CMLS, Cell. Mol. Life Sci. 57 (2000) 623–634
1420-682X/00/040623-12 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Review

New paradigms of CFTR chloride channel regulation

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Received 12 October 1999; accepted 31 December 1999

Abstract. The cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel controls salt and water transport across epithelial tissues. Alterations in the activity of this ion channel lead to two major human diseases: cystic fibrosis (low CFTR activity) and secretory diarrhea (excessive CFTR activity). The goal of this article is to review recent developments in our understanding of two aspects of CFTR biology: (i) interactions between CFTR domains (intramolecular interactions) that control the gating of this epithelial chloride channel and (ii) interactions between CFTR and other proteins (intermolecular interactions) that couple the activity of this ion channel to additional cellular processes in epithelial cells (e.g. membrane traffic). Clarifying the nature of these interactions may lead to the development of novel strategies for treating diseases that involve the CFTR chloride channel.

Key words. Cystic fibrosis; ion channels; membrane traffic; syntaxins; PDZ domains; epithelial cells; ABC transporters; cystic fibrosis transmembrane; conductance regulator.

Introduction: the pathobiology of CFTR

The cystic fibrosis transmembrane conductance regulator (CFTR) is a cyclic adenosine monophosphate (AMP)-regulated Cl^- channel that controls salt and water transport across epithelial tissues [1–3]. Cystic fibrosis, the most common lethal recessive genetic disorder among Caucasians, is caused by mutations in the *CFTR* gene that decrease either the biosynthesis or the ion channel activity of the CFTR protein [4, 5]. Most patients with cystic fibrosis eventually die from complications associated with lung infections. How reduced CFTR activity contributes to this lung pathology is a subject of considerable debate [6–9]. Current models of the etiology of cystic fibrosis focus on the connection between CFTR chloride channel dysfunction and the volume or salt composition of airway surface fluid [6–9]. However, the chloride channel property of CFTR is only one of several functional attributes of this molecule. There is emerging evidence that CFTR also participates in the regulation of other cellular processes such as parallel ion transport pathways and membrane traffic, as will be discussed later in this article. Defects in these processes could also contribute to the development of pathology in cystic fibrosis.

Whereas reduced CFTR activity causes cystic fibrosis, excessive activation of this chloride channel in gut leads to secretory diarrhea [10, 11]. CFTR resides at the apical membranes of secretory epithelial cells where it is normally inactive until phosphorylated by cyclic nucleotide-dependent protein kinases [12]. Activation of the CFTR channel by phosphorylation induces Cl^- secretion across the epithelium that in turn increases the electrical and osmotic driving forces for the parallel flows of Na^+ and water, respectively (fig. 1). In this way CFTR serves as the rate-limiting step for cyclic nucleotide-dependent salt and water secretion in the

intestine [12]. The putative physiological role of this fluid secretion is to lubricate the mucosal surface and to deliver secreted macromolecules (e.g. cytokines and antimicrobial peptides) to their sites of action within the intestinal lumen. However, bacterial toxins that induce cyclic nucleotide production in the intestine cause robust stimulation of CFTR channel activity that subsequently leads to massive salt and water secretion by gut epithelial cells [10–12]. In particular, cholera toxin elevates cyclic AMP production in epithelial cells leading to CFTR activation and fluid secretion in intestine. Heat-stable enterotoxin derived from *Escherichia coli* stimulates intestinal fluid and electrolyte secretion by elevating cyclic guanosine 5′-monophosphate (cGMP) production and subsequently activating cGMP-dependent protein kinase II, an apical membrane-targeted kinase that efficiently phosphorylates CFTR in vivo (reviewed in [13]). Thus, abnormally high CFTR activity can also have dire pathophysiological consequences, namely secretory diarrhea and dehydration. The involvement of CFTR in toxin-induced diarrhea has, in fact, led to the suggestion that the relatively high carrier frequency of mutant alleles in certain populations could be due to a heterozygote advantage against such bacterial pathogens [10].

The goal of this article is to review new developments in our understanding of the cellular and molecular mechanisms that control CFTR channel activity. Toward this end I will discuss three general topics: (i) interactions between CFTR domains that regulate channel gating (intramolecular interactions); (ii) interactions between CFTR and other proteins that couple the function of this chloride channel to additional cellular processes (intermolecular interactions) and (iii) the relevance of these paradigms of CFTR regulation to the development of new treatments for cystic fibrosis and secretory diarrhea. The reader is directed to a number of excellent review articles for information regarding other aspects of CFTR biology such as biosynthesis [14] and the genetics of cystic fibrosis [15, 16].

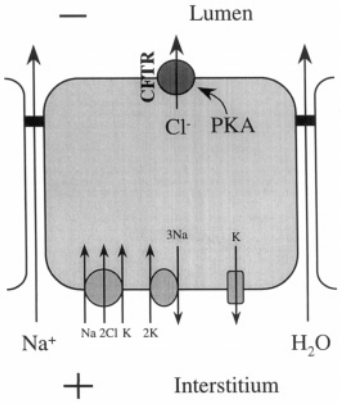

Figure 1. Model of a secretory epithelial cell in gut. CFTR channels are located at the apical (lumen-facing) membrane in series with three basolateral transporters: (i) Na^+/K^+ ATPase, (ii) $Na^+/Cl^-/K^+$ cotransporter and (iii) K^+ channels through which K^+ is recycled back into the interstitium. Water secretion is shown to take place between the cells, although it may take place through the cells as well.

