New insights into cystic fibrosis: molecular switches that regulate CFTR

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Abstract | Cystic fibrosis transmembrane conductance regulator (CFTR), a Cl–-selective ion channel, is a prototypic member of the ATP-binding cassette transporter superfamily that is expressed in several organs. In these organs, CFTR assembles into large, dynamic macromolecular complexes that contain signalling molecules, kinases, transport proteins, PDZ-domain-containing proteins, myosin motors, Rab GTPases, and SNAREs. Understanding how these complexes regulate the intracellular trafficking and activity of CFTR provides a unique insight into the aetiology of cystic fibrosis and other diseases.

The cystic fibrosis transmembrane conductance regulator (CFTR) is a member of the ATP-binding cassette (ABC) transporter superfamily. All ABC transporters bind to ATP and use its energy to drive the transport of several molecules across cell membranes. Mutations in ABC genes have been linked to many genetic diseases, including cystic fibrosis (associated with mutations in ABCC7, BOX 1), Dubin–Johnson Syndrome (associated with mutations in ABCC2), age-related macular degeneration (associated with mutations in ABCA4), Tangier disease (associated with mutations in ABCA1) and sitosterolaemia (associated with mutations in ABCG5 and ABCG8). For more information on the ABC transporter superfamily and for a comprehensive list of ABC-gene mutations that are associated with human diseases, see the Human ATP-Binding Cassette (ABC) Transporter Superfamily in Further Information.

Cystic fibrosis is a pernicious disease that presents as exocrine pancreatic insufficiency, an increase in sweat NaCl concentration, male infertility and airway disease. Although the life expectancy of individuals with cystic fibrosis has increased dramatically in the past three decades, the average age of death, which is caused by respiratory insufficiency, is ~37 years1–3. Because mutations in the CFTR gene cause cystic fibrosis, considerable effort has been made to understand the function and regulation of CFTR. The ultimate goal of these studies, many of which have focused on the molecular switches that regulate CFTR trafficking and activity, is to identify novel targets for drug discovery (for more information on cystic fibrosis, see the Cystic Fibrosis Foundation in Further Information).

CFTR is a plasma-membrane cyclic AMP-activated Cl– channel that is expressed in several functionally diverse tissues, including the kidney, pancreas, intestine, heart, vas deferens, sweat duct and lung4,5. In epithelial cells, CFTR mediates the secretion of Cl–. In addition to its role as a secretory Cl– channel, CFTR also regulates several transport proteins, including the epithelial sodium channel (ENaC), K+ channels, ATP-release mechanisms, anion exchangers, sodium-bicarbonate transporters, and aquaporin water channels6–13. CFTR and transport proteins form large macromolecular signalling complexes, which are regulated by molecular switches. CFTR and molecular switches mediate transcellular salt and water secretion into the lumen of kidney tubules, pancreatic ducts, and the intestine, but they also have important functions in pathophysiological conditions. CFTR might contribute to the enlargement of renal cysts in individuals with polycystic kidney disease14. In the intestine, CFTR-mediated Cl– secretion is responsible for secretory diarrhoea that is induced by *Vibrio cholera* and *Clostridium difficile*15, whereas in the heart, CFTR mediates protein kinase A (PKA)-stimulated Cl– currents, which might be protective against focal arrhythmias and cardiac ischaemia16.

CFTR-mediated Cl– secretion across many epithelial cells is regulated by modulating channel activity and by regulating the total number of CFTR channels in the membrane, which is achieved by the insertion and removal of CFTR channels from the plasma membrane. Mutations in CFTR affect the number of channels in the plasma membrane, channel activity and the intracellular trafficking of CFTR (BOX 2). Considerable effort is being made to examine the endoplasmic reticulum (ER) quality-control mechanisms that allow the export of wild-type CFTR but retain ΔF508-CFTR, the most common mutation in CFTR, and target it for degradation by the proteasomes17–20 (BOX 1). In the ER, molecular chaperones, including calnexin in the lumen of the
Molecular switches

Molecular switches are defined as molecules that exist in two different states: an activated state that regulates other molecules or signalling pathways, and an inactivated state that cannot regulate other molecules or downstream signalling pathways.

Epithelial sodium channel (ENaC). ENaC mediates sodium absorption in airway and kidney epithelial cells and sweat ducts. In airways and kidneys, CFTR represses ENaC, whereas in sweat ducts CFTR activates ENaC.

Dubin–Johnson Syndrome

An autosomal recessive genetic liver disease that is characterized by congenital hyperbilirubinaemia and is caused by mutations in ABCC2, the gene which encodes multidrug-resistance-related protein-2.

ER and the cytosolic heat-shock proteins HSP70 and HSP90, interact with CFTR. Although the interactions of these chaperones with wild-type CFTR are transient, longer-lived interactions with ΔF508-CFTR lead to the identification of the misfolded mutant protein and thereby target it for degradation. Because ΔF508-CFTR is partially functional as a Cl– channel, the goal of many research laboratories and biotechnology companies is to identify drugs that allow ΔF508-CFTR to fold properly and thereby escape the ER quality-control mechanism. Other drugs in cystic fibrosis clinical trials are focused on correcting the other phenotypes of this complex disease (BOX 2).

