Laboratory Investigation (2018) 98, 272–282

PATHOBIOLOGY IN FOCUS

NM23 proteins: innocent bystanders or local energy boosters for CFTR?

Richmond Muimo[1], Hani MM Alothaid[1] and Anil Mehta[2]

NM23 proteins NDPK-A and -B bind to the cystic fibrosis (CF) protein CFTR in different ways from kinases such as PKA, CK2 and AMPK or linkers to cell calcium such as calmodulin and annexins. NDPK-A (not -B) interacts with CFTR through reciprocal AMPK binding/control, whereas NDPK-B (not -A) binds directly to CFTR. NDPK-B can activate G proteins without ligand–receptor coupling, so perhaps NDPK-B's binding influences energy supply local to a nucleotide-binding site (NBD1) needed for CFTR to function. Curiously, CFTR (ABC-C7) is a member of the ATP-binding cassette (ABC) protein family that does not obey 'clan rules'; CFTR channels anions and is not a pump, regulates disparate processes, is itself regulated by multiple means and is so pleiotropic that it acts as a hub that orchestrates calcium signaling through its consorts such as calmodulin/annexins. Furthermore, its multiple partners make CFTR dance to different tunes in different cellular and subcellular locations as it recycles from the plasma membrane to endosomes. CFTR function in airway apical membranes is inhibited by smoking which has been dubbed 'acquired CF'. CFTR alone among family members possesses a trap for other proteins that it unfurls as a 'fish-net' and which bears consensus phosphorylation sites for many protein kinases, with PKA being the most canonical. Recently, the site of CFTR's commonest mutation has been proposed as a knock-in mutant that alters allosteric control of kinase CK2 by log orders of activity towards calmodulin and other substrates after CFTR fragmentation. This link from CK2 to calmodulin that binds the R region invokes molecular paths that control lumen formation, which is incomplete in the tracheas of some CF-affected babies. Thus, we are poised to understand the many roles of NDPK-A and -B in CFTR function and, especially lumen formation, which is defective in the gut and lungs of many CF babies.

Laboratory Investigation (2018) **98,** 272–282; doi:10.1038/labinvest.2017.121; published online 18 December 2017

Nucleoside diphosphate kinases (NDPKs/NME) are multifunctional proteins expressed as far apart as the amoeba *Dictyostelium* and modern humans. Humans have 10 NDPK genes, *NME* (*non-metastatic) 1–9* and *RP2*, retinitis pigmentosa 2 whereas simpler organisms typically have about one to three isoforms.[1,2] NDPKs are classified into two groups (I and II) based on their sequence identities and capacity for high energy phosphotransferase activity. Herein, we will refer to 'NDPK activity' as that role which is dependent on NDPK's internal phosphohistidine as a high energy source to effect change in another moiety. Thus, group I genes encode proteins with NDPK activity and comprise NME1–4 (also known as NDPK-A, -B, -C, and -D; or Nm23-H1–H4). These share higher sequence identity (58–88%) compared with group II, the latter additionally have low or no NDPK activity and share only about 22–44% sequence identity. Group I NDPKs (A–D) generate nucleoside triphosphates by covalently transferring the γ-phosphate from a donor nucleoside (or deoxy) triphosphate (NTP or dNTP, respectively) to an acceptor nucleoside (or deoxy) diphosphate (NDP or dNDP) in a ping-pong reaction using the high-energy phosphohistidine intermediate.[3,4] These NDPKs (perhaps as multimers) can also function as mammalian histidine protein kinases[5] but, curiously, also possess 3′–5′-exonuclease activity[6] which is important in DNA repair. This minireview provides a summary of the current knowledge of the functions of two isoforms from group I in airway epithelia, highlighting the role NDPKs might play in provision of energy for processes related to the protein whose mutation causes cystic fibrosis (CF).[7]

In the airway, ciliary clearance of inhaled contaminants entering the respiratory system prevents lung infections and is

[1]Department of Infection, Immunity and Cardiovascular Disease, The Medical School, University of Sheffield, Sheffield, UK and [2]Medical Research Institute, Ninewells Hospital and Medical School, University of Dundee, Dundee, UK
Correspondence: Dr R Muimo, PhD, Department of Infection, Immunity and Cardiovascular Disease The Medical School, Beech Hill Road, Sheffield S10 2RX, UK or Dr A Mehta, FRCP(Edin), Medical Research Institute, Ninewells Hospital and Medical School, University of Dundee, Dundee, DD1 9SY, UK.
E-mail: r.muimo@sheffield.ac.uk or a.mehta@dundee.ac.uk