Overview of CFTR structure

The CFTR chloride channel is a member of the growing family of ATP binding cassette (ABC) transporters [17, 18]. Most members of this protein family participate in the active transport of large solutes (e.g. drugs or peptides) across membranes [18–20]. Like other ABC transporters the CFTR protein consists of two putative transmembrane domains (TMDs) and two nucleotide binding domains (NBDs; see fig. 2). The TMDs contribute to the formation of the anion-selective pore within the CFTR molecule (reviewed in [21]). Chloride permeation through this pore is a diffusive process that is driven solely by the electrochemical potential gradient for this anion. In this regard, the CFTR chloride channel differs from most other ABC transporters that utilize the free energy liberated by adenosine triphosphate (ATP) hydrolysis at the NBDs to drive the uphill transport of substrate through the permeation pathway [18]. Instead, ATP hydrolysis by the CFTR NBDs probably controls channel gating by providing an energy source for transitions between closed and open states of the channel (see below). Another unique feature of CFTR is the large cytoplasmic regulatory (R) domain that is centrally located within the CFTR polypeptide [17] and that contains multiple sites for phosphorylation by cyclic AMP and cyclic GMP-dependent protein kinases. The R domain appears to have evolved as an inhibitory clamp that blocks ATP-dependent channel gating in the absence of appropriate physiologic stimuli (i.e. factors that elevate intracellular nucleotide levels), as will be discussed below.

CFTR regulation by intramolecular interactions

The NBDs

A large body of evidence indicates that the two NBDs play different but cooperative roles in controlling CFTR channel gating. Each of the CFTR NBDs possesses consensus sequences for ATP binding and hydrolysis (i.e. Walker A and B motifs), although the overall homology between the two NBDs is limited (less than 30% amino acid identity). Replacing an invariant lysine in the NBD1 Walker A motif with alanine (K464A), which inhibits ATP hydrolysis, decreases the rate of channel opening under phosphorylating conditions [22–24]. The analogous mutation in NBD2 (K1250A) generally prolongs the durations of channel openings (i.e. decreases the channel closing rate). Inter

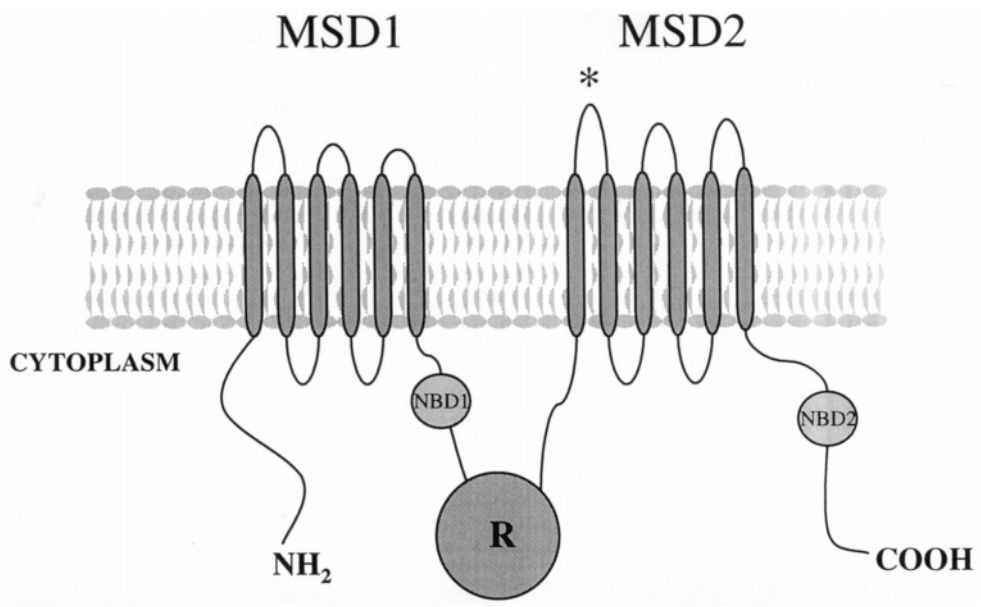

Figure 2. Putative CFTR topology. NBD: nucleotide binding domain; R: regulatory domain; MSD: membrane-spanning domain, each of which consists of six predicted transmembrane segments. Asterisk denotes glycosylation sites.

estingly, channel openings are also prolonged by nonhydrolyzable ATP analogs when they are added in the presence of MgATP [24–26]. Nonhydrolyzable analogs are unable to open the channel on their own. It has been argued that they bind to NBD2 and inhibit channel closing in a manner similar to that observed for the K1250A mutation (i.e. by inhibiting nucleotide hydrolysis at this domain). Based on the effects of such NBD mutations and nucleotide analogs on CFTR channel activity, several groups have proposed cyclic gating schemes [23, 25, 26] that share the following features: (i) dependence of CFTR gating on ATP hydrolysis, which presumably energizes transitions between closed and open states; (ii) a primary role for ATP hydrolysis at NBD1 in opening the channel and (iii) a primary role for NBD2 in closing the channel. According to this model, ATP-bound NBD2 serves as a kind of 'timer switch' that controls the durations of channel openings, openings that are terminated by ATP hydrolysis at this domain. Such a role for NBD2 would be analogous to the roles played by GTPases in controlling the duration of activation of their downstream effectors, as has been pointed out by several groups [21, 25, 27].