The purpose of this review is to present an overview of how dynamic macromolecular complexes that consist of molecular switches (including kinases, transporters, ion channels, myosin molecular motors, Ras GTPases, SNAREs, and PDZ-domain-containing proteins) regulate CFTR activity and intracellular trafficking. The ultimate goal of research in this area is to provide a basis for target-specific drug discovery to identify new drugs that can cure or treat patients with cystic fibrosis. Because similar dynamic macromolecular complexes regulate other ABC transporters, these studies provide an insight into the aetiology of diseases that are linked to other ABC transporters, and perhaps clues for potential new therapies for these diseases. Owing to the development of advanced technologies that allow high-throughput screening, our ability to identify and obtain structural information on proteins that interact with and regulate CFTR and our enhanced ability to rapidly modify and evaluate drugs, now is an exciting and promising time for cystic fibrosis drug discovery (BOX 2).

Many research programmes in academic institutions and most cystic fibrosis drug-discovery programmes in the biotechnology industry have been financed by the Cystic Fibrosis Foundation. This collaboration has been enormously successful, and has made cystic fibrosis research and drug discovery a paradigm for the drug-discovery programmes of other orphan diseases.

ABC transporter structure. ABC transporters usually contain two nucleotide-binding domains (NBD), and two transmembrane domains (TM), which contain several membrane-spanning α-helices (FIG. 1). However, some ABC transporters are composed of only one TM and one NBD. These so-called half transporters homodimerize or heterodimerize to form a functional transporter. CFTR is unique among ABC transporters because it also has a regulatory (R) domain that is phosphorylated by PKA and protein kinase C (PKC). CFTR contains several other domains that mediate protein–protein interactions, including a domain in the cytoplasmic N terminus that binds to syntaxin-1A (SYN1A) and synaptosome-associated protein, 23 kDa (SNAP23), and four domains in the C terminus — a PDZ-interacting domain, two endocytic motifs (the tyrosine-based motif YDST, and the dileucine motif LL), a protein phosphatase 2A (PP2A)-binding domain, and a domain that binds AMP kinase (AMPK; FIG. 1).

PDZ domains. PDZ domains are one of the most common modules that are found in mammalian proteins. PDZ domains consist of an 80–100 amino-acid sequence that mediates protein–protein interactions by binding to short peptide sequences, most often in the C termini of target proteins. PDZ domains mediate protein–protein interactions, cluster and colocalize transporters, channels and signalling proteins in specific subcellular domains, determine the polarized localization of many proteins, control channel and transporter function and regulate endocytic trafficking. Proteins that contain PDZ domains often contain other protein-interacting motifs (BOX 2).
The first PDZ-domain proteins identified as interacting with CFTR were NHERF1 (CAP70, CFTR-associated protein, 70 kDa, also known as EBP50, ezrin-binding protein, 50 kDa), NHERF2, CAP70 (CFTR-associated protein, 70 kDa, also known as NHERF3), NHERF4 and CFTR-associated ligand (CAL). 

PDZ-domain proteins assemble complexes of signaling modules (such as, ezrin, radixin, moesin (ERM)-binding domains and coiled-coil domains) and therefore can promote homotypic and heterotypic protein–protein interactions by binding to short peptide sequences that are most often in the C termini of the target proteins.

### Protein phosphatase-2 (PP2A)

A heterotrimetric protein phosphatase that interacts with and dephosphorylates CFTR.

### AMP kinase (AMPK)

A molecular switch that links ion transport to cellular metabolism. AMPK is activated when the AMP/ATP ratio increases.

### Ezrin

A member of the ezrin, radixin, moesin (ERM) family of actin-binding proteins. ERM proteins link the actin cytoskeleton to plasma-membrane proteins and function as signal transducers in responses that involve cytoskeletal remodelling. Ezrin has been implicated in metastatic tumour formation.

### Na+/H+ exchanger regulatory factor isoform-1 (NHERF1)

The first PDZ-domain protein that was identified to bind to and regulate CFTR. NHERF1 was originally named for its capacity to inhibit the Na+/H+ exchanger.

### CFTR-associated ligand (CAL)

A PDZ-domain protein that is expressed primarily in the trans-Golgi that regulates the intracellular trafficking of CFTR and several other transporters and receptors.

The ΔF508-CFTR (cystic fibrosis transmembrane conductance regulator) mutation: (a) retains CFTR in the endoplasmic reticulum (ER) where it is subsequently degraded by the proteosome, (b) reduces the capacity of CFTR to transport Cl− ions and (c) decreases the plasma-membrane half-life of CFTR in polarized human airway epithelial cells. 

Drug discovery for cystic fibrosis is focused on identifying drugs that allow ΔF508-CFTR to escape the ER99–102 and to activate ΔF508-CFTR channels that reach the plasma membrane.

Curcumin, a component of the spice turmeric, increases the expression of ΔF508-CFTR in the plasma membrane. However, its effect is controversial. Because the bioavailability of curcumin is low, studies are underway to increase its bioavailability. The levels of S-nitrosoglutathione (GSNO; a bronchodilator that is normally expressed in the lung) are reduced in patients with cystic fibrosis. GSNO also increases the plasma-membrane levels of ΔF508-CFTR. Accordingly, this compound is in pre-clinical trials as a therapeutic agent for cystic fibrosis.

