MECHANISMS OF DISEASE

New insights into cystic fibrosis: molecular switches that regulate CFTR

William B. Guggino and Bruce A. Stanton‡*

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.

Cystic fibrosis transmembrane conductance regulator (CFTR). A Cl^--selective ion channel that is activated by protein kinase A, and is a regulator of other ion channels and transporters.

ATP-binding cassette (ABC) transporters
A family of transport proteins that bind ATP and use its energy to transport molecules across cell membranes. Examples include the multidrug-resistance-related proteins MRP2 and MRP4, P-glycoprotein, ABCA1 and ABCA7.

Cystic fibrosis
An autosomal recessive genetic disease that is caused by mutations in CFTR.

*Department of Physiology and Pediatrics, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA.
‡Department of Physiology, Dartmouth Medical School, Hanover, New Hampshire, USA.
Correspondence to B.A.S.
e-mail:bas@Dartmouth.edu
doi:10.1038/nrm1949

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 years[1–3]. 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 lung[4,5]. 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 channels[6–13]. CFTR and transport proteins form large macromolecular signalling complexes, which are regulated by molecular switches. CFTR and molecular switches mediate transepithelial 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 disease[14]. 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 ischaemia[16].

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 proteasome[17–20] (BOX 1). In the ER, molecular chaperones, including calnexin in the lumen of the

Box 1 | **Cystic fibrosis is an autosomal recessive disease**

Cystic fibrosis, one of the most common lethal genetic diseases in Caucasians, is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene[1,17,18]. The CFTR gene, which is expressed on chromosome 7, encodes a 1480 amino-acid residue protein. Approximately 1 in 20 Caucasians are carriers for mutations in CFTR (there are estimated to be ~10,000,000 carriers in the United States); however, carrier frequency is much less in Hispanic, Asian and African-American populations. There are approximately 30,000 patients with cystic fibrosis in the United States and an estimated 60,000 affected individuals worldwide. Although cystic fibrosis is classified as an orphan disease, individuals who are carriers for CFTR mutations have an increased incidence of nasal polyposis, allergic bronchopulmonary aspergillosis, diffuse bronchiectasis, azoospermia, congenital bilateral absence of the vas deferens (which causes male infertility), chronic pancreatitis and chronic rhinosinusitis[2,3]. Therefore, it is becoming increasingly clear that mutations in CFTR are associated with significant morbidity.

Mutations in the CFTR gene disrupt CFTR function by five different mechanisms[17]. Class I mutations lead to premature transcription termination that results in an unstable truncated CFTR transcript or no CFTR expression. Missense mutations (Class II), including ΔF508-CFTR, cause protein misfolding that leads to the retention of the misfolded protein in the endoplasmic reticulum and premature degradation. Class III mutations result in the reduced capacity of CFTR to secrete Cl^- due to abnormal channel activation by ATP. Class IV mutations cause a reduced capacity to conduct Cl^- across membranes, whereas class V mutations cause abnormal or alternative splicing, which reduces the amount of functional protein. Significant reduction (>90%) of functional CFTR in the plasma membrane of airway epithelial cells results in a defect in Cl^- secretion, hyperabsorption of sodium and other changes that reduce the capacity of cilia to clear bacteria from the airways[17,18]. This effect results in chronic infection with *Pseudomonas aeruginosa* and an enhanced inflammatory response that ultimately leads to a decrease in respiratory function.

Over 1,400 mutations have been identified in the CFTR gene (for a comprehensive list see the Cystic Fibrosis Mutation Database in Further Information), however, the most common mutation is ΔF508-CFTR. Approximately 70% of individuals with cystic fibrosis are homozygous for the ΔF508-CFTR mutation, and ~90% of patients with cystic fibrosis have at least one ΔF508-CFTR allele. Because of the prevalence of the ΔF508-CFTR mutation and because all of the other mutations are relatively rare, the research and drug-discovery programmes have focused on ΔF508-CFTR.

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.

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 inhibits ENaC, whereas in sweat ducts CFTR activates ENaC.

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.

CFTR activity in the plasma membrane

Many cells regulate Cl^- secretion by modulating CFTR Cl^--channel activity through several signalling mechanisms, including those involving phosphorylation and dephosphorylation. This section discusses the mechanisms that regulate the activity of the CFTR channel. It also discusses the regulation of the molecular switches that are essential for the assembly of kinases and phosphatases into dynamic signalling complexes that amplify signals and increase the fidelity and specificity of signalling.

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)[19]. 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 YDSI, 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[21–25]. 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[23]. Proteins that contain PDZ domains often contain other protein-interacting

Ras GTPase
The Ras GTPase superfamily of small monomeric G proteins are GDP–GTP regulated molecular switches.

SNAREs
(Soluble *N*-ethylmalemide-sensitive factor attachment protein receptors). These proteins mediate membrane fusion and vesicle trafficking by assembling into complexes that link vesicle-associated SNAREs (v-SNAREs) with SNAREs on target membranes (t-SNAREs).

PDZ domain
PDZ domains, which are named after the three proteins in which this domain was first described (PSD95, Dlg, and ZO-1), are 80–100 amino-acid modules that mediate 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 heterotrimeric 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.

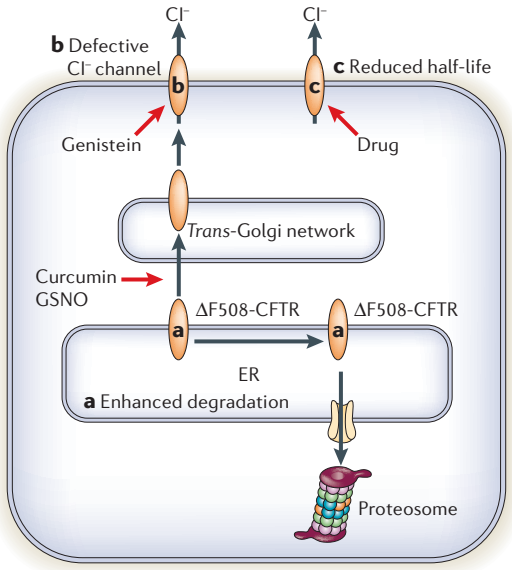
Na^+/H^+ exchanger regulatory factor isoform-1
(NHERF1). The first PDZ-domain protein that was identified to bind to and regulate CFTR. NHERF1 was originally named for its capacity to inhibit the Na^+/H^+ exchanger.

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.

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[1,17,18,92]. Drug discovery for cystic fibrosis is focused on identifying drugs that allow ΔF508-CFTR to escape the ER[99–102] and to activate ΔF508-CFTR channels that reach the plasma membrane[101].