The two NBDs probably cooperate to regulate CFTR gating in addition to serving distinct roles in the gating process. For example, mutations in one NBD can have profound effects on the overall ATPase activity of purified CFTR [28]. In addition, the catalytic activity of CFTR exhibits positive cooperativity for ATP under phosphorylating conditions [28]. Cross-talk between NBDs would be consistent with the observation that the K1250A mutation in NBD2 inhibits the rate of channel opening (presumably governed by NBD1) as well as increases the durations of channel openings [23]. Such cross-talk between the CFTR NBDs would also be consistent with the evidence that the NBDs of several other ABC transporters must oligomerize into dimers or tetramers to form transport-competent ATPases [20, 29, 30]. Conceivably, the chloride channel activity of CFTR also depends upon oligomerization of its two NBDs, although biochemical evidence for a direct physical interaction between these two domains is currently lacking. For more detailed discussions of the roles played by the NBDs in controlling CFTR gating the reader is directed to two excellent reviews [21, 31].

It should be noted that not all evidence supports the notion that CFTR channel gating is tightly coupled to ATP hydrolysis. For example, Ramjeesingh et al. [32] have reported that the gating of purified CFTR that has been incorporated into synthetic lipid bilayers is only modestly affected by NBD mutations that dramatically inhibit the ATPase activity of this preparation. This apparently loose coupling between CFTR gating and ATP hydrolysis may be warning us that there is still much to learn about how the ion channel activity of CFTR is linked to its catalytic activity.

The R domain controls nucleotide-dependent gating

How does the large cytoplasmic R domain, which is unique to CFTR, contribute to the gating process? This domain contains multiple consensus sites for PKA

phosphorylation, at least five of which are phosphorylated in vivo [33–36]. Phosphorylation at most of these sites stimulates chloride channel activity, although at least one other site that is phosphorylated in vivo appears to be inhibitory [37]. CFTR also contains many PKC sites, some of which are in the R domain [17], and there is evidence that prior phosphorylation of the channel by PKC primes CFTR for subsequent phosphorylation and activation by PKA [38, 39]. The mechanism for this priming effect of PKC is unknown. The elimination of PKA sites from the R domain generally leads to a decrease in the rate of channel opening under phosphorylating conditions [40, 41], which implies that the phosphorylated R domain plays a major role in controlling the activation rate of the channel. In addition, the durations of channel openings are prolonged at high levels of CFTR phosphorylation, as is the susceptibility of the channel to being locked open by nonhydrolyzable analogs such as 5′-adenylylimidodiphosphate [26]. Thus, phosphorylation of the R domain by PKA has multiple and substantial effects on channel gating.

Two additional lines of evidence indicate that the R domain controls CFTR gating by modulating the functional activities of the NBDs. First, eliminating PKA phosphorylation sites from this domain leads to a decrease in the ATP sensitivity of CFTR channel activity (i.e. an increase in the concentration of ATP required to open the channel) under phosphorylating conditions [40, 41]. Second, the ATPase activity of purified CFTR is stimulated by phosphorylation due primarily to an increase in the apparent affinity for ATP [28]. These observations imply that the unphosphorylated R domain clamps CFTR to low levels of activity by reducing the sensitivity of this channel to ATP. Inhibition by the R domain is then relieved by phosphorylation through an unknown mechanism. The R domain presumably mediates its effects on the ATP sensitivity of channel gating through an interaction (direct or indirect) with one or both NBDs, although the details of such interactions remain to be determined.