Genistein, a flavinoid, activates G551D-CFTR channels — which are present in the plasma membrane, but are inactive — and ΔF508-CFTR channels. Therefore, genistein might be useful in individuals with the G551D mutation, and might enhance ΔF508-CFTR-mediated Cl− secretion in patients who also receive a drug that increases the membrane expression of ΔF508-CFTR.

Other strategies to correct defective Cl− secretion in the airways of patients with cystic fibrosis include the use of compacted cDNA to introduce a normal copy of the gene into cystic fibrosis airways, the use of aminoglycosides to block the apical actin cytoskeleton in polarized epithelial cells39–43 and the use of antioxidants such as N-acetylcysteine and N-acetylcystine in patients with cystic fibrosis.

Several drugs are in development to reduce airway sodium absorption44–49. For an up-to-date list of drugs in development for cystic fibrosis go to Cystic Fibrosis Foundation – Clinical Trials & Clinical Studies in Further information.

### Box 2 | Drug discovery for cystic fibrosis

The ΔF508-CFTR (cystic fibrosis transmembrane conductance regulator) mutation: (a) retains CFTR in the endoplasmic reticulum (ER) where it is subsequently degraded by the proteosome, (b) reduces the capacity of CFTR to transport Cl− ions and (c) decreases the plasma-membrane half-life of CFTR in polarized human airway epithelial cells. 

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The sequence of the PDZ-interacting domain in CFTR is Asp-Thr-Arg-Leu, and it mediates the binding of CFTR to several PDZ-domain-containing proteins, including Na+/H+ exchanger regulatory factor isoform-1 (NHERF1), also known as EBP50, ezrin-binding protein, 50 kDa), NHERF2, CAP70 (CFTR-associated protein, 70 kDa, also known as NHERF3), NHERF4 and CFTR-associated ligand (CAL).31–35,37. NHERF1 and NHERF2 have a C-terminal ERM-binding domain, which interacts with ezrin, radixin, or moesin to tether NHERF-binding proteins to the apical actin cytoskeleton in polarized epithelial cells.

Results from fluorescent recovery after photobleaching (FRAP) studies are consistent with the view that PDZ-domain proteins interact with CFTR in the plasma membrane, and these same studies show that the interaction between CFTR and PDZ-domain proteins is highly dynamic and occurs on a time scale of seconds or milliseconds.

NHERF1 and CAP70 increase the single-channel activity of CFTR and stimulate CFTR Cl− permeability.31–33. It has been suggested that NHERF1 and CAP70 induce CFTR dimerization and that dimer formation facilitates CFTR intermolecular interactions, which alter channel conformation and activity [33,34] (Fig. 1). Dimerization of CFTR might facilitate interaction between the R domains, which increases the activity of CFTR channels.44. However, the role of PDZ-domain proteins in promoting the formation of CFTR dimers is controversial. For example, in a recent study, neither NHERF1 nor NHERF2 were found to induce the formation of CFTR dimers. By contrast, PKA promotes the formation of CFTR dimers.

Although biochemical, imaging and electrophysiological studies showed that CFTR might exist either as a monomer or as a dimer in various organs, the physiological significance of CFTR monomers and dimers has not been fully understood. As noted above, dimer formation might increase CFTR-channel activity, which would also increase Cl− secretion. It has also been suggested that PKA-induced dimerization of CFTR might inhibit the endocytic retrieval of CFTR from the plasma membrane, which would increase the number...
of active (that is, phosphorylated) CFTR channels in the plasma membrane. According to this hypothesis, the formation of CFTR dimers would stabilize CFTR in the plasma membrane; this suggestion is consistent with the observation that PKA reduces the endocytic removal of CFTR from the plasma membrane. The corollary of this hypothesis is that following the dephosphorylation of the channel, CFTR currents would decline because the activity of the channel would decrease and monomeric CFTR would be removed from the plasma membrane by endocytosis. Clearly, more studies are required to determine the physiological role of PDZ-domain proteins and PKA in the regulation of CFTR-dimer formation.

Regulation of CFTR by phosphorylation. PKA activates CFTR by phosphorylating its R domain. This physical association is facilitated by an interaction between CFTR, the PDZ-domain proteins NHERF1 or NHERF2, and ezrin, which binds to the regulatory subunit (RII) of PKA and to the submembrane actin-filamentous network. These interactions, however, are highly dynamic and regulated. Ezrin is a molecular switch that, when activated by PKC, phosphatidylinositol bisphosphate (PIP2) and actin, is essential for the efficient activation of CFTR by PKA.

However, PKA can also negatively regulate CFTR. PKA phosphorylation of the R domain inhibits the binding of PKC to NHERF1, which reduces channel dimerization and, thereby, CFTR-channel activity. Other proteins, including adenylyl cyclases, phosphatases and CAMP-specific phosphodiesterases, are also recruited to CFTR-containing signalling complexes by PDZ-domain proteins and their interacting partners.

These multiprotein signalling complexes provide spatial and temporal specificity for the biological effects that are mediated through the CAMP-PKA pathway. The observation that cAMP and PKA activate CFTRs that lack the PDZ-interacting domain does not mitigate the importance of PDZ-domain proteins in the regulation of CFTR activity or of NHERF1 multiprotein signalling complexes in the PKA-mediated activation of CFTR. Indeed, when cAMP levels are elevated by cocktails that contain forskolin, IBMX (an inhibitor of phosphodiesterases) and non-hydrolysable analogues of cAMP, there is sufficient cAMP and PKA in the cytosol to phosphorylate CFTR. However, under physiological conditions where endogenous agonists increase cAMP and PKA levels in microdomains, the close physical association between CFTR and PKA, facilitated by NHERF1 and ezrin, is essential for the efficient activation of CFTR by PKA.