Curcumin, a component of the spice tumeric, 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 trails 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[103], the use of aminoglycosides to increase the production of CFTR in patients with Class I stop mutations[104], and the identification of drugs that activate other, normally inactive, Cl^- channels in the airway. Also, because sodium absorption is increased in patients with cystic fibrosis, several drugs are in development to reduce airway sodium absorption[18,101]. 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.

modules (such as, ezrin, radixin, moesin (ERM)-binding domains and coiled-coil domains) and therefore can promote homotypic and heterotypic protein–protein interactions[21–25]. Many ABC transporters, including CFTR, ABCA1, ABCA7 and the multidrug-resistance-related proteins MRP2 and MRP4 have PDZ-binding motifs at their C termini[26].

PDZ-domain proteins assemble complexes of signalling proteins that ensure high fidelity and efficient signal transduction and thereby regulate CFTR-channel activity and plasma-membrane protein localization. This section will review PDZ regulation of CFTR activity and Cl^- secretion, whereas the regulation of CFTR localization in the plasma membrane by PDZ-domain proteins will be discussed below.

The sequence of the PDZ-interacting domain in the C terminus of human 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)[21–25,27]. 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[28,29] (FIG. 1). 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[30].

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[31,32] (FIG. 1). Dimerization of CFTR might facilitate interaction between the R domains, which increases the activity of CFTR channels[34]. 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[35].

Although biochemical, imaging and electrophysiological studies showed that CFTR might exist either as a monomer or as a dimer[19,31,35–40], 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

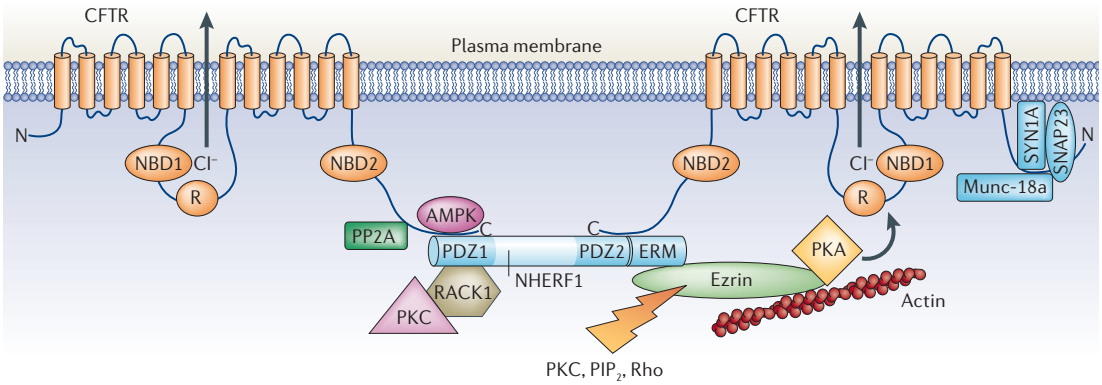


Figure 1 | **Molecular switches that regulate CFTR activity in the plasma membrane.** Several proteins interact directly or indirectly with the cystic fibrosis transmembrane conductance regulator (CFTR), including protein phosphatase-2A (PP2A), AMP kinase (AMPK), syntaxin-1A (SYN1A), synaptosome-associated protein, 23 kDa (SNAP23) and Munc-18a. These proteins inhibit channel activity and reduce CFTR-mediated Cl^- secretion across the apical plasma membrane in epithelial cells. Other CFTR-interacting proteins that enhance CFTR activity, either directly or indirectly, include Na^+/H^+ exchanger regulatory factor isoform-1 (NHERF1), receptor for activated C-kinase-1 (RACK1), protein kinase C (PKC), protein kinase A (PKA) and ezrin. ERM, ezrin, radixin, moesin binding domain; NBD, nucleotide-binding domain; PIP_2, phosphatidylinositol bisphosphate; R, regulatory domain.

of active (that is, phosphorylated) CFTR channels in the plasma membrane[19]. 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[41,42]. 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[28,43] (FIG. 1). These interactions, however, are highly dynamic and regulated. Ezrin is a molecular switch that, when activated by PKC, phosphatidylinositol bisphosphate (PIP_2) and Rho GTPases, binds to NHERF1 and increases the affinity of the PDZ2 domain of NHEFR1 for CFTR. This might facilitate CFTR dimerization and the formation of a microdomain in which PKA can efficiently phosphorylate CFTR[44].

However, PKA can also negatively regulate CFTR. PKA phosphorylation of the R domain inhibits the binding of CFTR to NHERF1, which reduces channel dimerization and, thereby, CFTR-channel activity[45]. Other proteins, including adenylate cyclases, phosphatases and cAMP-specific phosphodiesterases, are also recruited to CFTR-containing signalling complexes by PDZ-domain proteins and their interacting partners[46]. 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[46], 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 interaction facilitates the efficient dephosphorylation of CFTR[47,48] (FIG. 1). NHERFs also recruit phospholipase C (PLC), which has a C-terminal PDZ-binding motif, to this signalling complex. The β1- and β2-isoforms of PLC interact with NHERF1, whereas the β3-isoform interacts with NHERF2 (REF. 49). Activated PLC promotes the PKC-mediated phosphorylation of the second PDZ domain in NHERF1 — this modification blocks the stimulatory effect of NHERF1 on CFTR[50]. Moreover, it has been shown that PKCξ can be tethered to NHERF1 by RACK1 (a receptor for activated C-kinase), which binds to the PDZ1 domain in NHERF1 (REFS 51,52) (FIG. 1). Taken together, these findings indicate that CFTR-interacting proteins, including PKA, PP2A and PKC, regulate the phosphorylation state of CFTR, and this regulation depends on PDZ-domain proteins to function as molecular glue.

Fluorescent recovery after photobleaching
A fluorescent-based confocal-microscopy imaging technique that is used to monitor protein trafficking and to study protein–protein interactions in cells.

Endocytosis
A process by which extracellular material and membrane-resident proteins are taken up by cells.

Rho GTPase
Members of the Ras GTPase superfamily that regulate signalling networks that influence actin organization, the cell cycle, and gene expression.