Received 20 May 2017; revised 25 August 2017; accepted 12 September 2017

dependent upon transport of chloride and other anions into the lumen of the airways, accompanied by water and mucus movement. In CF, a disease mostly caused by just one triplet nucleotide deletion in the CF gene (the cystic fibrosis transmembrane conductance regulator, CFTR), lack of normal fluid, and mucus transport results in an abnormal airway surface liquid layer that accumulates thick mucus with frequent lung infections from a host of unusual microorganisms.[8–11] Cell calcium plays a critical role in exocytotic mucus release and recently, CFTR has been shown to bind calmodulin, which might link anion transport to the viscid mucus found in CF airways.[12] The CFTR anion channel also recycles using endosomes to and fro from the apical surface of many epithelial cells and is a key hub that generates a macromolecular complex including both sodium and potassium channels, anion exchangers, transporters that export cAMP, and other regulatory molecules.[13] We will return to this theme of CFTR as an input and output hub later in the review when we consider the molecular events leading to embryonic tracheogenesis where calcium-dependent exocytosis is used (in fetal life) to build a tracheal lumen.[14]

We have been interested in the constitutive protein phosphorylation of membrane proteins purified from airway epithelia and its relationship to ion transport and CFTR function for a number of years.[15–18] Our work was initiated by our discovery that, provided sodium was absent *in vitro*, the chloride concentration could act as a signal to purified apical membranes by regulating acid-labile phosphorylation of a number of soluble and membrane-bound proteins.[19] In CF, it is established that mutation of CFTR causes a very low residence time and high degradation rate for the mutant (F508-deleted) protein which somehow enhances the function of epithelial sodium channels in a chloride-dependent manner.[20] Later, we identified that chloride-sensitive proteins such as NDPK and annexin A1 are phosphorylated on histidine residues (see summary in Figure 1); for annexin A1 this occurs near the conserved calcium-binding domain of this regulator of inflammation and steroid signaling.[15,17,21–23] Inhibition of NDPK by non-hydrolyzable nucleoside analogs abrogated histidine phosphorylation of annexin A1.[15] Other members of the annexin family are also implicated in CF disease and these will be discussed below but we note that independent confirmation of the complex role of annexin AI in CF pathophysiology has been reported in the last few years.[24]

CFTR is a member of the ATP-binding cassette (ABC) transporter/pump superfamily, which comprises membrane-anchored and 'scissor shaped' transmembrane proteins that use the energy from adenosine triphosphate (ATP) cleavage to translocate a range of molecules across many different cellular membranes. ABCs are one of the largest and oldest protein families, with members found in all phyla. Over 40 members are involved in essential processes in eukaryotic cells where their mutation causes disparate human diseases.[25–27] They are multi-domain, and often self-assemble in lower orders in a multi-subunit manner comprising transmembrane domains (akin to scissor blades) bound to paired cytosolic clamps for ATP-binding domains bearing at least one