If the primary role of the unphosphorylated R domain is to inhibit ATP-dependent gating, then deleting this domain should result in a channel that does not require PKA for its activity. The effects of deleting large portions of this domain on CFTR gating are qualitatively consistent with this prediction, but with some interesting twists. In particular, those R domain deletion constructs that are functional (not all are) generally do exhibit channel activity in the presence of MgATP alone [40, 42, 43]. This is unlike wild-type CFTR, which is active only when phosphorylated. However, the level of constitutive activity exhibited by these constructs is only 10–20% of the activity exhibited by wild-type CFTR under maximally activating conditions [P_o (open probability) of 0.05–0.15 vs maximal wild-type P_o of 0.4–0.6]. In addition, two groups have reported that adding recombinant R domain peptide to these mutant channels does not block their basal, PKA-independent activity, as might be expected if the R domain were a simple blocker of CFTR channel activity [40, 43]. Rather, this peptide confers upon these R domain deletion mutants a modest responsiveness to subsequent PKA addition (i.e. a 50–100% increase in P_o over basal levels). These observations have led to the conclusion that the R domain has two activities [40, 43]: (i) an inhibitory activity that can be relieved by phosphorylation or by deletion of this domain and (ii) a separate stimulatory activity that predominates under phosphorylating conditions. It should be noted that this conclusion rests on data that were generated using deletion mutants and R domain peptides that do not fully mimic wild-type behavior (e.g. even in the presence of recombinant R domain peptide and maximal PKA activity, the P_os of the R domain deletion mutants are less than 50% of wild-type P_o). Nevertheless, the available evidence indicates that the R domain plays a critical, although perhaps complex, role in controlling the level of CFTR channel activity. And since phosphorylating this domain increases the ATP sensitivity of channel gating, it seems likely that the R domain exerts its influence on channel activity via functional interactions with the NBDs.

Cytoplasmic loops and NH_2 terminal tail also modulate CFTR channel gating

Are the NBDs and the R domain the sole determinants of CFTR gating? In addition to these domains, the CFTR polypeptide contains two cytoplasmically oriented tails and four cytoplasmic loops (fig. 2), regions that could also contribute to the gating process. Indeed, a number of CF-associated mutations in cytoplasmic loops 1, 3 and 4 that substantially affect channel gating have been described [44–47]. For example, a loop 1 mutation (E193K) markedly reduces CFTR channel P_o by decreasing the rate of channel opening (44). Loop 3 and 4 mutations, on the other hand, reduce P_o in large part by decreasing open channel lifetime [45, 47]. How these mutations affect channel gating is unknown. Conceivably, these cytoplasmic loops participate in coupling the catalytic activity of the NBDs to structural rearrangements in the transmembrane domains that underlie channel gating, as has been proposed [47].

Recent evidence from our laboratory indicates that the amino terminal tail (N-tail) is also an important component of the CFTR gating machinery [48]. The functional activity of a CFTR deletion construct that lacks this tail is markedly stimulated by the addition of recombinant N-tail peptide. This regulatory activity maps to a cluster of acidic residues in the N-tail, residues that are strictly

conserved across species (aa D47,E51,E54,D58). The substitution of these negatively charged residues with alanine eliminates the stimulatory effect of the N-tail peptide on the activity of the N-tail deletion mutant. Furthermore, these same N-tail mutations, when introduced into the intact CFTR polypeptide, inhibit steady-state CFTR channel activity. CFTR gating appears to be tightly controlled by this cluster of acidic residues, since the successive elimination of negative charge from this region of the N-tail leads to a graded reduction in steady-state activity. These N-tail mutations influence CFTR gating in part by destabilizing the activated state of the channel. In particular, the N-tail mutants exhibit reduced open channel burst durations in excised membrane patches under conditions that maximally activate the wild-type channel, and markedly accelerated deactivation kinetics in intact *Xenopus* oocytes following washout of a cyclic AMP cocktail.

The amino terminal tail appears to exert its effects on CFTR chloride channel gating via an interaction with the R domain [48]. A recombinant peptide (a.a. 595–855) containing the R domain plus the distal portion of NBD1 binds to N-tail peptide in vitro with moderately high affinity. (The domain boundaries of NBD1 and the R domain have not been precisely mapped; estimates of the carboxy terminal boundary of NBD1 vary from residue 589 [17] to 670 [29].) When phosphorylated in vivo this R domain construct retains its ability to physically interact with N-tail peptide, if anything, with a slightly greater affinity than that exhibited by the unphosphorylated R domain. Importantly, the binding interaction between the R domain and N-tail is proportionately inhibited by those N-tail mutations that disrupt channel gating. Moreover, these N-tail mutations have no effect on the constitutive, PKA-independent channel activity of an R domain deletion construct. Thus, the N-tail controls CFTR gating in the presence, but not absence, of an intact R domain.

These findings support a model in which CFTR chloride channel activity is stabilized by an interaction between the amino terminal tail and the R domain and/or NBD1. In this regard, the amino terminal tails of several types of K^+ channels also participate in intramolecular interactions that modulate gating; for example, the amino terminal tails of certain Shaker-type K^+ channel subunits neutralize the blocking activity of the N-type inactivation domain in these channels [49]. Perhaps the N-tail of CFTR stabilizes channel activity by controlling access of the phosphorylated R domain to inhibitory or stimulatory sites within the channel, such as the NBDs. These findings also implicate the amino terminal tail as a potentially interesting target for physiologic regulators and pharmacologic modulators of CFTR channel gating. In particular, the amino terminal tail also serves to physically couple the CFTR chloride channel to components of the membrane traffic machinery (see below). These dual activities of the amino terminal tail raise the interesting possibility that CFTR gating and CFTR traffic are coordinately regulated processes.