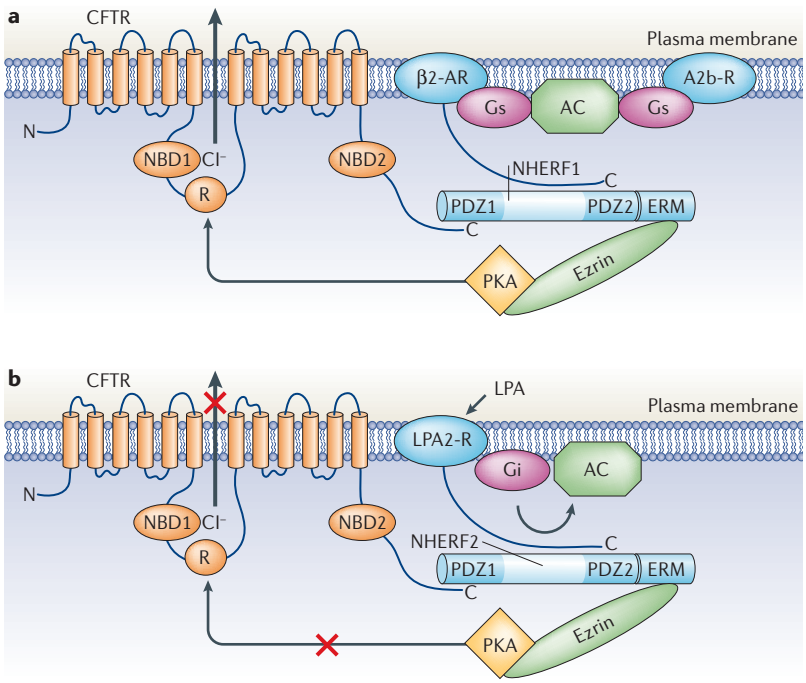


Figure 2 | **Regulation of CFTR activity in the plasma membrane of epithelial cells.** **a** | The adenosine 2b receptor (A2b-R) and the β2-adrenergic receptor (β2-AR) activate cystic fibrosis transmembrane conductance regulator (CFTR) in the apical plasma membrane of epithelial cells. A macromolecular 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. **b** | The lysophosphatidic-acid-2 receptor (LPA2-R) in the apical membrane inhibits CFTR activity. A macromolecular signalling complex that is composed of CFTR, NHERF2, ezrin, PKA, as well as AC and inhibitory GTP-binding protein (Gi) inactivates CFTR. Activation of LPA2-R reduces cAMP and PKA phosphorylation of 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.

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[45] (FIG. 2a). NHERF1 binds to CFTR (through the PDZ1 domain) and to β2-AR (through the PDZ2 domain), which is located in the apical plasma membrane of airway epithelial cells (FIG. 2a). When activated by an agonist, β2-AR increases the levels of cAMP, which activates PKA and thereby activates CFTR. The assembly of this macromolecular complex, which is facilitated by NHERF1, organizes key elements of the signalling pathway, including the β2-AR, stimulatory GTP-binding protein (Gs), adenylate cyclase, ezrin and PKA, that promote the efficient and rapid activation of CFTR by β2-AR agonists[45,53,54]. NHERF1 also has an important role in the organization of the signalling complex that facilitates adenosine activation of CFTR through the activation of A2b-R and PKA[54] (FIG. 2a).

By contrast, the lysophosphatidic acid-2 receptor (LPA2-R) in the apical membrane of intestinal epithelial cells inhibits CFTR[55]. 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[55]. Therefore, the consumption of LPA-rich foods (such as eggs) might be an alternative method for treating some forms of secretory diarrhoea — 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)[35,56–59]. 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 (FIG. 1). 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 insertion of CFTR into the plasma membrane from an intracellular vesicular pool[58]. 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[57,59,60]. 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[61]. 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[62–66], which is activated by an increase in the AMP/ATP ratio during cell stress and anoxia. AMPK activity is elevated in cells that have

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[66].

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 (FIG. 1). 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 into a 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[5]. PKA is involved in this insertion and removal of CFTR from the plasma membrane[4,5,42,67–74]. 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[5,75,76]. 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[75,76]. 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[75,76].

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 (FIG. 3). Importantly, 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[77] (FIG. 3). 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[77].

CAL, a PDZ-domain protein with two coiled-coil domains and one PDZ domain, also regulates CFTR trafficking[78–81] (FIG. 3). 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[78–81]. 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(REF. 82). 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[78,83] (FIG. 3). 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[78]. Moreover, the activation of TC10 reverses the