Several other proteins that interact with CFTR, either directly or indirectly, regulate its channel activity. PP2A binds to amino acids 1451–1476 of CFTR, and this observation that PKA activates CFTRs that lack the PDZ-interacting domain does not mitigate the importance of PDZ-domain proteins in the regulation of CFTR activity or of NHERF1 multiprotein signalling complexes in the PKA-mediated activation of CFTR. Indeed, when cAMP levels are elevated by cocktails that contain forskolin, IBMX (an inhibitor of phosphodiesterases) and non-hydrolysable analogues of cAMP, there is sufficient cAMP and PKA in the cytosol to phosphorylate CFTR. However, under physiological conditions where endogenous agonists increase cAMP and PKA levels in microdomains, the close physical association between CFTR and PKA, facilitated by NHERF1 and ezrin, is essential for the efficient activation of CFTR by PKA.
By contrast, the lysophosphaticid acid-2 receptor (LPA2-R) in the apical membrane of intestinal epithelial cells inhibits CFTR. NHERF2 binds to CFTR (through the PDZ1 domain) and to the LPA2-R (through the PDZ2 domain), which is located in the apical plasma membrane of intestinal epithelial cells (fig. 2b). When activated by an agonist, the LPA2-R reduces cAMP levels through the inhibitory GTP-binding protein (Gi), which suppresses PKA-mediated activation of CFTR. The assembly of this macromolecular complex, which is facilitated by NHERF2, organizes key elements of the signalling pathway, including LPA2-R, Gi, adenylate cyclase and ezrin, that promote the efficient and rapid inhibition of CFTR by LPA2-R agonists. Therefore, the consumption of LPA-rich foods (such as eggs) might be an alternative method for treating some forms of secretory diarrhea — especially those that activate CFTR-mediated NaCl and fluid secretion in the intestine.

**SNAREs regulate CFTR-channel gating.** SNAREs mediate membrane fusion and vesicle trafficking by assembling into complexes that link vesicle-associated SNAREs (v-SNAREs) with SNAREs on target membranes (t-SNAREs). Two t-SNAREs, SNAP23 and SYN1A, a plasma-membrane anchored protein, bind to the N terminus of CFTR (specifically at amino acids 1–79) and to each other, and cooperatively reduce the capacity of PKA to activate CFTR. The N terminus of CFTR modulates PKA-mediated CFTR activation by interacting with the R domain and NBD1 of CFTR. The SNAP23–SYN1A complex might sterically block the N terminus from interacting with the R domain and NBD1 of CFTR, which would result in a decrease in channel activity. In some cells, such as oocytes, SYN1A also modulates CFTR currents by negatively regulating the formation of CFTR into the plasma membrane from an intracellular vesicular pool. However, there are no data to support a role for SYN1A in the regulation of exocytosis in epithelial cells. Interestingly, Munc-18a, a syntaxin-binding protein, which prevents SYN1A from interacting with CFTR, allows PKA to stimulate CFTR. Another member of the t-SNARE subfamily, SYN8, which is involved in vesicle trafficking from early endosomes to late endosomes and in homotypic fusion in late endosomes, binds to the N terminus and perhaps to the R domain of CFTR and inhibits exocytosis, thereby reducing the levels of CFTR in the plasma membrane. Therefore, SNAREs have an important physiological role in the regulation of CFTR activity, and the resulting capacity of epithelial cells to secrete Cl–.

**AMPK regulates CFTR.** The physical interaction between AMPK and CFTR is another example of how macromolecular complexes and molecular switches regulate and integrate several essential biological processes. AMPK is a member of the metabolite-sensing serine/threonine kinase family, which is activated by an increase in the AMP/ATP ratio during cell stress and anoxia. AMPK activity is elevated in cells that have

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**Figure 2 | Regulation of CFTR activity in the plasma membrane of epithelial cells.**

**a** | The adenosine 2b receptor (A2b-R) and the β2-adrenergic receptor (β2-AR) activate cystic fibrosis transmembrane conductance regulator (CFTR) in the apical plasma membrane of epithelial cells. A macromolecular signalling complex that is composed of CFTR, Na+/H+ exchanger regulatory factor isoform-1 (NHERF1), ezrin, protein kinase A (PKA), A2b-R and β2-AR, as well as adenylate cyclase (AC) and stimulatory GTP-binding protein (Gs), activates CFTR-channel activity. Activation of A2b-R or β2-AR elevates cyclic AMP, which activates PKA, a kinase that is tethered by ezrin and located near the regulatory (R) domain of CFTR. PKA phosphorylates the R domain and activates CFTR. The assembly of this macromolecular complex, which is facilitated by NHERF1, organizes key elements of the signalling pathway, including LPA2-R, Gi, adenylate cyclase and ezrin, that promote the efficient and rapid inhibition of CFTR by LPA2-R agonists. Therefore, the consumption of LPA-rich foods (such as eggs) might be an alternative method for treating some forms of secretory diarrhea — especially those that activate CFTR-mediated NaCl and fluid secretion in the intestine.