In summary, CFTR channel gating is regulated by a complex interplay among multiple cytoplasmic domains: NBD1, NBD2, the R domain, the amino terminal tail and several cytoplasmic loops. The involvement of multiple domains in the gating process may offer advantages with respect to the physiologic regulation of this channel. For example, the availability of multiple sites for modulating channel gating would allow for the integration of simultaneous regulatory inputs at these sites. On the other hand, the complexity of the gating process increases the challenge to workers in this field who are attempting to characterize this process. The essential issues with respect to CFTR gating have moved beyond the realm of classical ion channel physiology, and now concern the order and structural basis of those intramolecular interactions that underlie this process.

CFTR regulation by intermolecular interactions

An emerging theme in the CFTR field is the notion that this chloride channel is coupled to additional cellular processes by multiple protein-protein interactions. Of particular interest are two classes of proteins that physically interact with the opposing tails of this chloride channel (see fig. 3): (i) syntaxin 1A, a component of the membrane traffic machinery that binds to the amino terminal tail of CFTR and (ii) PDZ-domain-containing proteins that bind to the carboxy terminal tail of this ion channel.

Syntaxin interactions at the amino terminal tail of CFTR: coupling ion transport to membrane traffic

Syntaxin 1A was originally identified in brain where it is an essential component of the protein complex that mediates the fusion of the synaptic vesicle with the presynaptic plasma membrane [50–52]. This integral membrane protein is a member of a large family of proteins that regulate membrane fusion in diverse tissues referred to as T-SNARES or Q-SNARES (reviewed in [53, 54]). Syntaxin 1A protein is also expressed in gut and airway epithelial cells, although at much lower levels than in brain [55]. The coexpression of syntaxin 1A with CFTR in heterologous expression systems such as *Xenopus* oocytes leads to an inhibition of CFTR-mediated currents [55]. Moreover, CFTR can be rescued from this inhibition by multiple reagents, including (i) Munc-18a, a high-affinity syntaxin-binding

protein [56, 57] that is also expressed in gut epithelial cells [55]; (ii) soluble syntaxin 1A peptides; and (iii) botulinum neurotoxin C1, an endoprotease that cleaves membrane-anchored syntaxin 1A [51]. Importantly, each of these reagents also markedly stimulates CFTR-mediated chloride currents in various colonic epithelial cell lines that normally express CFTR and syntaxin 1A [55, 58]. Thus, syntaxin 1A appears to tonically inhibit CFTR activity in cultured gut epithelial cells.

The negative modulation of CFTR currents by syntaxin 1A is at least in part mediated by a direct physical interaction between these membrane proteins [58]. It would seem plausible that syntaxin 1A could remotely modulate CFTR function by regulating membrane fusion events that subsequently influence the numbers of CFTR channels at the cell surface, given its participation in vesicle fusion reactions at the synapse [50–54]. However, the interaction between these proteins is more direct. In particular, syntaxin 1A (but no other syntaxin isoform that was tested) binds to the amino terminal tail of CFTR with a 1:1 stoichiometry [58]. This binding interaction is inhibited by Munc-18a, which itself is unable to bind to CFTR. Furthermore, the functional interaction between CFTR and syntaxin 1A is eliminated either by deleting the amino terminal tail from CFTR or by blocking this interaction with recombinant amino terminal tail peptide. The direct interaction between syntaxin 1A and the amino terminal tail of CFTR raises the interesting possibility that syntaxin 1A modulates the channel gating properties of CFTR, especially since this tail controls gating via an interaction with the R domain.

What is the physiological or pathophysiologic relevance of this protein-protein interaction in epithelial cells? This interaction could fine-tune CFTR activity in response to signals that up- or downregulate its binding to syntaxin 1A (e.g. Munc-18 or kinases that modulate the CFTR-syntaxin 1A interaction). In addition, if CFTR activity in epithelial cells is normally limited by its interaction with syntaxin 1A, then reagents that block this interaction may augment the activities of partial-loss-of-function CFTR mutants that cause cystic fibrosis.

In broader terms, the interaction between the CFTR chloride channel and syntaxin 1A may serve to coordinate the regulation of ion transport with the regulation of membrane traffic in epithelial cells. In this regard, it is interesting to note that syntaxin 1A also physically interacts with voltage-gated calcium channels in neurons [59, 60] and in neuroendocrine cells [61], i.e. cells that engage in regulated membrane traffic. Syntaxin 1A modulates the gating of N-type [62] and L-type calcium channels [61] in heterologous expression systems. In vivo, these interactions appear to be important for optimizing the efficiency of calcium-evoked secretion by physically tethering the secretory vesicles to sites of calcium influx [61, 63]. Thus, the interactions between syntaxin 1A and calcium channels serve to spatially and temporally couple the regulation of membrane traffic to the regulation of ion transport in neurons and neuroendocrine cells.

