system. Clearly, it will be important to develop methods for the study of the ATPase activity of CFTR in native epithelial cell membranes in order to establish the role of the lipids and proteins which normally provide the context for this activity.

We found that, as with its chloride channel activity, CFTR ATPase function is dependent upon phosphorylation. While constitutive ATPase activity by CFTR can be measured prior to treatment by exogenous PKA, this basal level of activity is abrogated by the phosphatase, PP2A, a phosphatase which has been

shown to dephosphorylate recombinant CFTR in an independent set of studies (Berger *et al.*, 1993). Phosphorylation by addition of PKA enhanced the ATPase activity of CFTR by two- to threefold and this stimulatory effect was related to a reduction in apparent K_m from 1 to 0.3 mM rather than a change in the V_{max} of the reaction. Hence, phosphorylation probably acts to stimulate ATP hydrolysis by increasing the affinity of CFTR for ATP. The shape of the function describing ATP dependence of ATPase activity changed from hyperbolic, for the partially phosphorylated protein, to sigmoidal for the PKA-treated, fully phosphorylated protein, suggestive of the induction of cooperativity between catalytic sites.

We have yet to determine the structural basis for CFTR ATPase activity. It will be of interest to determine if the structural basis for catalytic activity of CFTR is comparable to P-glycoprotein. While both nucleotide binding domains (NBDs) of P-glycoprotein appear capable of hydrolyzing ATP when expressed separately, in the intact molecule, the two NBDs likely function in a cooperative fashion (Loo and Clarke, 1995). Loo and Clarke have shown that mutations within ATP binding motifs of either NBD totally abrogates the ATPase activity of the intact molecule. Further, kinetic analyses of the ATPase activity of intact P-glycoprotein reveals a single apparent K_m, suggesting that there may be one catalytic site. Senior and colleagues have formulated a model for the catalytic activity of P-glycoprotein whereby the structural basis for ATPase activity may alternate between each nucleotide-binding domain (Senior *et al.*, 1995b) Hence, we plan to compare the catalytic activity of each NBD of CFTR independently and also within the context of the entire molecule. The role of the first nucleotide-binding domain (NBD1) was assessed in our studies of the catalytic activity of a mutation, CFTRG551D, which lies between the Walker A and Walker B nucleotide-binding motifs of NBD1 within a linker motif (D-X-[G/A]-G-Q). This linker motif is conserved among several ATPase and GTPases and is thought to participate in nucleotide binding and hydrolysis (Manavalan *et al.*, 1995). In the present studies, we determined that purified, reconstituted CFTRG551D protein exhibits altered ATPase activity. It is not yet clear whether defective ATPase activity by this mutant is due to an alteration in ATP affinity and/or ATP turnover; however, studies assessing nucleotide binding to a synthetic peptide corresponding to the variant NBD1 possessing the mutation G551D, suggest that this mutation may act to decrease binding affinity for nucleotide (Hartman *et al.*, 1992). Our results suggest that the first nucleotide-binding fold is essential for the ATPase activity of CFTR and support the findings of Ko and Pedersen (1995) who reported that a fusion protein containing a large fragment of NBF1 was capable of binding and hydrolyzing ATP. We have not yet determined whether ATP is hydrolyzed by NBD2 or if the two NBDs need to interact with one another to generate maximal catalytic rates. So far, data from studies of synthetic peptides corresponding to NBD2 suggest that this domain is capable of binding ATP, but as yet there is no direct biochemical evidence that it can hydrolyze ATP (Ko *et al.*, 1994). These questions will be addressed in our future studies of purified CFTR protein possessing mutations within the Walker A, Walker B, and linker region in the NBD2. We also plan to define the structural bases for the stimulative effects of phosphorylation on catalytic activity. Hypothetically, there are structures within the R domain which are inhibitory to ATP binding and hydrolysis until phosphorylated.