** Activation of CFTR in epithelial cells.** The β2-adrenergic receptor (β2-AR) and the adenosine 2b receptor (A2b-R), which both have important physiological roles in regulating CFTR-mediated Cl– secretion in human airways, assemble into a signalling complex that regulates CFTR activity. The β2-AR binds to CFTR (through the PDZ1 domain) and to β2-AR (through the PDZ2 domain), which is located in the apical plasma membrane of epithelial cells. When activated by an agonist, β2-AR increases the levels of AMP, which activates PKA, a kinase that is tethered by ezrin and located near the regulatory (R) domain of CFTR. PKA phosphorylates the R domain and activates CFTR. The close association of the proteins in these signalling complexes creates microdomains that are essential for the activation and inhibition of CFTR activity. ERM, ezrin, radixin, moesin binding domain; NBD, nucleotide-binding domain; R, regulatory domain.

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**Exocytosis**

The movement of vesicles inside the cell to the plasma membrane, where the vesicles fuse with the membrane. By fusing, they release the contents of the vesicle into the extracellular space and they insert proteins that were in the vesicle membrane into the plasma membrane.
been isolated from patients with cystic fibrosis, and pharmacological activation of AMPK inhibits the secretion of pro-inflammatory cytokines. This observation is consistent with the hypothesis that AMPK activation in cystic fibrosis airway cells is an adaptive response that reduces inflammation.

Initial studies showed that CFTR binds to AMPK and that this interaction might be essential for AMPK-mediated phosphorylation of CFTR, which reduces Cl⁻ secretion by inhibiting channel activity without affecting the number of CFTR channels in the plasma membrane. Because CFTR is activated by and consumes ATP, AMPK inhibition of CFTR minimizes the depletion of ATP during ischaemia. Therefore, therapies that activate AMPK in cystic fibrosis airways might prove beneficial for the reduction of ischaemia and airway inflammation, which are main causes of cystic fibrosis morbidity. However, any drug that activates AMPK might also inhibit CFTR, an effect that would exacerbate the symptoms of cystic fibrosis. Therefore, drugs that modulate AMPK would ideally inhibit inflammation, but not CFTR-channel activity.

**Intracellular trafficking of CFTR**

A second mechanism that increases CFTR-mediated Cl⁻ secretion in epithelial cells is the addition of CFTR channels to the apical plasma membrane from an intracellular vesicular pool. This section reviews the regulation of intracellular trafficking of CFTR between intracellular vesicles and the plasma membrane in some epithelial cells, including the intestine. The insertion of CFTR into the apical plasma membrane of the intestine, which is facilitated by bacterial toxins that cause secretory diarrhoea, converts the intestine from an absorptive to an secretory epithelium. Compelling evidence shows that the number of CFTR channels in the apical plasma membrane of polarized epithelial cells — and therefore the rate of transepithelial Cl⁻ secretion — is determined in part by the regulation of the endocytic retrieval of CFTR from the plasma membrane and the exocytic insertion of CFTR into the plasma membrane by intracellular vesicles. PKA is involved in this insertion and removal of CFTR from the plasma membrane. Interestingly, in many cells, the ability of PKA to regulate endocytosis and exocytosis depends on the expression of wild-type CFTR. In cells from patients with cystic fibrosis, PKA fails to alter endocytosis or exocytosis. This finding indicates that CFTR might regulate its own trafficking. By contrast, there is no direct evidence to support the view that PKA affects the levels of the CFTR-channel in the plasma membrane of airway epithelial cells. In fact, studies that have directly measured the levels of CFTR in airway epithelial cells report no change in the CFTR levels in the plasma membrane following stimulation with PKA. It has been suggested that the inability to show PKA-mediated regulation of CFTR trafficking might be due to the overexpression of CFTR; however, this observation was made in cells that express low levels of endogenous CFTR.

As proteins that interact with and regulate CFTR activity and trafficking are cell-type specific, it is probable that the capacity of PKA to regulate CFTR trafficking requires a unique set of cell-type-specific signalling and regulatory complexes. Therefore, cells that respond to PKA by increasing the plasma-membrane levels of CFTR, such as the intestinal cells, might have the requisite proteins to facilitate the trafficking of CFTR, whereas airway epithelial cells that do not manifest an increase in CFTR levels in response to PKA might not express the essential proteins that are needed to facilitate channel insertion.

**PDZ proteins, SNAREs and Rho GTPases.** A complex of proteins, which includes the PDZ-domain proteins NHERF1 and CAL as well as the SNARE SYN6 and the Rho GTPase TC10, regulates the levels of CFTR in the plasma membrane. Interestingly, several pathogens might cause secretory diarrhoea by causing the dysregulation of these highly dynamic protein complexes in intestinal epithelial cells.

The endocytosis and recycling of CFTR are regulated in a highly dynamic manner to affect plasma-membrane localization of CFTR, and therefore affect the capacity of the cell to transport Cl⁻ as well as the capacity of CFTR to regulate the function of other ion transporters and channels. PDZ-domain proteins regulate the exocytosis of CFTR. The deletion of the PDZ-interacting domain of CFTR inhibits the exocytosis of CFTR to the plasma membrane, but has no effect on the endocytic retrieval of CFTR from the membrane. Efficient exocytosis of CFTR requires that NHERF1 binds to both the C terminus of CFTR and to actin through the ERM domain of NHERF1, which binds to ezrin.