The regulation of fluid and electrolyte transport is also coupled (at least temporally) to the regulation of membrane traffic in CFTR-expressing epithelial cells. For

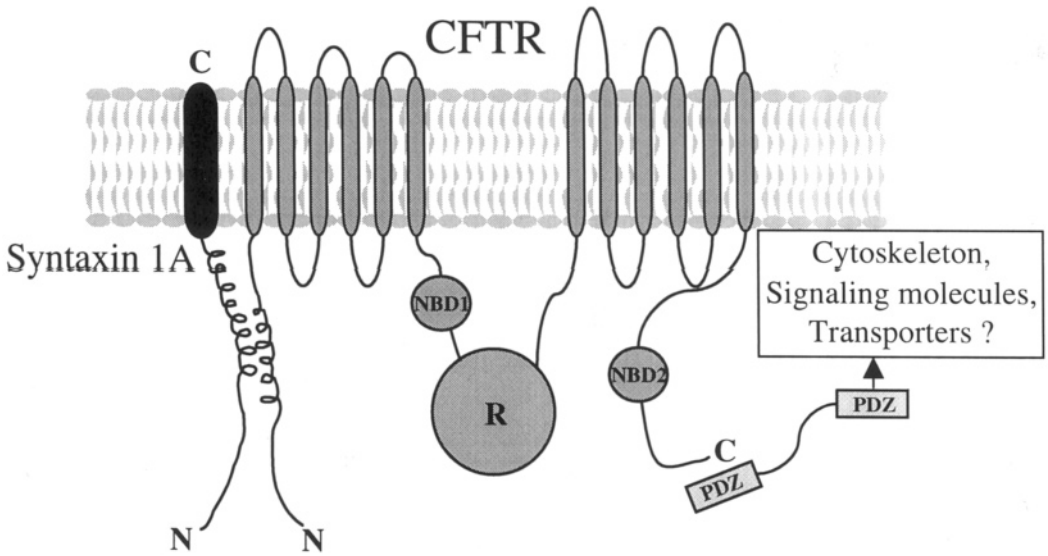

Figure 3. Schematic illustration of CFTR-binding partners at the amino terminal and carboxy terminal tails. Syntaxin 1A and the amino terminal tail of CFTR are predicted to form α helices that may be relevant to the binding interaction between these proteins.