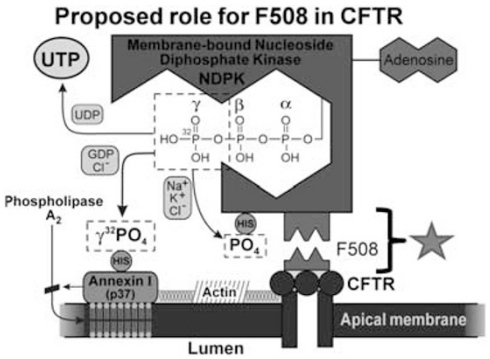

Figure 1 Reducing knowledge gaps between NDPK, Annexins and CFTR. Since this model was first proposed in 1998 for the F508 site, our new data show that deletion of F508 residue from CFTR knocks in a new binding site for CK2 and mis-controls CK2 allosterically along with ATP concentration by log orders. It has recently been proposed[109] that many members of the Annexin gene family produce proteins that are virulence factors in pathogen–host interactions. These include the commonest pathogen in cystic fibrosis (*Pseudomonas aeruginosa*) and notable viruses such as HPV type 16, CMV, hepatitis C, and many others. In addition, it is becoming clear that pathogens can 'summon' cytosolic annexins to the plasma membrane by subverting cell signals and can even promote the extracellular expulsion of such proteins by unknown means. In this context, there seems to be an interaction between extracellular receptors for annexins related to the Formyl Receptor family and the recruitment of host neutrophils. It is well established that such neutrophils accumulate to excess in CF lungs and our work has reported that annexins such as annexin A2 and CFTR are found in the same complex.[18] Since we have also reported that annexins can receive phosphohistidine in their core structure[17] and that NDPK promotes p-His generation, then it seems a reasonable hypothesis that annexins, NDPK, and CFTR are to be expected to co-locate. Our view is that this is a fertile area for future discovery given that annexins bring cell calcium sensing, membrane fusion events, and modification of the host cytoskeleton during pathogen invasion into the picture. Since we have found that both annexin function and NDPK function are defective in CF cells, perhaps one idea for future work is that host defense might be enhanced when annexin and NDPK dysfunction is induced in a carrier of CF which in turn might explain why so many Europeans carry the common CF mutation. For example, we recently have related kinase CK2 directly to CFTR stability and CFTR function[59] through the C terminus of CFTR at a site proposed by Ostedgaard *et al*[63] to be important in CFTR functioning. That site near T1471 in the CFTR tail lies very close to the binding site for AMPK. Indeed we have also found that NDPK and AMPK function towards CFTR are related to one another.[86] Recently, albeit in another tissue, annexin 1 and CK2 have been proposed to be related to one another,[110] which provides independent confirmation that processes linked to cell injury, CK2, and annexin function are part of a common signaling pathway. The star (★) refers to factors to consider in the interaction such as the role of fragments derived from this part of CFTR after its breakup in the membrane to generate a new deletion-induced F508del-based allosteric binding site for kinase CK2 that mis-controls both CK2 and substrate level phosphorylation towards proteins such as calmodulin and regulators of dephosphorylation including protein phosphatase one (PP1) and controllers of protein synthesis such as factors controlling chain initiation on the ribosome.

membrane-associated ATPase domain. Such domains trap two ATP molecules between their two cognate, inverted and opposed halves. CFTR is driven by one ATP-binding site acting as a non-hydrolyzing 'degenerate' ATP holding 'bridge-like' domain with the other site permitting free hydrolysis that enables domain shifts to facilitate pore access. The relationship between ATP binding and channel gating is a very complex process as reviewed recently by Zwick *et al.*[7] However, these models take no account of the binding of CFTR-associated proteins that drive a cAMP or calcium-dependent CFTR activation[12,18] or to the possibility that CFTR might also become a (protease) split molecule that could recombine its N- and C-terminal fragments[28] in the membrane to create an array of different CFTR halves that add to the diversity of CFTR molecules in the membrane. It is accepted that hydrolysis of clamped ATP drives conformational changes in the translocator 'blades' structure, fueling the transport of biological substrates across the cellular membranes.[26,27] Thus a typical structure of an ABC transporter comprises two transmembrane domains (TMDs), which are formed by six transmembrane α-helices with the pump energy supplied from two opposed cytoplasmic nucleotide-binding domains (NBDs)[26] bridged by two spatially separated ATP molecules. However, CFTR is a most unusual member of the ABC transporter family in that it is the only member that forms a transmembrane ion channel in which the ATP-driven changes in the cytosolic domains of the protein conformation result in the opening and closing of a distant gate at the entrance to the anion pore, allowing for the flow of pre-dehydrated anions (chloride, bicarbonate and other anions) down the prevailing electrochemical gradient.[29,30] In that sense, CFTR is a regulated slave to the direction of the gradient of potential which means that anions can flow in or out of the cell on an 'as required' basis, even against a concentration gradient. Hence, CFTR is the equivalent of an 'if then else' statement in the central processing unit of a computer program controlling a complex circuit bearing many other components and it is these regulators that form the focus of our review. This complexity idea provides a backdrop to the many roles of CFTR which seems to create causal actions at a distance from the channel through its many partners.