CAL, a PDZ-domain protein with two coiled-coil domains and one PDZ domain, also regulates CFTR trafficking. The overexpression of CAL causes a reduction in the number of CFTR channels in the plasma membrane and facilitates the trafficking of CFTR to lysosomes. This effect of CAL overexpression can be reversed by the overexpression of NHERF1. Moreover, ΔF508-CFTR can be coaxed to the plasma membrane by the overexpression of NHERF1. Therefore, the NHERF1 and CAL macromolecular complexes might be viable targets for cystic fibrosis drug discovery.

Recent studies have begun to examine how CAL regulates CFTR trafficking. Two proteins, specifically SYN6, which tethers CAL to the trans-Golgi, and TC10, bind to the second coiled-coil domain of CAL and modulate its capacity to regulate CFTR trafficking. SYN6 is a SNARE that is involved in vesicular transport between the trans-Golgi and endosomes. TC10 is a member of the Rho family of GTPases, a family of molecular switches that mediate cytoskeletal rearrangements, activation of signal-transduction cascades and activation of gene transcription. When activated by GTP, TC10 facilitates the targeting of CFTR-containing vesicles to the plasma membrane. Moreover, the activation of TC10 reverses the
Polarization of CFTR by PDZ-domain proteins. The role of the PDZ-interacting domain in regulating the polarized localization of CFTR to the apical plasma membrane of polarized epithelial cells is controversial. Initial studies showed that the deletion of the PDZ-interacting domain of CFTR (Thr-Arg-Leu) resulted in the loss of polarization of CFTR to the apical plasma membrane of kidney and human airway epithelial cells that express wild-type CFTR. Pulse-chase studies in combination with domain-selective cell-surface biotinylation studies showed that newly synthesized wild-type CFTR and CFTR that lacks the PDZ-interacting domain were targeted from the TGN to the apical and basolateral membranes in a non-polarized fashion. However, the half-life of wild-type CFTR in the apical plasma membrane was considerably longer than the half-life in the basolateral membrane. Moreover, the deletion of the PDZ-interacting domain dramatically reduced the half-life of CFTR in the apical membrane but had no effect on the half-life of CFTR in the basolateral membrane. These results show that CFTR interaction with NHERF1, which is localized to the apical, but not the basolateral, region of the cells, is important for the polarized localization of CFTR in the apical plasma membrane. Therefore, in the steady-state, CFTR polarizes to the apical plasma membrane of epithelial cells because the CFTR–NHERF1 interaction stabilizes and retains CFTR in the apical plasma membrane. However, the CFTR that traffics to the basolateral membrane does not interact with NHERF1 and is not retained in this membrane domain.

By contrast, other studies have concluded that the PDZ-interacting domain of CFTR does not regulate the apical polarization of CFTR. In these studies, the deletion of the C-terminal PDZ-interacting domain of CFTR had no effect on the apical polarization of CFTR in human airway epithelial cells isolated from a patient with cystic fibrosis. The deletion of the C-terminal six amino acids of CFTR also failed to alter the apical plasma-membrane localization of CFTR in several cell types, including kidney cells, pancreatic cells that were isolated from a patient with cystic fibrosis, airway epithelial cells and intestinal cells. The controversy concerning the role of the PDZ-interacting domain in determining CFTR polarization might be due to subtle differences in experimental design or the cell types that were used, however, recent data indicate another explanation.

Studies using the C-terminal tail of CFTR showed that the PDZ-binding motif of CFTR is essential, but not sufficient on its own, to localize CFTR to the apical membrane of kidney cells. Also, at least two other regions of the C terminus (amino acids 1370–1394 and 1404–1425) are required for the polarization of CFTR to the apical plasma membrane. These apical-localization signals might interact with the PDZ-interacting motif, PDZ proteins, and perhaps with other molecular switches. Therefore, the presence of multiple localization signals in the C terminus of CFTR and the possibility that other molecular switches might interact with the PDZ-interacting domain of CFTR is important for the polarized localization of CFTR to the apical plasma membrane.
with these signals indicate: that the cellular localization of CFTR is highly regulated, that the trafficking signals in CFTR might be redundant and possibly hierarchical, and that the variable levels of interacting proteins might be cell-type dependent. These possibilities could explain why the deletion of the PDZ-interacting domain alone does not always alter the function and localization of CFTR and they provide testable hypotheses that could be addressed in future studies.

**Rab GTPases.** Rab GTPases are catalysts that have many vital functions, including cargo selection during transport vesicles formation. They allow motor proteins to interact with membranes and to facilitate vesicle motility, and they interact with a host of other proteins to assure that transport vesicles dock and fuse with high fidelity to the appropriate target membranes. Many Rab GTPases regulate the intracellular transport and the plasma-membrane localization of CFTR. Rab5 regulates the trafficking of CFTR from the plasma membrane to early endosomes. From early endosomes, CFTR is recycled back to the plasma membrane through recycling endosomes that are controlled by Rab11 and RME1, a protein that is required for the release of cargo from recycling endosomes to the plasma membrane. Myosin-Vb, a molecular motor that drives vesicular transport on actin tracks and binds to Rab11, is also required for the efficient exocytosis of CFTR. The disruption of the interaction between NHERF1 and CFTR or between NHERF1, Rab11, myosin-Vb and actin inhibits CFTR exocytosis. Also, CFTR can be recycled from early endosomes to the trans-Golgi, a process that is regulated by Rab11.