EVIDENCE FOR COUPLING OF CATALYTIC AND TRANSPORT FUNCTIONS OF CFTR

It was first shown by Anderson and Welsh (1991a) and subsequently by Carson and Welsh (1993), in patch clamp studies of transfected cells that ATP hydrolysis is required for channel opening by phosphorylated CFTR. The probability of channel opening exhibited a complex dependence on ATP concentration, which was described using a model whereby nucleotides interact with CFTR at two sites to affect channel gating, i.e., transitions between the open and closed conductance levels. Evidence provided by Baukrowitz *et al.* (1994) and Gunderson and Kopito (1994) supported the proposal that ATP hydrolysis was not only required for channel gating to the open state but also for gating to the closed state, as nonhydrolyzable nucleotide analogs such as AMP-PNP and analogues of the transition state inorganic phosphate inhibited channel closure. Further, Doumanis *et al.* (1996) showed that both channel opening and channel closure exhibit a dependence on magnesium ion, an essential co-factor in most hydrolytic reactions. Hence, these patch clamp studies favored a model in which ATP hydrolysis at one site fueled channel opening whereas ATP hydrolysis at a distinct second site led to channel closing. If this model is valid, one would predict that the rate of channel

gating would be tightly coupled to the rate of ATP hydrolysis by CFTR.

Our studies using purified protein permitted direct comparison of the rates of channel gating with rates of ATP hydrolysis by CFTR. Planar bilayer studies allowed us to quantitate the rate of channel gating by purified, reconstituted protein and verified patch clamp studies which showed that CFTR exhibits a "bursting" pattern of gating (Winter *et al.*, 1994; Gunderson and Kopito, 1994). We found that both the transition rate to the open burst, i.e., the bursting rate, and the transition rate to the interburst, closed state, were dependent on ATP concentration (Fig. 1b). The opening or bursting rates and closing rates of CFTR channel activity respond differently to increasing ATP concentrations. The effective bursting rate increased and the closing rate decreased with increasing ATP concentration. This observation contrasts with the reported effects of magnesium ion titration on channel gating, in which increasing Mg^{2+} concentrations cause increases in both the opening and closing rates of the CFTR channel (Doumanis *et al.*, 1996). We have established that magnesium ion is required for CFTR ATPase activity (Li *et al.*, 1996b); hence, we interpret the above results to support a model for CFTR gating whereby there are both nonhydrolytic and hydrolytic roles for ATP in