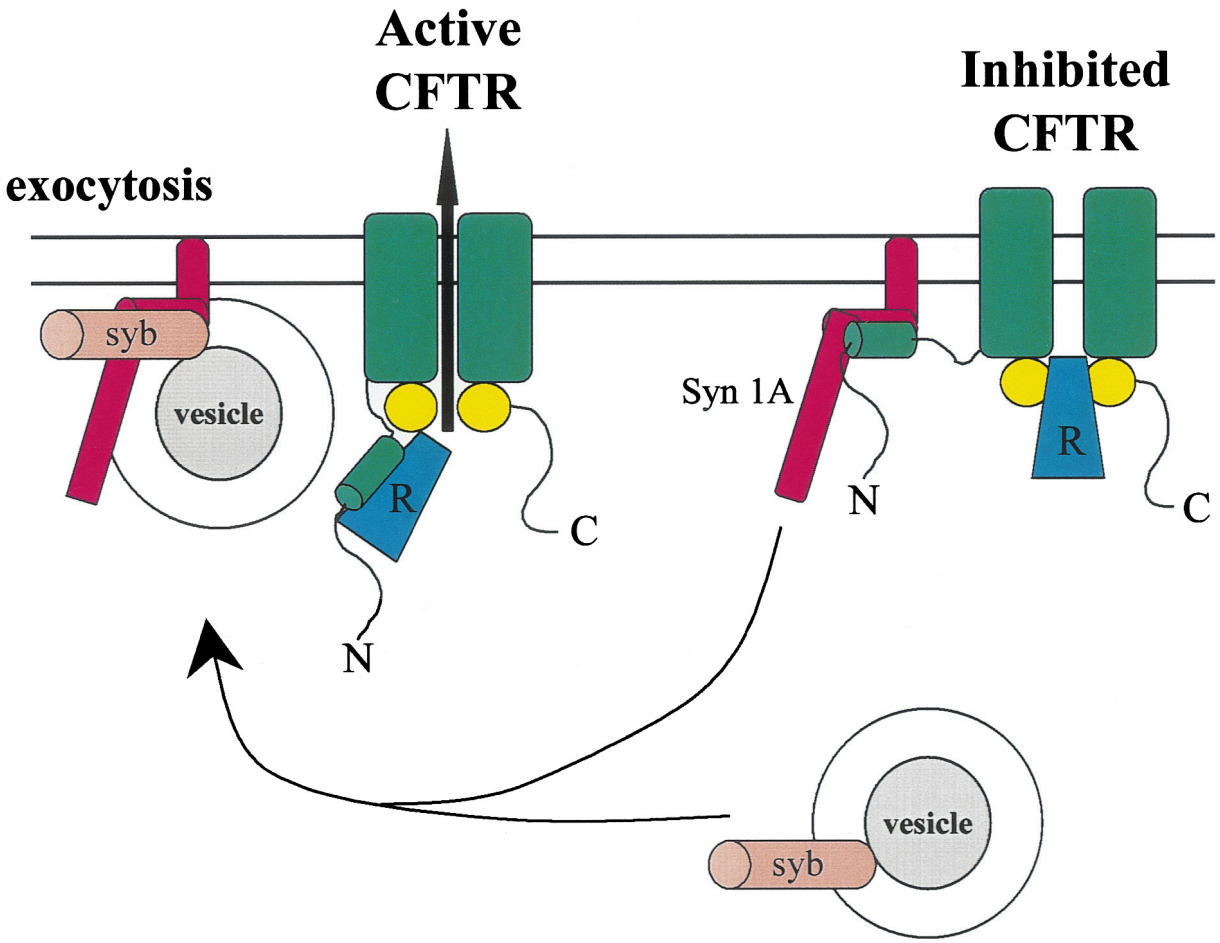

Figure 4. Speculative model of the coupling between CFTR activity and apical protein secretion. Syntaxin 1A (syn1A) could reduce CFTR activity at the plasma membrane by binding to the amino terminal tail and disrupting the interaction between this tail and the R domain. How disrupting this interaction decreases CFTR activity is unknown, but could involve increasing the access of the R domain to inhibitory sites in the channel (e.g. binding sites within the NBDs). The delivery of secretory vesicles with V-SNARES such as synaptobrevin (syb) to the plasma membrane could rescue CFTR from this inhibition by recruiting syntaxin 1A into SNARE complexes that mediate membrane fusion. This model is consistent with the dynamic nature of the syntaxin 1A-CFTR interaction [55, 58] and the stability of SNARE complexes [53, 54].

example, the same factors that stimulate CFTR chloride channel activity and thereby fluid secretion in gut epithelial cells (e.g. cholera toxin via cyclic AMP) also inhibit apical membrane endocytosis [64, 65] and stimulate the secretion of proteins that are important for mucosal homeostasis [66–68]. Indeed, there is some evidence to indicate that CFTR itself may be cargo in cyclic-AMP-regulated membrane traffic pathways in epithelial cells [69–71], pathways that could modulate the numbers of CFTR channels at the cell surface and in intracellular compartments. The extent to which the surface expression of CFTR is acutely regulated by cAMP is a controversial issue (for example, see [72, 73]), in part because this phenomenon appears to vary considerably between cell types and expression systems. One intriguing possibility is that both the intracellular location of CFTR [71] and its functional activity in certain intracellular compartments are regulated by interactions between cell-type-specific components of the membrane traffic machinery (e.g. syntaxin A) and the amino terminal tail of this ion channel. Such dual regulation of CFTR traffic and channel gating could optimize CFTR chloride channel activity at specific intracellular locations. Another possibility is that, as transport vesicles are delivered to the cell surface, they deliver syntaxin binding proteins (i.e. R-SNARES or V-SNARES [54]) that recruit syntaxin 1A away from CFTR Cl^- channels already present at the apical membrane. In this way, CFTR activity could be increased in proportion to the rate of apical protein secretion in

epithelial cells. A model for such coupling between CFTR activity and outbound membrane traffic is illustrated in figure 4.

The physical interaction between CFTR and syntaxin 1A also may enable this ion channel to be an active participant in the regulation of membrane traffic in epithelial cells. It may be relevant that the CFTR binding site on syntaxin 1A maps to that domain that is most critical for the participation of syntaxin 1A in membrane fusion reactions (i.e. the third helical domain, or H3 domain; [58, 74]). This observation implies that CFTR could regulate the availability of syntaxin 1A for membrane fusion reactions in certain cell types or compartments (i.e. this functional interaction may be bidirectional). In this regard, the expression of recombinant CFTR in some cell types alters the kinetics and regulation of endocytosis and exocytosis [66, 75, 76], although not all cells appear to be susceptible to this effect of CFTR [77]. It is conceivable that CFTR provides regulatory input into some membrane traffic pathways in part because of its physical interactions with the membrane traffic machinery.

In summary, interactions between CFTR and components of the membrane traffic machinery such as syntaxin 1A may help couple the regulation of salt and water transport to the regulation of protein traffic in epithelial cells. Such interactions between ion channels and syntaxins may be a more general theme in biology, as evidenced by the recent identification of a plant syntaxin that regulates chloride channels and potassium channels in stomatal guard cells [78]. Physical and functional interactions between ion channels and syntaxins would provide a direct means to couple the regulation of ion transport to the regulation of membrane traffic in those tissues for which coordinating these processes is physiologically advantageous.