CFTR PROTEIN STRUCTURE REMAINS TO BE FULLY ELUCIDATED

The CFTR protein comprises a regulatory domain (R domain or R region), two transmembrane domains, TMD1 and TMD2, and two homologous (but non-identical) inverted and opposed nucleotide-binding domains, NBD1 and NBD2.[25,31–33] Electrophysiology suggests that the ion channel formed between the TMD domains of the CFTR protein opens when two different ATP molecules are bound to CFTR's two NBD domains. Conversely, closing of the channel gate follows a cycle of ATP hydrolysis, which leads to a different type of NBD1–NBD2 interaction, and thus to the termination of the flow of anions through the channel.[29] This complex gating cycle has a second 'if then else controller' in that the protein kinase-mediated phosphorylation of an unstructured cytoplasmic R domain of CFTR is required to permit the channel-gating cycle. This unique (to ABC proteins) cytoplasmic R domain interacts with the two NBDs and is an integral part of CFTR protein that links NBD and TMD domains. The R region has multiple adjacent target sites for protein kinase A (PKA, a cAMP-activated protein kinase) phosphorylation which imparts a net double-digit negative charge to the protein.[31,34,35] Thus, the central domain structure organization of the single-polypeptide 1480-amino-acid long wild-type CFTR protein encompasses TMD2–NBD2–R–TMD1–NBD1.[10,35] How such a system operates in an intact cell is not agreed with some recent data even suggesting that NBDs may be permissive for CFTR phosphorylation.[7] We will return to the N-terminus and the C terminus later when we (respectively) consider t-SNARES of the syntaxin family and protein kinases such as CK2 and AMPK along with linkers from the DTRL motif at the end of CFTR to the cytoskeleton such as ezrin. Interestingly, the origins of the capture and insertion of the R domain in CFTR are unknown as no significant sequence homology with other proteins has been found to date.[36] PKA phosphorylation sites are highly conserved among species, and the R region has been shown to contain the majority of those sites.[36,37] This region may be likened to a net or web structure that is highly plastic with subregions of disorder and coil where transitions occur between these states when targeted by PKA and other protein kinases. One idea is that a randomly fluctuating R region becomes locally ordered with coil structures after regulation to permit the binding of other proteins to assemble a hub (or hubs). Thus, the R region acts as a molecular fly trap for passing cellular protein prey after protein phosphorylation imparts order through multiple negative charges. Thus, CFTR does not act alone and a recent review of the data suggests that calmodulin is attracted to CFTR by this R region.[12] This R region may lie to the side of the structure based on atomic maps.[38] Interestingly, recent evidence from three-dimensional structures of both zebrafish (*Danio rerio*) and human CFTR from cryo-electron microscopy at a medium resolution of 3.7 Å in the absence of ATP reveals (a) a funnel-shaped anion conduction pathway lined by positive charged residues, (b) a single gate at the extracellular surface, and with (c) the dephosphorylated regulatory domain located between (and not to the side of) the two NBDs preventing dimerization and channel opening.[39,40] The authors suggest that spontaneous disengagement of the R domain from its docking site in the intracellular vestibule likely leads to phosphorylation by PKA, correlating the channel gating with ATP hydrolysis rates. Comparative analysis of above conformations with zebrafish CFTR in its phosphorylated and ATP-bound form shows large conformational changes after phosphorylation and ATP binding that promote channel function including NBD dimerization,

disengagement of the R domain from its inhibitory position, and opening of the pore to the cytosol but not the extracellular space.[41] Although these recent advances have provided such important and invaluable insights into CFTR function, further work remains to be done since CFTR function is also impacted by a complex of CFTR-associated proteins.

A key step in CFTR's regulatory functions is a consequence of targeting of PKA to CFTR. This approximates PKA, certain Na+/H+ exchanger regulatory factor isoforms (NHERF1 or NHERF2), and the cytoskeleton through the A kinase anchoring protein AKAP78 (ezrin, a member of the ERM protein family). The last two moieties play regulatory and structural roles in the stabilization and relocation of many plasma membrane-bound proteins. For example, ERM proteins act as a bridges-cum-molecular switches which when activated by PKC, PIP2, and Rho-GTPases promoting interactions with NHEFR1 so as to bind more tightly with CFTR. This may cause dimerization of CFTR and formation of a micro-domain where PKA can more efficiently activate CFTR.[42] ERM proteins are also implicated (along with phosphorylation by kinase CK2) in optimally targeting sodium channels to their correct locations in the cytoskeleton of neurons. This is consistent with local substrate channeling of the constitutive activity of kinases such as NDPK and CK2 inside a new hub structure located near CFTR that may be shielded from the cytoplasm. Importantly, CK2 is also essential for sodium channel function[20] by phosphorylating two different sites and others suggest that NDPK and CK2 may interact *in vitro*, but this remains to be confirmed.