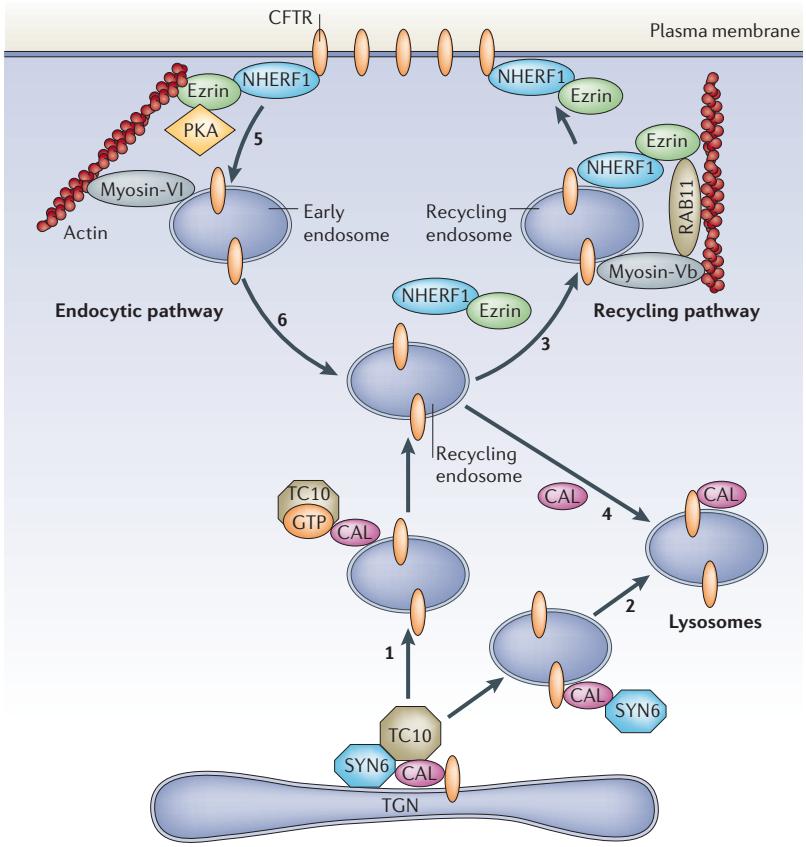


Figure 3 | **Model describing how molecular switches regulate CFTR trafficking.** **1** | The second coiled-coil domain of CAL (cystic fibrosis transmembrane conductance regulator (CFTR)-associated ligand) binds to syntaxin-6 (SYN6), which tethers CAL to the *trans*-Golgi network (TGN). The Rho GTPase TC10 also binds to the second coiled-coil domain of CAL. When activated by GTP, TC10 facilitates the entry of CFTR (depicted as an orange oval) into the exocytic pathway and CFTR is inserted into the plasma membrane (through step **3**). **2** | SYN6 facilitates the trafficking of CAL–CFTR from TGN to lysosomes. **3** | In recycling endosomes, if the Na^+/H^+ exchanger regulatory factor isoform-1 (NHERF1)–ezrin complex displaces CAL from interacting with CFTR, then CFTR-containing endosomes are delivered to the apical membrane. This process is facilitated by myosin-Vb, a molecular motor that drives vesicle transport to the plus end of F-actin (A. Swiatecka-Urban and B. S., unpublished observations) and the GTPase RAB11. CAL and NHERF1 compete for the PDZ-interacting domain of CFTR at some sites in the cell, probably in recycling endosomes. **4** | However, if CFTR binds to CAL, it drives CFTR from endosomes to lysosomes. **5** | CFTR in the plasma membrane is stabilized by NHERF1 and ezrin, which binds to the submembrane actin network. **6** | When NHERF1 dissociates from CFTR, CFTR is endocytosed, perhaps due to protein kinase A (PKA)-mediated phosphorylation of the threonine in the PDZ-interacting domain in the C terminus of CFTR, which reduces the affinity of CFTR for NHERF1. This allows CFTR to bind to the adaptor protein-2 (AP2)–clathrin complex, which facilitates the endocytic removal of CFTR from the membrane. The endocytosis of CFTR requires myosin-VI, a molecular motor that drives cargo to the minus end of F-actin[105].

CAL-mediated degradation of CFTR and increases the levels of CFTR in the plasma membrane[78]. Toxin B, which is secreted by *C. difficile* and causes secretory diarrhoea, activates Rho GTPases. It is therefore tempting to speculate that the activation of TC10 by toxin B might facilitate the targeting of CFTR-containing vesicles to the plasma membrane and thereby increase CFTR-mediated secretory diarrhoea.

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[33,77,84–88]. 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[87,88]. 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 *trans*-Golgi to the apical and basolateral membranes in a non-polarized fashion[77]. 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[84]. 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[33]. 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[85,86]. 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

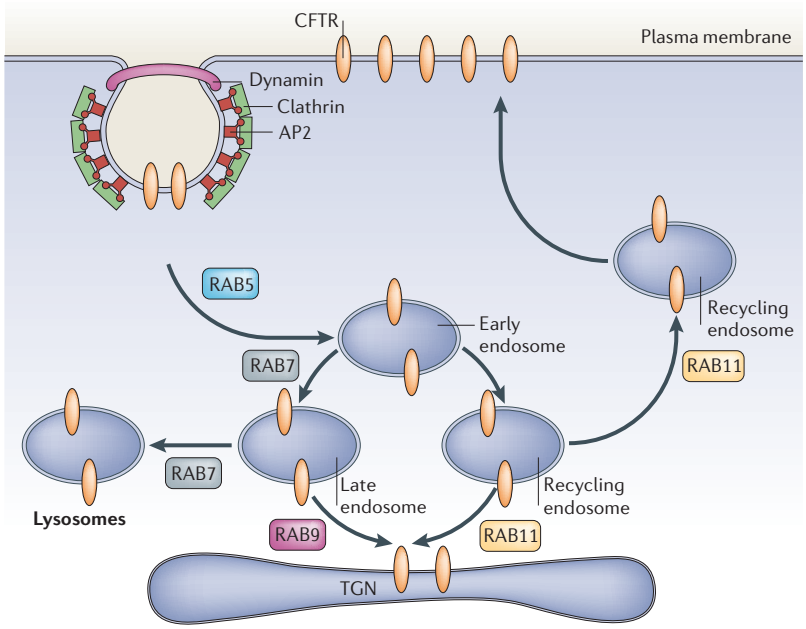


Figure 4 | **Rab GTPases regulate CFTR trafficking, and thereby the localization of CFTR in the plasma membrane.** Cystic fibrosis transmembrane conductance regulator (CFTR; depicted as orange ovals) is endocytosed from the apical membrane by a clathrin-dependent process that involves dynamin, which is required for vesicle formation, and adaptor protein-2 (AP2), which binds to clathrin and an endocytic motif (YDSI) in the C terminus of CFTR. Several members of the Rab family of GTPases have been shown to control CFTR trafficking. RAB5 promotes endocytosis and RAB7 enhances the degradation of CFTR by facilitating the trafficking of CFTR to lysosomes. RAB9 mediates CFTR trafficking from late endosomes to the *trans*-Golgi network (TGN). RAB11 promotes CFTR trafficking from recycling endosomes to either the plasma membrane or TGN. The overexpression of RAB11 causes ΔF508-CFTR to traffic to the 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[89]. Many Rab GTPases regulate the intracellular transport and the plasma-membrane localization of CFTR[90–92] (FIG. 4). 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[90]. 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 (A. Swiatecka-Urban and B. S., unpublished observations). 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[91,92]. 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 increasing 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[13]. 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[6]. 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 transporters 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[6]. 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

Rab GTPases
Members of the Ras GTPase superfamily that control endocytosis, vesicle trafficking, endosome fusion and exocytosis.

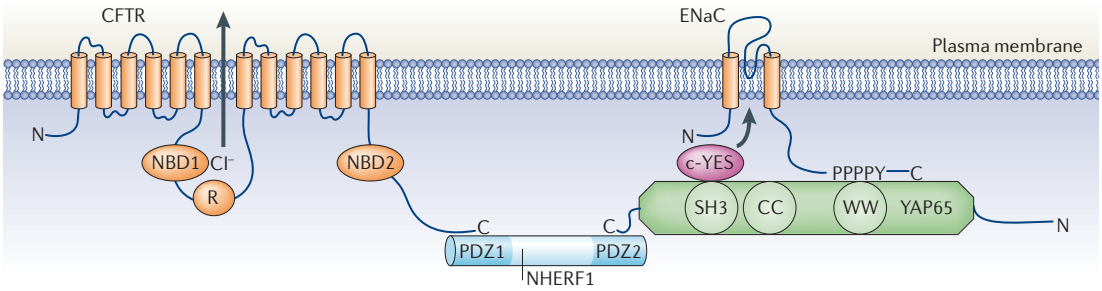


Figure 5 | **NHERF1 mediates the protein–protein interaction between CFTR and ENaC and facilitates reciprocal regulation between these channels.** The interaction between cystic fibrosis transmembrane conductance regulator (CFTR) and the epithelial sodium channel (ENaC) might involve a large dynamic signalling complex that consists of PDZ-domain proteins and kinases. CFTR binds to the PDZ1 domain of Na^+/H^+ exchanger regulatory factor isoform-1 (NHERF1). The YES-associated protein-65 (YAP65) binds to the PDZ2 domain of NHERF1. The WW domain of YAP65 binds to the PY motif in the C termini of three ENaC subunits (only one subunit is shown in the figure). Because the Src-homology-3 (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. CC, coiled-coil domain; NBD, nucleotide-binding domain; R, regulatory domain.