**RAB7** controls the movement of CFTR from early endosomes to late endosomes, and also facilitates the trafficking of CFTR to lysosomes. Alternatively, Rab9 can drive CFTR from late endosomes to the trans-Golgi. From the trans-Golgi, CFTR can re-enter the secretory pathway and be delivered to the plasma membrane. Regulation of CFTR trafficking that is carried out by Rab GTPases has been exploited to increase the expression of ΔF508-CFTR in the plasma membrane. Although the biological role of these molecular switches in regulating CFTR function, and possibly the trafficking of ΔF508-CFTR, has not been fully elucidated, Rab GTPases are potential targets that might be used to identify new drugs to treat cystic fibrosis and secretory diarrhoea.

**Interactions between CFTR and other channels**

As noted above, CFTR regulates several other transport proteins, including ENaC, K+ channels (such as ROMK1 and ROMK2), ATP-release mechanisms, anion exchangers, and aquaporin water channels. Although little is known about the molecular mechanisms whereby CFTR regulates these transporters, it is becoming increasingly clear that such regulation involves the formation of macromolecular signalling complexes that are dynamically regulated by molecular switches, including PDZ-domain proteins. For example, NHERF1 and NHERF2 increase the physical interaction between ROMK2 (renal outer medullary K+ channel, a K+ channel that has an important role in renal function) and CFTR. The NHERF-facilitated interaction between ROMK2 and CFTR enhances glibenclamide-induced activation of ROMK2.

NHERF1 is crucial for the reciprocal regulation between CFTR and the SLC26 family of chloride-bicarbonate exchangers. The binding of CFTR and SLC26 transporters to PDZ domains in NHERF1 facilitates the interaction between the R domain of CFTR and the STAS domain (named after sulfate transporter and anti-sigma-factor antagonist) of the SLC26 transporters, which increases the ion-transport activity of both transporters. Moreover, the interaction between the R domain and STAS domains is regulated by PKA-mediated phosphorylation of the R domain. Therefore, the regulation of chloride absorption and bicarbonate secretion by pancreatic ducts, mediated by SLC26 transporters, depends on the formation of a macromolecular complex that is facilitated by NHERF1 and PKA-induced phosphorylation of CFTR. The disruption of this complex by mutations in CFTR results in defective chloride and bicarbonate transport in the pancreatic duct of patients with cystic fibrosis. Although the biological significance of the coordinated...
regulation by molecular switches is not always clear, when this coordinated regulation is disrupted during diseases such as cystic fibrosis, multiple phenotypes are affected.

**Reciprocal regulation of CFTR and ENaC.** NHERF1 also mediates, at least indirectly, the interaction between CFTR and ENaC, and might facilitate the reciprocal regulation of these channels. CFTR down-regulates ENaC (except in sweat ducts where CFTR activates ENaC), whereas ENaC activates CFTR. This interaction between CFTR and ENaC is biologically relevant because the balance between CFTR-mediated Cl⁻ secretion and ENaC-mediated Na⁺ reabsorption regulates the net amount of salt and water in airway periciliary fluid, and thereby the capacity to clear bacteria and other noxious agents from the lungs. It is tempting to speculate that the coordinated regulation of CFTR and ENaC involves a large dynamic signalling complex that is composed of PDZ-domain proteins and kinases. NHERF1, through its PDZ1 domain, binds to CFTR and to the YES-associated protein-65 (YAP65) through its PDZ2 domain. YAP65 has several other protein-binding modules, including a WW domain, a Src-homology-3 (SH3) domain, and a coiled-coil motif, that are important for ‘signalplex formation’. The WW domain binds to the PY motif in the C terminus of all three ENaC subunits. Because the SH3 domain of YAP65 interacts with the non-receptor tyrosine kinase c-YES, a member of the c-Src kinase family, and because c-Src is a potent inhibitor of ENaC, it is probable that c-YES mediates the CFTR inhibition of ENaC-channel activity.

Challenges that lie ahead

The past several years have seen an explosion of knowledge regarding the identification and elucidation of the molecular switches that regulate CFTR activity. Many studies have identified ‘drugable’ targets, and several new compounds, based on the results of these studies, are in clinical trials. However, it is clear that we have only scratched the surface of CFTR regulation. To gain a complete understanding of CFTR, and to identify more targets for cystic fibrosis therapy, we must identify all of the CFTR-interacting proteins and understand how these proteins are dynamically regulated by protein–protein interactions and by post-translational mechanisms, including phosphorylation, dephosphorylation and ubiquitylation. Moreover, it will be important to show that protein–protein interactions, which are identified in heterologous cells, also occur in vivo. Studies in knockout mice (for example, the knockout of NHERF1) to examine the effect of genes on CFTR function and expression would be particularly valuable. It is also essential to understand how mutations in CFTR affect the formation and localization of signalling complexes and whether alterations in signalling-complex formation account for the numerous phenotypic changes that are observed in patients with cystic fibrosis. To achieve these goals, mass spectroscopy will be particularly useful, especially in the identification of the CFTR interactome for wild-type and mutant forms of CFTR. The identification of the CFTR-binding partners will show how CFTR functions as an ion channel and as a regulator of other transporters, and will help to identify new drug targets for cystic fibrosis and other diseases that are caused by CFTR mutations. Because similar dynamic macromolecular complexes regulate other ABC transporters, these studies will also provide an insight into the aetiology of many other diseases that are linked to other ABC transporters.
Reviews macromolecular-complex regulation of CFTR.