MECHANISM OF ACTION OF THE CFTR ANION CHANNEL

It has been proposed that pre-dehydrated, negatively charged chloride ions accumulate around the positively charged sections of the TMDs that form the CFTR channel's pore. Ions such as chloride can then flow freely through the pore upon opening of the channel's gate.[43–45] The course of events is initiated by the phosphorylation of the R domain of CFTR at multiple sites by an anchored PKA enzyme,[46,47] accompanied by the binding of ATP molecules to the two different ATP-binding sites on the inner faces of the cytoplasmic NBDs of the CFTR. ATP has been shown to be required for the opening of the phosphorylated CFTR channels. Channels do not open in the absence of ATP and the open channels close within 1 s of removal of ATP.[12,30,47,48] The binding of ATP to the NBDs of phosphorylated CFTR promotes the initiation of the channel-gating cycle.[49] In most epithelial cells, the normal ATP concentration of ~2 mM is thought to be sufficient to ensure channel activation. Additionally, it has been observed that the opening and closing of the CFTR channel is a cyclical process, with the activated channels showing typical opening durations of only hundreds of milliseconds.[49,50] According to a currently accepted model, this results in conformational changes, which in turn leads to the opening of the channel.[51] This channel-gating cycle is terminated by the subsequent hydrolysis of the ATP promoted by the enzymatic activity of one NBD, upon which the ADP is released. Interestingly, ADP release is not reversible (ADP is not turned into ATP in the gating cycle under experimental patch clamp conditions *in vitro*). Moreover, the energy released during the ATP hydrolysis reaction is not used for the chloride 'pumping' or transport.[49,52,53] It is instead proposed that the channel gating of CFTR can be promoted by non-hydrolyzable ATP analogs, for example, the AMP-PNP.[54,55] Confusingly, it has been demonstrated, that apart from having an ATPase activity (ATP+H(2)O –> ADP+Pi), the CFTR enzyme may also manifest adenylate kinase activity (ATP+AMP ⇆ 2 ADP), which has been proposed to be essential to the normal CFTR activity in the epithelial cells of the lungs.[46,56,57] These workers propose that both ATP and AMP interact with CFTR, albeit at separate binding sites, and that those nucleotides could mutually influence their interaction such that CFTR acts like an adenylate kinase.[58] This remains a controversial idea but is consistent with data linking the AMP-activated kinase (AMPK) to the inhibitory control of CFTR channel regulation. Additionally, the cell essential protein kinase CK2, formerly casein kinase 2, is dominantly permissive over PKA in CFTR channel opening. For example, using two independent approaches with different *in vivo* preparations, coupled to data when human CFTR is expressed in different model systems, inhibition of CK2 prevents activated PKA from maintaining an open channel. Indeed, D.N. Sheppard and colleagues observed prompt closure occurs after about 90 s of CK2 inhibition in patch clamp experiments and despite adequate concentrations of ATP. Importantly, when a mutated and inhibitor-insensitive version of CK2 is present alongside CFTR, pharmacological CK2 inhibition can no longer overcome PKA activation of CFTR. These new recent data point to an inability to maintain an open CFTR when CK2 (despite active PKA) is inhibited, suggesting that yet another control point may be operant during anion transport.[59]

The thermodynamic basis of the CFTR channel-gating cycle is still not understood, with contradictory opinions. Although the available data support the assertion that dimerization and trapping of ATP between opposed and inverted NBD1 and NBD2 domains drives the process of channel opening, and that the ATP hydrolysis leading to the disruption of the NBD1–NBD2 interaction, results in channel closing, it is not clear how conformational changes in NBDs are translated into channel gating. Some studies have suggested that the process of the CFTR channel opening is temperature-dependent, while the closing of the channel was suggested to be only weakly dependent on ambient temperature.[54,60] Thus the event of the ATP binding, rather than ATP hydrolysis, has been suggested to be responsible for the opening of the CFTR channel.[60] However, the measurements of the contribution of the enthalpy and entropy to the energetic states of the open and closed state of the channel, which were obtained by different groups (albeit using different subcellular cell purification methods and