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[7,8,93–95] (FIG. 5). 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[18]. 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[96]. 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[97]. 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[98], c-YES might mediate CFTR inhibition of ENaC-channel activity. Therefore, NHERF1 might assemble CFTR and ENaC into a signalling complex that would include YAP65 and a c-Src kinase that would mediate the inhibition of ENaC by CFTR.Mutations in CFTR would exclude CFTR from this complex and so enhance ENaC-mediated fluid absorption, causing a subsequent reduction in the depth of the periciliary fluid, and thereby a decrease in mucociliary clearance. This chain of events would ultimately facilitate the chronic infection of the airways by bacteria.

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.

1. Davis, P. B. Cystic fibrosis since 1938. *Am. J. Respir. Crit. Care Med.* **173**, 475–482 (2006).
2. Wang, X., Kim, J., McWilliams, R. & Cutting, G. R. Increased prevalence of chronic rhinosinusitis in carriers of a cystic fibrosis mutation. *Arch. Otolaryngol. Head Neck Surg.* **131**, 237–240 (2005).
3. Cohn, J. A. *et al.* Increased risk of idiopathic chronic pancreatitis in cystic fibrosis carriers. *Human Mutat.* **26**, 303–307 (2005).
4. Bradbury, N. A. Intracellular CFTR: localization and function. *Physiol. Rev.* **79**, S175–S191 (1999).
5. Bertrand, C. A. & Frizzell, R. A. The role of regulated CFTR trafficking in epithelial secretion. *Am. J. Physiol. Cell Physiol.* **285**, C1–C18 (2003).
An excellent review of PKA regulation of CFTR trafficking.
6. Ko, S. B. *et al.* Gating of CFTR by the STAS domain of SLC26 transporters. *Nature Cell Biol.* **6**, 343–350 (2004).
7. Ji, H. L. *et al.* The cytosolic termini of the β- and γ-ENaC subunits are involved in the functional interactions between cystic fibrosis transmembrane conductance regulator and epithelial sodium channel. *J. Biol. Chem.* **275**, 27947–27956 (2000).
8. Jiang, Q. S. *et al.* Epithelial sodium channels regulate cystic fibrosis transmembrane conductance regulator chloride channels in *Xenopus* oocytes. *J. Biol. Chem.* **275**, 13266–13274 (2000).
9. Stutts, M. J., Rossier, B. C. & Boucher, R. C. Cystic fibrosis transmembrane conductance regulator inverts protein kinase A-mediated regulation of epithelial sodium channel single channel kinetics. *J. Biol. Chem.* **272**, 14037–14040 (1997).
Describes the functional interaction between CFTR and ENaC.
10. Cheung, K. H., Leung, C. T., Leung, G. P. & Wong, P. Y. Synergistic effects of cystic fibrosis transmembrane conductance regulator and aquaporin-9 in the rat epididymis. *Biol. Reprod.* **68**, 1505–1510 (2003).
11. Shumaker, H., Amlal, H., Frizzell, R., Ulrich, C. D., II & Soleimani, M. CFTR drives Na^+–$NHCO_3^-$ cotransport in pancreatic duct cells: a basis for defective HCO_3^- secretion in CF. *Am. J. Physiol. Cell Physiol.* **276**, C16–C25 (1999).
12. Schwiebert, E. M. *et al.* CFTR regulates outwardly rectifying chloride channels through an autocrine mechanism involving ATP. *Cell* **81**, 1063–1073 (1995).
13. Yoo, D. *et al.* Assembly and trafficking of a multiprotein ROMK (Kir 1.1) channel complex by PDZ interactions. *J. Biol. Chem.* **279**, 6863–6873 (2004).
14. Stanton, B. A. Cystic fibrosis transmembrane conductance regulator (CFTR) and renal function. *Wien Klin. Wochenschr.* **109**, 457–464 (1997).
15. Thiagarajah, J. R. & Verkman, A. S. CFTR pharmacology and its role in intestinal fluid secretion. *Curr. Opin. Pharmacol.* **3**, 594–599 (2003).
16. Duan, D. Y. *et al.* Functional role of anion channels in cardiac diseases. *Acta Pharmacol. Sin.* **26**, 265–278 (2005).
17. Gibson, R. L., Burns, J. L. & Ramsey, B. W. Pathophysiology and management of pulmonary infections in cystic fibrosis. *Am. J. Respir. Crit. Care Med.* **168**, 918–951 (2003).
18. Boucher, R. C. New concepts of the pathogenesis of cystic fibrosis lung disease. *Eur. Respir. J.* **23**, 146–158 (2004).
19. Riordan, J. R. Assembly of functional CFTR chloride channels. *Annu. Rev. Physiol.* **67**, 701–718 (2005).
An excellent review of CFTR-dimer formation.
20. Welch, W. J. Role of quality control pathways in human diseases involving protein misfolding. *Semin. Cell Dev. Biol.* **15**, 31–38 (2004).
21. Shenolikar, S., Voltz, J. W., Cunningham, R. & Weinman, E. J. Regulation of ion transport by the NHERF family of PDZ proteins. *Physiology (Bethesda)* **19**, 362–369 (2004).
22. Brone, B. & Eggermont, J. PDZ proteins retain and regulate membrane transporters in polarized epithelial cell membranes. *Am. J. Physiol. Cell Physiol.* **288**, C20–C29 (2005).
23. Guggino, W. B. The cystic fibrosis transmembrane regulator forms macromolecular complexes with PDZ domain scaffold proteins. *Proc. Am. Thorac. Soc.* **1**, 28–32 (2004).
A review of PDZ regulation of CFTR trafficking and function.
24. Li, C. & Naren, A. P. Macromolecular complexes of cystic fibrosis transmembrane conductance regulator and its interacting partners. *Pharmacol. Ther.* **108**, 208–223 (2005).
Reviews macromolecular-complex regulation of CFTR.
25. Wang, S. S., Raab, R. W., Schatz, P. J., Guggino, W. B. & Li, M. Peptide binding consensus of the NHE-RF-PDZ1 domain matches the C-terminal sequence of cystic fibrosis transmembrane conductance regulator (CFTR). *FEBS Lett.* **427**, 103–108 (1998).
One of the first papers to describe interactions between PDZ-domain proteins and CFTR.
26. Hegedus, T. *et al.* C-terminal phosphorylation of MRP2 modulates its interaction with PDZ proteins. *Biochem. Biophys. Res. Commun.* **302**, 454–461 (2003).
27. Thelin, W. R., Hodson, C. A. & Milgram, S. L. Beyond the brush border: NHERF4 blazes new NHERF turf. *J. Physiol.* **567**, 13–19 (2005).
28. Sun, F. *et al.* E3KARP mediates the association of ezrin and protein kinase A with the cystic fibrosis transmembrane conductance regulator in airway cells. *J. Biol. Chem.* **275**, 29539–29546 (2000).
29. Short, D. B. *et al.* An apical PDZ protein anchors the cystic fibrosis transmembrane conductance regulator to the cytoskeleton. *J. Biol. Chem.* **273**, 19797–19801 (1998).
30. Haggie, P. M., Stanton, B. A. & Verkman, A. S. Increased diffusional mobility of CFTR at the plasma membrane after deletion of its C-terminal PDZ binding motif. *J. Biol. Chem.* **279**, 5494–5500 (2004).
31. Wang, S., Yue, H., Derin, R. B., Guggino, W. B. & Li, M. Accessory protein facilitated CFTR–CFTR interaction, a molecular mechanism to potentiate the chloride channel activity. *Cell* **103**, 169–179 (2000).
32. Raghuram, V., Mak, D. O. D. & Foskett, J. K. Regulation of cystic fibrosis transmembrane conductance regulator single-channel gating by bivalent PDZ-domain-mediated interaction. *Proc. Natl Acad. Sci. USA* **98**, 1300–1305 (2001).