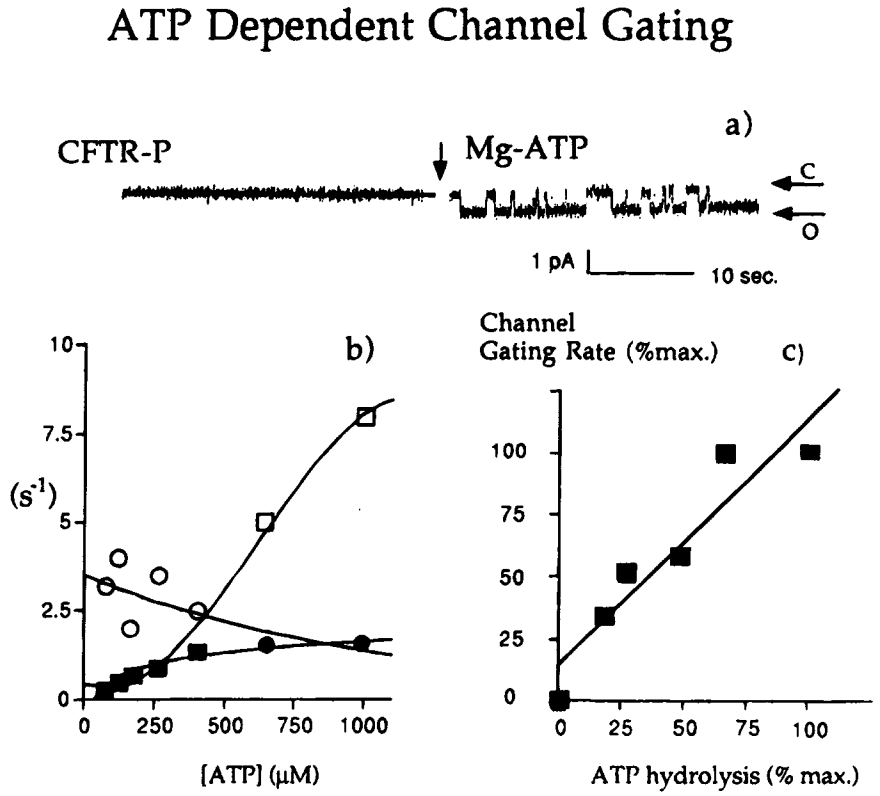

Fig. 1. ATP-dependent gating of the CFTR channel. (a) activation of a purified, PKA-phosphorylated CFTR channel by the addition of 1 mM Mg-ATP. In this experiment, purified CFTR, reconstituted in phospholipid liposomes, was phosphorylated by treatment with the catalytic subunit of PKA (75 units) plus 1 mM MgATP. Subsequently, the proteoliposomes containing phosphorylated CFTR were passed through a gel filtration column to remove PKA and collected in the void volume. The proteoliposomes were added to the *cis* compartment of our bilayer chamber to assess channel activity. Clearly, the phosphorylated protein is not channel competent until Mg-ATP is added, and once stimulated the channel exhibits slow gating from the closed (c) to the open (o) configuration. (b) ATP dependence of both the channel opening rate to a burst ("bursting rate") (squares) and the channel closing rate (circles). Assuming that these two rates are dependent on one another, the overall gating rate will be limited by the slower of these two rates at each concentration of ATP (blackened symbols). The overall gating rates were fitted using a Michaelis–Menten equation to yield an apparent K_m for ATP of 367 μM. (c) Linear relationship (r = 0.95) between the channel gating rate by CFTR and the rate at which CFTR hydrolyzes ATP.

CFTR channel gating. Elevations in ATP concentration may lead to a decrease in the closing rate of CFTR due to an effect caused by ATP binding rather than hydrolysis. This interpretation is consistent with previous reports which show that the nonhydrolyzable analogue of ATP, AMP-PNP, stabilizes the channel open state (Hwang *et al.* 1994; Gunderson and Kopito, 1994). The transitions between the channel burst state and the channel closed state are not independent; hence, at low concentrations of ATP (<300 μM), the overall transition rate will be limited by the slow rate of channel opening and at higher ATP concentrations (>500 μM) the slower closing rate will limit gating. The limiting gating rates could be fitted with the Michaelis–Menten equation to yield an apparent K_m for ATP of 360 μM and a maximum gating rate of approximately 2 transitions per second.

Our data support the hypothesis that channel gating and ATP hydrolysis by CFTR are directly coupled. In Table I, we show that the apparent K_m values for ATP are similar for both functions, approximately 300 μM. In Fig. 1c, we show that there is a linear relationship between the overall rate of channel gating and the rate of ATP hydrolysis determined at varying ATP concentrations, when both rates are expressed as a percentage of the maximum, suggesting that there is a direct relationship between these two activities. With respect to the absolute rates, we found that our estimation of the maximum overall rate of CFTR gating exceeds our approximation of the maximum rate of ATP hydrolysis by CFTR by approximately 5- to 10-fold. This discrepancy could indicate that the coupling between ATP hydrolysis and channel gating is not tight. Hence, the hydrolysis of one molecule of ATP may lead to multiple gating events. Alternatively, the discrepancy may be due to a faulty assumption inherent in our quantitation of ATPase activity. Whereas we quantitate the rate of channel gating from measurements of single, channel-competent molecules of CFTR, the rates of ATP hydrolysis are determined as an average from a batch of proteoliposomes or a population of CFTR molecules. We suggested in our original description of the purification and reconstitution schemes for CFTR (Bear *et al.*, 1992) that the proportion of functionally reconstituted CFTR molecules relative to nonfunctional molecules may be as low as 1:10. Therefore, the turnover number for ATPase activity of CFTR could be as high as 2/s and may be more tightly coupled to channel gating than our quantitation suggests. Ultimately, we have to devise methods for measuring the hydrolysis of ATP by a single CFTR molecule in order to define the coupling ratio for these two functions. Such a goal may not be outside our reach forever as such methods are being currently being refined in studies of myosin (Warshaw, 1996).