PDZ domain-mediated protein interactions at the carboxy terminal tail of CFTR

Whereas the amino terminal tail couples CFTR to the membrane traffic machinery, the extreme carboxy terminal tail binds to proteins that possess binding modules referred to as PDZ (for PSD-95, discs large, ZO-1) domains. Proteins that possess PDZ domains are often multivalent (i.e. they contain multiple PDZ domains) and can promote homotypic and heterotypic protein-protein interactions in a variety of tissues [79]. Such interactions can facilitate the clustering of ion channels within microdomains at the cell surface, and the assembly of signaling complexes at the plasma membrane. Several PDZ-domain-containing proteins have been shown to bind with high affinity both in vitro and in vivo to a consensus sequence (TRL) at the extreme C terminus of CFTR (e.g. NHERF or EBP50; see [80–82]). Based on studies from other systems, it seems reasonable to propose that PDZ-domain-mediated interactions at the C terminal tail of CFTR could link this ion channel to a variety of proteins, including signaling molecules (i.e. kinases and phosphatases), cytoskeletal elements and other transport proteins. For example, it has been argued that interactions between CFTR and EBP50 could anchor this ion channel to the apical membrane cytoskeleton, since EBP50 is an apically localized ezrin-binding protein [82]. In support of this hypothesis, Moyer et al. [83] have recently reported that deletion of the carboxy terminal TRL sequence results in the mislocalization of CFTR in airway and kidney epithelial cells. Thus, one of the functional roles of PDZ-domain-mediated interactions may be to help localize CFTR chloride channels to the apical membranes of polarized epithelial cells.

It is tempting to speculate that PDZ-domain-mediated interactions also could facilitate cross-talk between CFTR chloride channels and parallel ion transport pathways. In addition to its role as a chloride channel, CFTR exerts modulatory influences over a variety of other transport proteins and processes, including epithelial Na^+ channels [84], outwardly rectifying Cl^- channels [85, 86], apical K^+ channels from renal epithelial cells [87], aquaporin water channels [88], Cl^-/HCO_3^- exchangers [89] and ATP release mechanisms [90, 91]. How this one ion channel can regulate the activities of so many other transport proteins is unknown. One possibility is that CFTR interacts directly or indirectly with other transport molecules in macromolecular complexes that are assembled by PDZ-domain-containing scaffolding proteins. An example of such a macromolecular signaling complex is the INAD complex in *Drosophila* retina [92], which includes at least two distinct ion channels that are coupled to multiple signaling molecules by a scaffolding protein with five PDZ domains. Conceivably, CFTR is a component of similar macromolecular complexes in epithelial cells, where it can functionally and perhaps physically interact with other transport molecules. And, since there are large numbers of proteins that either possess PDZ domains or that can bind to these domains in various tissues, CFTR could engage in combinatorial interactions with a wide variety of transporters and signaling molecules in a tissue-specific manner.

Relevance of these interactions to treating cystic fibrosis and secretory diarrhea

Can these new paradigms of CFTR regulation lead to novel strategies for treating diseases that involve this chloride channel? With respect to the regulation of CFTR gating by the amino terminal cytoplasmic tail,

this domain now becomes an interesting target for pharmacologic modulators of CFTR activity. High-throughput assays of the binding interaction between the amino terminal tail and the R domain could be developed as a means to screen small molecule libraries for organic compounds that inhibit or stabilize this interaction. In addition, defining the structural basis of the physical interaction between the amino tail and its docking site by nuclear magnetic resonance (NMR) or crystallographic methods could set the stage for the rational design of drugs to perturb this interdomain interaction. Blockers of the interaction between the tail and the R domain might be expected to inhibit CFTR channel activity, and could serve as novel lead compounds for treating secretory diarrheas. Compounds that stabilize this interdomain interaction might augment chloride channel activity in cases of cystic fibrosis that are caused by partial-loss-of-function mutations in the CF gene. The advantage of this interdomain interaction as a drug target, versus the channel pore, for example, would be the potential for identifying highly specific modulators of CFTR activity. The CFTR pore would be expected to share some topological similarities with other chloride channels and transporters; indeed, there is no known pore blocker that is specific for the CFTR channel. Conversely, the R domain and its binding site in the amino terminal tail are unique to CFTR.

Protein-protein interactions that involve the CFTR chloride channel also may be worth considering as targets for pharmacologic intervention. Indeed, peptides that block the syntaxin 1A-CFTR interaction can increase the functional activity of the most common CF-associated mutant (ΔF508) in a tissue culture model of epithelial cells [58]. However, more caution is warranted in this case, since disrupting these intermolecular interactions may have unanticipated effects on epithelial cell physiology. It may be that these interactions serve not only to regulate the channel activity of CFTR but also to enable the CFTR molecule to participate in other cellular processes such as the regulation of membrane traffic or the modulation of parallel ion transport processes (as noted above). In our view, this ion channel is probably connected to multiple cellular processes via networks of regulatory interactions. Blocking these intermolecular interactions may have multiple functional consequences.

In summary, the functional activity of the CFTR chloride channel appears to be linked to other cellular processes via distinct protein-protein interactions at the opposing cytoplasmic tails. In addition, the gating of this ion channel is controlled by a complex set of intramolecular interactions. Resolving the nature and functional relevance of these intramolecular and intermolecular interactions will improve our understanding of how this ion channel is regulated under normal circumstances, and will shed light on the various physiologic roles that CFTR plays in epithelial cells. Ultimately, such information may lead to new strategies for treating diseases that involve the CFTR chloride channel.

Acknowledgements. The author thanks Drs Estelle Cormet-Boyaka, Anjaparavanda Naren, David Nelson, Erik Schwiebert and Eric Sorscher for critically reading this manuscript. I also thank Dr Cormet-Boyaka for her help with the figures. Our work is supported primarily by the National Institutes of Health (DK51868 and DK50830).

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