Shows that AMPK inhibits CFTR-channel activity.


Acknowledgements

Due to space limitations the authors regret that they could not cite all of the outstanding papers that have contributed to this field. The authors thank A. Swiatecka-Urban for comments on this review. National Institutes of Health Grants and Research Development Program grants from the Cystic Fibrosis Foundation supported research in the author's laboratories.

Competing interests statement

The authors declare no competing financial interests.

DATABASES

The following terms in this article are linked online to: OMIM: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM

ABCA1 | ABCA4 | ABCC2 | ABCC7 | ABCC8


UniprotKB: http://ca.expasy.org/sprot

AB2 | B3X | SNAP23 | SYN1A | SYN6 | SYN8 | TC10 | YAP6

FURTHER INFORMATION

William Guggino’s homepage: http://www.guggino.org

Bruce Stanton’s homepage: http://www.dartmouth.edu/~physiology/faculty/stanton.html

http://www.mayoclinic.org/health/cystic-fibrosis/D-20032431/


Cystic Fibrosis Foundation: http://wwwcff.org/about_the_cff/foundation/50th_anniversary/

Cystic Fibrosis Mutation Database: http://www.genet.sickkids.on.ca/cff/

Cystic Fibrosis Foundation – Clinical Trials & Clinical Studies: http://www.cff.org/research/cysticfifis/clinicaltrials/

Access to this links box is available online.
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Toc Blurb
Cystic fibrosis transmembrane conductance regulator (CFTR), a Cl\textsuperscript{−}-ion channel, assembles into dynamic macromolecular complexes. Understanding how these complexes regulate the intracellular trafficking and activity of CFTR provides a unique insight into the aetiology of cystic fibrosis and other diseases.

Online summary
- Cystic fibrosis transmembrane conductance regulator (CFTR) is a member of the ATP-binding cassette (ABC) transporter superfamily. Mutations in ABC genes have been linked to many genetic diseases including cystic fibrosis (associated with mutations in \(ABCC7\)), Dubin–Johnson Syndrome (associated with mutations in \(ABCC2\)), age-related macular degeneration (associated with mutations in \(ABCA4\)), Tangier disease (associated with mutations in \(ABCA1\)) and sitosterolaemia (associated with mutations in \(ABCG5\) and \(ABCG8\)).

- CFTR is a plasma membrane, cAMP-activated Cl\textsuperscript{−} channel that is expressed in functionally diverse tissues, including kidney, pancreas, intestine, heart, vas deferens, sweat duct and lung. In epithelial cells, CFTR mediates Cl\textsuperscript{−} secretion.

- Mutations in CFTR cause cystic fibrosis, whereas the activation of CFTR in the intestine causes secretory diarrhoea. Therefore the function and regulation of CFTR are intensively investigated.

- CFTR assembles into large, dynamic macromolecular complexes that contain signalling molecules, kinases, transport proteins, PDZ-domain-containing proteins, myosin motors, Rab GTPases, and SNAREs. These molecular switches ensure the fidelity of signalling, amplify signals, enable efficient signalling and facilitate the interaction of CFTR with other transport proteins.

- Understanding how molecular switches and macromolecular signalling complexes regulate the intracellular trafficking and activity of CFTR provides a unique insight into the aetiology of cystic fibrosis and other diseases that involve CFTR-mediated Cl\textsuperscript{−} secretion, including secretory diarrhoea that is caused by \textit{Vibrio cholera} and \textit{Clostridium difficile} toxins.

- This review discusses how molecular switches and macromolecular complexes regulate CFTR and speculates about some promising areas for additional study in this dynamic and rapidly moving area of research.

Online links

\(ABCA1\)

\(ABCA4\)

\(ABCC2\)

\(ABCC7\)

\(ABCG5\)

UniProtKB: http://ca.expasy.org/sprot

A2b-R
http://ca.expasy.org/uniprot/P29275

\(\beta2AR\)
http://ca.expasy.org/uniprot/P07550

SNAP23
http://ca.expasy.org/uniprot/O00161

SYN1A
http://ca.expasy.org/uniprot/Q16623

SYN6
http://ca.expasy.org/uniprot/O43752

SYN8
http://ca.expasy.org/uniprot/Q9UNK0

TC10
http://ca.expasy.org/uniprot/O14559

YAP65
http://ca.expasy.org/uniprot/P46937

William Guggino’s homepage: http://www.guggino.org

Bruce Stanton’s homepage: http://www.dartmouth.edu/~physiol/faculty/stanton.html

Cystic Fibrosis Foundation: http://www.cff.org/about_the_cf_foundation/50th_anniversary/


Cystic Fibrosis Mutation Database: http://www.genet.sickkids.on.ca/cftr/

Cystic Fibrosis Foundation – Clinical Trials & Clinical Studies: http://www.cff.org/research/clinical_trials/#CF-specific