33. Benharouga, M. *et al.* The role of the C terminus and Na^+/H^+ exchanger regulatory factor in the functional expression of cystic fibrosis transmembrane conductance regulator in nonpolarized cells and epithelia. *J. Biol. Chem.* **278**, 22079–22089 (2003).
34. Gupta, S., Xie, J., Ma, J. & Davis, P. B. Intermolecular interaction between R domains of cystic fibrosis transmembrane conductance regulator. *Am. J. Respir. Cell Mol. Biol.* **30**, 242–248 (2004).
35. Li, C., Roy, K., Dandridge, K. & Naren, A. P. Molecular assembly of cystic fibrosis transmembrane conductance regulator in plasma membrane. *J. Biol. Chem.* **279**, 24673–24684 (2004).
36. Rosenberg, M. F., Kamis, A. B., Aleksandrov, L. A., Ford, R. C. & Riordan, J. R. Purification and crystallization of the cystic fibrosis transmembrane conductance regulator (CFTR). *J. Biol. Chem.* **279**, 39051–39057 (2004).
37. Zerhusen, B., Zhao, J. Y., Xie, J. X., Davis, P. B. & Ma, J. J. A single conductance pore for chloride ions formed by two cystic fibrosis transmembrane conductance regulator molecules. *J. Biol. Chem.* **274**, 7627–7630 (1999).
38. Eskandari, S., Wright, E. M., Kreman, M., Starace, D. M. & Zampighi, G. A. Structural analysis of cloned plasma membrane proteins by freeze-fracture electron microscopy. *Proc. Natl Acad. Sci. USA* **95**, 11235–11240 (1998).
39. Chen, J. H., Chang, X. B., Aleksandrov, A. A. & Riordan, J. R. CFTR is a monomer: biochemical and functional evidence. *J. Membr. Biol.* **188**, 55–71 (2002).
40. Ramjeesingh, M., Kidd, J. F., Huan, L. J., Wang, Y. & Bear, C. E. Dimeric cystic fibrosis transmembrane conductance regulator exists in the plasma membrane. *Biochem. J.* **374**, 793–797 (2003).
41. Prince, L. S., Workman, R. B. Jr. & Marchase, R. B. Rapid endocytosis of the cystic fibrosis transmembrane conductance regulator chloride channel. *Proc. Natl Acad. Sci. USA* **91**, 5192–5196 (1994).
42. Lukacs, G. L., Segal, G., Kartner, N., Grinstein, S. & Zhang, F. Constitutive internalization of cystic fibrosis transmembrane conductance regulator occurs via clathrin-dependent endocytosis and is regulated by protein phosphorylation. *Biochem. J.* **328**, 353–361 (1997).
43. Huang, P., Trotter, K., Boucher, R. C., Milgram, S. L. & Stutts, M. J. PKA holoenzyme is functionally coupled to CFTR by AKAPs. *Am. J. Physiol. Cell Physiol.* **278**, C417–C422 (2000).
References 43 and 44 describe PKA anchoring in a microdomain that contains CFTR.
44. Sun, F., Hug, M. J., Bradbury, N. A. & Frizzell, R. A. Protein kinase A associates with cystic fibrosis transmembrane conductance regulator via an interaction with ezrin. *J. Biol. Chem.* **275**, 14360–14366 (2000).
45. Naren, A. P. *et al.* A macromolecular complex of β2 adrenergic receptor, CFTR, and ezrin/radixin/moesin-binding phosphoprotein 50 is regulated by PKA. *Proc. Natl Acad. Sci. USA* **100**, 342–346 (2003).
46. Tasken, K. & Aandahl, E. M. Localized effects of cAMP mediated by distinct routes of protein kinase A. *Physiol. Rev.* **84**, 137–167 (2004).
47. Thelin, W. R. *et al.* The cystic fibrosis transmembrane conductance regulator is regulated by a direct interaction with the protein phosphatase 2A. *J. Biol. Chem.* **280**, 41512–41520 (2005).
48. Vastiau, A. *et al.* Interaction of the protein phosphatase 2A with the regulatory domain of the cystic fibrosis transmembrane conductance regulator channel. *FEBS Lett.* **579**, 3392–3396 (2005).
49. Suh, P. G., Hwang, J. I., Ryu, S. H., Donowitz, M. & Kim, J. H. Breakthroughs and views – the roles of PDZ-containing proteins in PLC-β-mediated signaling. *Biochem. Biophys. Res. Commun.* **288**, 1–7 (2001).
50. Raghuram, V., Hormuth, H. & Foskett, J. K. A kinase-regulated mechanism controls CFTR channel gating by disrupting bivalent PDZ domain interactions. *Proc. Natl Acad. Sci. USA* **100**, 9620–9625 (2003).
51. Liedtke, C. M., Yun, C. H., Kyle, N. & Wang, D. PKC-ε dependent regulation of CFTR involves binding to RACK1, a receptor for activated C kinase, and RACK1 binding to NHERF1. *J. Biol. Chem.* **277**, 22925–22933 (2002).
52. Liedtke, C. M., Raghuram, V., Yun, C. C. & Wang, X. Role of a PDZ1 domain of NHERF1 in the binding of airway epithelial RACK1 to NHERF1. *Am. J. Physiol. Cell Physiol.* **286**, C1037–C1044 (2004).
53. Huang, P. *et al.* Compartmentalized autocrine signaling to cystic fibrosis transmembrane conductance regulator at the apical membrane of airway epithelial cells. *Proc. Natl Acad. Sci. USA* **98**, 14120–14125 (2001).
54. Taouil, K. *et al.* Stimulation of β2-adrenergic receptor increases cystic fibrosis transmembrane conductance regulator expression in human airway epithelial cells through a cAMP/protein kinase A-independent pathway. *J. Biol. Chem.* **278**, 17320–17327 (2003).
55. Li, C. *et al.* Lysophosphatidic acid inhibits cholera toxin-induced secretory diarrhea through CFTR-dependent protein interactions. *J. Exp. Med.* **202**, 975–986 (2005).
56. Cormet-Boyaka, E. *et al.* CFTR chloride channels are regulated by a SNAP-23/syntaxin 1A complex. *Proc. Natl Acad. Sci. USA* **99**, 12477–12482 (2002).
57. Chang, S. Y. *et al.* Mechanisms of CFTR regulation by syntaxin 1A and PKA. *J. Cell Sci.* **115**, 783–791 (2002).
58. Peters, K. W., Qi, J. J., Johnson, J. P., Watkins, S. C. & Frizzell, R. A. Role of snare proteins in CFTR and ENaC trafficking. *Pflugers Arch.* **443** (Suppl. 1), S65–S69 (2001).
59. Ganeshan, R., Di, A., Nelson, D. J., Quick, M. W. & Kirk, K. L. The interaction between syntaxin 1A and cystic fibrosis transmembrane conductance regulator Cl^- channels is mechanistically distinct from syntaxin 1A–SNARE interactions. *J. Biol. Chem.* **278**, 2876–2885 (2003).
60. Naren, A. P. *et al.* Regulation of CFTR chloride channels by syntaxin and Munc 18 isoforms. *Nature* **390**, 302–305 (1997).
61. Bilan, F. *et al.* Syntaxin 8 impairs trafficking of cystic fibrosis transmembrane conductance regulator (CFTR) and inhibits its channel activity. *J. Cell Sci.* **117**, 1923–1935 (2004).
62. Hallows, K. R., Raghuram, V., Kemp, B. E., Witters, L. A. & Foskett, J. K. Inhibition of cystic fibrosis transmembrane conductance regulator by novel interaction with the metabolic sensor AMP-activated protein kinase. *J. Clin. Invest.* **105**, 1711–1721 (2000).
Shows that AMPK inhibits CFTR-channel activity.
63. Hallows, K. R., McCane, J. E., Kemp, B. E., Witters, L. A. & Foskett, J. K. Regulation of channel gating by AMP-activated protein kinase modulates cystic fibrosis transmembrane conductance regulator activity in lung submucosal cells. *J. Biol. Chem.* **278**, 998–1004 (2003).
64. Hallows, K. R. Emerging role of AMP-activated protein kinase in coupling membrane transport to cellular metabolism. *Curr. Opin. Nephrol. Hypertens.* **14**, 464–471 (2005).