Table I. Comparison of Catalytic and Channel Properties of CFTR[a]

Activity	Turnover number or transition number	K_m (app)	Regulation
Catalytic	0.22/s	300 μM	PKA/PP2A
Channel gating	1–2/s	360 μM	PKA/PP2A

[a] The values for turnover number (molecules of ATP hydrolyzed by each CFTR molecule per second), transition number (gating events per second), and apparent K_m (ATP) were determined using experimental data described in Li *et al.* (1996). Phosphorylation by PKA enhances, whereas dephosphorylation by PP2A abrogates, channel gating (Berger *et al.*, 1993) and ATPase activity (Li *et al.*, 1996).

Further evidence for coupling between the catalytic and channel functions of CFTR is found in our studies of the disease-causing CFTR variant, CFTRG551D. Both the catalytic and channel gating activities of CFTRG551D are impaired relative to the wild type protein. The ATPase activity of CFTRG551D is less than 10% of the normal protein and in the lipid bilayer, channel openings of the mutant protein are rare and brief. Possibly, the linker motif in which G551D resides in NBD1 is critical for the coupling of these functions.

We predict that the R domain also has an important role in coupling the catalytic and transport function of CFTR as both activities are dependent upon phosphorylation. As previously mentioned, our kinetic analysis of CFTR ATPase activity suggests that phosphorylation of CFTR by PKA increases its affinity for ATP, possibly by engaging the cooperative function of a second catalyic site (Li *et al.*, 1996b). Likewise, Hwang *et al.* (1994) have suggested that a high level of PKA phosphorylation appears to unmask a second site at which nucleotides interact to alter channel gating. In Fig. 2, we have depicted a model which represents how the ATPase activity of CFTR may be coupled to channel opening through phosphorylation. The left-hand side of the picture depicts the closed CFTR channel. This protein may be partially phosphorylated in this state and capable of weak hydrolytic activity; however, this weak ATPase activity is not sufficient to stimulate the conformational change in the transmem-