65. Hallows, K. R. *et al.* Upregulation of AMP-activated kinase by dysfunctional CFTR in cystic fibrosis airway epithelial cells mitigates excessive inflammation. *J. Biol. Chem.* **281**, 4231–4241 (2005).
Suggests that AMPK might reduce inflammation in patients with cystic fibrosis.
66. Walker, J., Jijon H. B., Churchill T., Kulka M. & Madsen KL. Activation of AMP-activated protein kinase reduces cAMP-mediated epithelial chloride secretion. *Am. J. Physiol. Gastrointest. Liver Physiol.* **285**, G850–G856 (2003).
67. Bradbury, N. A. *et al.* Regulation of plasma membrane recycling by CFTR. *Science* **256**, 530–532 (1992).
68. Bradbury, N. A., Jilling, T., Kirk, K. L. & Bridges, R. J. Regulated endocytosis in a chloride secretory epithelial cell line. *Am. J. Physiol.* **262**, C752–C759 (1992).
69. Lehrich, R. W., Aller, S. G., Webster, P., Marino, C. R. & Forrest, J. N. Jr. Vasoactive intestinal peptide, forskolin, and genistein increase apical CFTR trafficking in the rectal gland of the spiny dogfish, *Squalus acanthias*. Acute regulation of CFTR trafficking of CFTR in an intact epithelium. *J. Clin. Invest.* **101**, 737–747 (1998).
70. Morris, R. G., Tousson, A., Benos, D. J. & Schafer, J. A. Microtubule disruption inhibits AVT-stimulated Cl^- secretion but not Na^+ reabsorption in A6 cells. *Am. J. Physiol. Renal Physiol.* **274**, F300–F314 (1998).
71. Ameen, N. A. *et al.* Subcellular distribution of CFTR in rat intestine supports a physiologic role for CFTR regulation by vesicle traffic. *Histochem. Cell Biol.* **114**, 219–228 (2000).
72. Howard, M. *et al.* Epitope tagging permits cell surface detection of functional CFTR. *Am. J. Physiol. Cell Physiol.* **269**, C1565–C1576 (1995).
73. Peters, K. W., Qi, J. J., Watkins, S. C. & Frizzell, R. A. Syntaxin 1A inhibits regulated CFTR trafficking in *Xenopus* oocytes. *Am. J. Physiol. Cell Physiol.* **277**, C174–C180 (1999).
74. Prince, L. S. *et al.* Efficient endocytosis of the cystic fibrosis transmembrane conductance regulator requires a tyrosine-based signal. *J. Biol. Chem.* **274**, 3602–3609 (1999).
75. Loffing, J., Moyer, B. D., McCoy, D. & Stanton, B. A. Exocytosis is not involved in activation of Cl^- secretion via CFTR in Calu-3 airway epithelial cells. *Am. J. Physiol. Cell Physiol.* **275**, C913–C920 (1998).
76. Denning, G. M., Ostedgaard, L. S., Cheng, S. H., Smith, A. E. & Welsh, M. J. Localization of cystic fibrosis transmembrane conductance regulator in chloride secretory epithelia. *J. Clin. Invest.* **89**, 339–349 (1992).
77. Swiatecka-Urban, A. *et al.* PDZ domain interaction controls the endocytic recycling of the cystic fibrosis transmembrane conductance regulator. *J. Biol. Chem.* **277**, 40099–40105 (2002).
Shows that the PDZ-interacting domain in CFTR regulates CTFR endocytic recycling.
78. Cheng, J., Wang, H. & Guggino, W. B. Regulation of cystic fibrosis transmembrane regulator trafficking and protein expression by a Rho family small GTPase TC10. *J. Biol. Chem.* **280**, 3731–3739 (2005).
79. Cheng, J. *et al.* A Golgi-associated PDZ domain protein modulates cystic fibrosis transmembrane regulator plasma membrane expression. *J. Biol. Chem.* **277**, 3520–3529 (2002).
First evidence showing that CAL regulates CFTR trafficking.
80. Cheng, J., Wang, H. & Guggino, W. B. Modulation of mature cystic fibrosis transmembrane regulator protein by the PDZ domain protein CAL. *J. Biol. Chem.* **279**, 1892–1898 (2004).
81. Gentzsch, M. *et al.* The PDZ-binding chloride channel ClC-3B localizes to the Golgi and associates with cystic fibrosis transmembrane conductance regulator-interacting PDZ proteins. *J. Biol. Chem.* **278**, 6440–6449 (2003).
82. Guerra, L. *et al.* Na^+/H^+ exchanger regulatory factor isoform 1 overexpression modulates cystic fibrosis transmembrane conductance regulator (CFTR) expression and activity in human airway 16HBE14o- cells and rescues ΔF508 CFTR functional expression in cystic fibrosis cells. *J. Biol. Chem.* **280**, 40925–40933 (2005).
83. Charest, A., Lane, K., McMahon, K. & Housman, D. E. Association of a novel PDZ domain-containing peripheral Golgi protein with the Q-snare (Q-soluble *N*-ethylmaleimide-sensitive fusion protein (NSF) attachment protein receptor protein syntaxin 6. *J. Biol. Chem.* **276**, 29456–29465 (2001).
84. Ostedgaard, L. S. *et al.* Effects of C-terminal deletions on cystic fibrosis transmembrane conductance regulator function in cystic fibrosis airway epithelia. *Proc. Natl Acad. Sci. USA* **100**, 1937–1942 (2003).