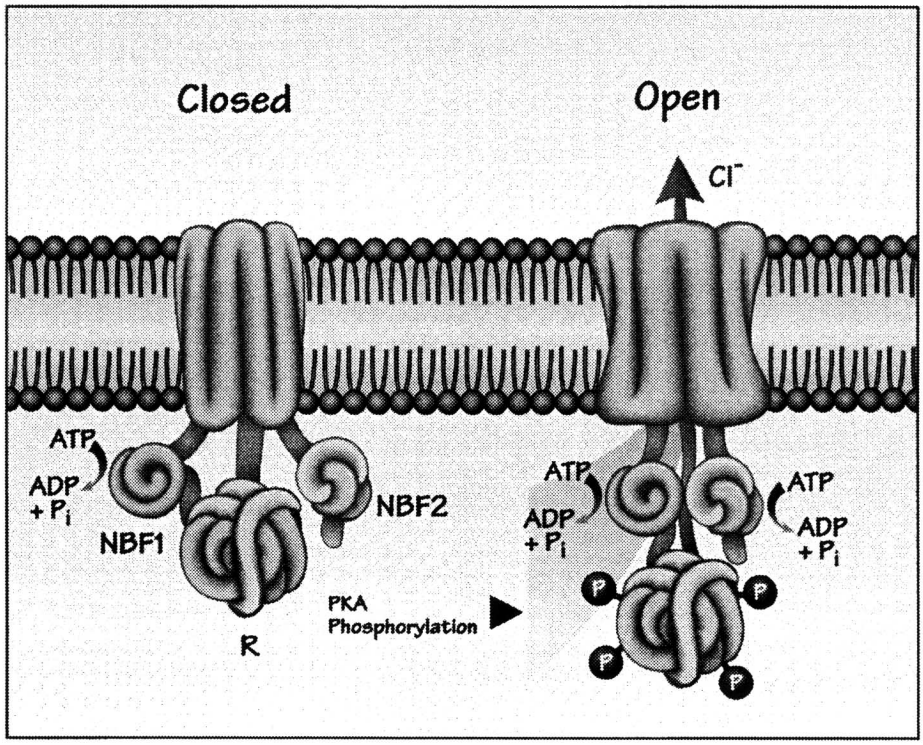

Fig. 2. Model for coupling between CFTR channel and ATPase activities. In this cartoon, the left-hand side of the picture depicts the closed CFTR channel. This protein may be partially phosphorylated and capable of weak hydrolytic activity; however, this weak ATPase activity is not sufficient to stimulate the conformational change in the transmembrane segments necessary for channel opening. On the other hand, if higher levels of phosphorylation of the R domain are induced by PKA, ATPase activity by CFTR is increased beyond a threshold required to induce a conformational changes in the pore region of the protein leading to opening of the channel. Artwork by S. Diamant, bio.art incorp.

brane segments necessary for channel opening. On the other hand, if higher levels of phosphorylation of the R domain are induced by PKA, ATPase activity by CFTR is increased beyond a threshold required to induce a conformational changes in the pore region of the protein leading to opening of the channel. We speculate that phosphorylation may enhance ATPase activity by facilitating the functional and/or structural interaction of the two nucleotide-binding domains.

Finally, it is clear that we still have much to learn about the properties of the CFTR protein before we can comprehend its mechanism of action. Once we have achieved a more sophisticated understanding of this protein, then we may address some of the questions pertaining to the role of this protein in the biology of the epithelial cell. As we pointed out at the outset of this review, there are many questions of immediate interest to cell biologists that we can only treat in a cursory fashion at this time. For example, why is this apical chloride channel regulated in such a complex manner? To our knowledge, there is no other ion channel which requires the hydrolysis of ATP for the operation of its gating mechanism. At this stage in our investigations, CFTR appears as a unique hybrid between an ion channel and an ion-translocating ATPase. Like the P-type ATPases, Na^+/K^+ ATPase, and Ca^{2+} ATPase, CFTR likely utilizes ATP to drive a conformational change permissive to its transport function. However, there are many dissimilarities between CFTR and these transporters. Unlike the two P-type ATPases, there is no evidence to suggest that a "phospho-intermediate" is involved in the transport cycle. Further, the ion-translocating ATPases can act to move their transport substrates against a concentration gradient, whereas chloride ion moves down its electrochemical gradient through open CFTR. Finally, the stoichiometry for coupling between ATP hydrolysis and transport for the two types of protein is different. Three sodium ions are transported out of the cell and two potassium ions are translocated inward for each

ATP molecule hydrolyzed by the Na^+/K^+ ATPase and completion of the transport cycle. For CFTR, if our predictions prove correct, two molecules of ATP need to be hydrolyzed for completion of the gating scheme from the closed to the open channel and back to the closed channel state again. Hence, the hydrolytic rate sets the timing of this reaction. During the complete gating reaction, chloride ion will flux through the CFTR channel at a rate of approximately a million ions per second. Although ATP is hydrolyzed by the Na^+/K^+ ATPase at a rate which far exceeds the catalytic activity CFTR (Racker, 1985), its transport capacity is much less than that of CFTR. Hence, CFTR appears to combine the rapid ion throughput rates of a channel with a timing mechanism inherent in ATPases.

As pointed out by Quinton and his colleagues several years ago, (Quinton and Reddy, 1992), coupling an ATP-dependent gating mechanism to the CFTR channel activity may confer exquisite sensitivity to the metabolic status of the cell. Continuous chloride secretion is likely to be expensive in terms of cellular energy, and the unique gating mechanism of CFTR will limit secretion if intracellular ATP concentrations (Quinton and Reddy, 1992) sink below a certain optimal level. Our measurement of the catalytic activity of CFTR revealed a K_m for ATP of approximately 300 μM, suggesting that this activity may be susceptible to changes in cellular ATP which are well within the physiological range. Therefore, the activity of the CFTR chloride channel and its role in secretion is intimately linked to the metabolic status of the epithelial cells in which it resides.

ACKNOWLEDGMENTS

We would like to acknowledge the important contributions of other members of our laboratory, Marek Hewryk and Ling-Jun Huan. These studies were supported by grants from the Canadian Cystic Fibrosis Foundation and NIH-SCOR (USA), No. 5 P50 DK 49096. C.E.B. is an MRC Scientist.

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