85. Milewski, M. I., Lopez, A., Jurkowska, M., Larusch, J. & Cutting, G. R. PDZ-binding motifs are unable to ensure correct polarized protein distribution in the absence of additional localization signals. *FEBS Lett.* **579**, 483–487 (2005).
86. Milewski, M. I. *et al.* A PDZ-binding motif is essential but not sufficient to localize the C terminus of CFTR to the apical membrane. *J. Cell Sci.* **114**, 719–726 (2001).
87. Moyer, B. D. *et al.* The PDZ interacting domain of CFTR is required for functional expression in the apical plasma membrane. *J. Biol. Chem.* **275**, 27069–27074 (2000).
88. Moyer, B. D. *et al.* A PDZ-interacting domain in CFTR is an apical membrane polarization signal. *J. Clin. Invest.* **104**, 1353–1361 (1999).
89. Wennerberg, K., Rossman, K. L. & Der, C. J. The Ras superfamily at a glance. *J. Cell Sci.* **118**, 843–846 (2005).
90. Picciano, J. A., Ameen, N., Grant, B. D. & Bradbury, N. A. Rme-1 regulates the recycling of the cystic fibrosis transmembrane conductance regulator. *Am. J. Physiol. Cell Physiol.* **285**, C1009–C1018 (2003).
91. Gentzsch, M. *et al.* Endocytic trafficking routes of wild type and ΔF508 cystic fibrosis transmembrane conductance regulator. *Mol. Biol. Cell* **15**, 2684–2696 (2004).
Shows the rescue of ΔF508-CFTR by RAB11.
92. Swiatecka-Urban, A. *et al.* The short apical membrane half-life of rescued ΔF508-cystic fibrosis transmembrane conductance regulator (CFTR) results from accelerated endocytosis of ΔF508-CFTR in polarized human airway epithelial cells. *J. Biol. Chem.* **280**, 36762–36772 (2005).
93. Mall, M., Hipper, A., Greger, R. & Kunzelmann, K. Wild type but not ΔF508 CFTR inhibits Na^+ conductance when coexpressed in *Xenopus* oocytes. *FEBS Lett.* **381**, 47–52 (1996).
94. Stutts, M. J. *et al.* CFTR as a cAMP-dependent regulator of sodium channels. *Science* **269**, 847–850 (1995).
95. Reddy, M. M., Light, M. J. & Quinton, P. M. Activation of the epithelial Na^+ channel (ENaC) requires CFTR Cl^- channel function. *Nature* **402**, 301–304 (1999).
96. Mohler, P. J. *et al.* Yes-associated protein 65 localizes $p62^{c\text{-}Yes}$ to the apical compartment of airway epithelia by association with EBP50. *J. Cell Biol.* **147**, 879–890 (1999).
97. Pirozzi, G. *et al.* Identification of novel human WW domain-containing proteins by cloning of ligand targets. *J. Biol. Chem.* **272**, 14611–14616 (1997).
98. Gilmore, E. S., Stutts, M. J. & Milgram, S. L. Src family kinases mediate epithelial Na^+ channel inhibition by endothelin. *J. Biol. Chem.* **276**, 42610–42617 (2001).
99. Pedemonte, N. *et al.* Small-molecule correctors of defective ΔF508-CFTR cellular processing identified by high-throughput screening. *J. Clin. Invest.* **115**, 2564–2571 (2005).
Excellent example of cystic fibrosis drug discovery by an academic laboratory using high-throughput screening.
100. Egan, M. E. *et al.* Curcumin, a major constituent of turmeric, corrects cystic fibrosis defects. *Science* **304**, 600–602 (2004).
101. Roomans, G. M. Pharmacological approaches to correcting the ion transport defect in cystic fibrosis. *Am. J. Respir. Med.* **2**, 413–431 (2003).
102. Zaman, K. *et al. S*-nitrosoglutathione increases cystic fibrosis transmembrane regulator maturation. *Biochem. Biophys. Res. Commun.* **284**, 65–70 (2001).
103. Konstan, M. W. *et al.* Compacted DNA nanoparticles administered to the nasal mucosa of cystic fibrosis subjects are safe and demonstrate partial to complete cystic fibrosis transmembrane regulator reconstitution. *Hum. Gene Ther.* **15**, 1255–1269 (2004).
104. Clancy, J. P. *et al.* Evidence that systemic gentamicin suppresses premature stop mutations in patients with cystic fibrosis. *Am. J. Respir. Crit. Care Med.* **163**, 1683–1692 (2001).
105. Swiatecka-Urban, A. *et al.* Myosin VI regulates endocytosis of the cystic fibrosis transmembrane conductance regulator. *J. Biol. Chem.* **279**, 38025–38031 (2004).

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* | *ABCG5*
UniProtKB: http://ca.expasy.org/sprot
A2b-R | β2AR | SNAP23 |SYN1A | SYN6 | SYN8 |TC10 | YAP65

FURTHER INFORMATION
William Guggino's homepage: http://www.guggino.org
Bruce Stanton's homepage: http://www.dartmouth.edu/~physiol/faculty/stanton.html
The Human ATP-Binding Cassette (ABC) Transporter Superfamily: http://www.ncbi.nlm.nih.gov/books/bv.fcgi?call=bv.View..ShowTOC&rid=mono_001.TOC&depth=2
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
Access to this links box is available online.