electrophysiological methods) are inconsistent and will require further elucidation. The channel gating of CFTR is not a thermodynamic 'near to' equilibrium reversible process, and thus the non-equilibrium thermodynamic analysis may provide more consistent data and allow better understanding of the energy profile of the gating mechanism of CFTR.[49,61] It has been suggested that the large activation entropy and activation enthalpy for channel opening may mean that the conformational transition at the gate may spread across domains while the channel is still closed and after dimerization of NBD domains is in place.[49,61] On the other hand, the small activation entropy for closing the channel could be more in line with the dissolution of a single phosphate bond in a trapped ATP molecule.[61] This would suggest that the channel closing is a process that does not involve the substantial conformational changes of the protein, and that indeed, this transitional step precedes the disruption of the NBD1–NBD2 dimer.[52,61,62] Such a controller domain might include the C-terminal tail that is also implicated in the gating of the pore because deletion of an acidic stretch in the tail close to the binding site for AMPK (see below) alters channel gating.[63] Interestingly, there is a site immediately adjacent to this acidic tail that is stoichiometrically phosphorylated by kinase CK2 at T1471 when peptides corresponding to this tail are used as CK2 substrates. Mutation of this T1471 site in full-length CFTR is also implicated in the fragmentation of the protein. For example, a phosphomimic such as T1471D drastically attenuates the expected wild type CFTR fracture pattern into N and C terminal halves.[28,64] Thus at the time of writing, the molecular mechanisms by which the conformational changes due to the NBDs dimerization lead to the CFTR's channel opening remain clouded (see reference 7 for example) and in particular, how a supposedly remote tail domain of CFTR might 'talk' to the pore is a puzzle that will need a better picture of the full length of the CFTR structure that is currently lacking at atomic resolution. The combined data predict that the negatively charged region of the tail when augmented further by the addition of (CK2-transferred) negative phosphate charge will manifest an interaction with the pore. In this context, an earlier proposal that CK2 and NDPK can interact with each *in vitro* remains an intriguing challenge for future work. This is especially interesting given that the transition between the monomeric and multimeric states of CK2 is dependent on ionic strength. It is tempting to speculate that just as the R region of CFTR has been proposed to be a trap for proteins such as calmodulin,[12] perhaps multiple C-terminal interacting proteins whose expression might differ in different cell types[65] might also increase the functional tethering of CFTR in different cell contexts. Perhaps consideration should be given to the hierarchy of potential steric restrictions when multiple protein partners try to bind to the tail of CFTR.

In a recent study with other ABC proteins, it has been demonstrated that the ATPase and the adenylate kinase activities are coupled such as in the ABC lipid A flippase MsbA, and in the LmrA and TmrAB family members. It may be that this feature could represent a general characteristic of the ABC transporters that might have been retained by CFTR.[66] Recent authors have shown that the MsbA, a typical ABC pump, is able to catalyze both the ATP hydrolysis and the adenylate kinase reactions, and that the coupling could have a cyclic character. Cycling may depend on concurrent physiological conditions, in which the ATPase activity would be most prominent under the normal physiological conditions, and the adenylate kinase activity would be most prominent under low ATP/high ADP cellular level. Coupling of the two activities (perhaps with AMP-kinase) would thus provide a mechanism that would allow flexibility for the ABC transporter to function under a variety of physiological conditions, with adenylate kinase playing a role during physiological stress (such as the ATP depletion). In this context, it is important to remember that the cell metabolic sensor 'AMP-kinase' is a misnomer because this complex protein is both an AMP-stimulated and ADP-sustained kinase that binds to the C terminus of CFTR and phosphorylates the R region to keep CFTR shut at baseline. NDPK-A is central to this effect and PKA overcomes this blockade (see below). Thus, it is possible that the coupled ATPase-adenylate kinase mechanism could be a general feature of ABC transporters, including the CFTR, and could represent a physiologically relevant feature of those proteins and protein complexes, representing their mode of functioning under the conditions of energy depletion.[56,58,62] A key unexplained aspect of CFTR function is the discrepancy between the slow rates of ATP hydrolysis observed with purified CFTR and the fast gating kinetics of channel opening.[67–69] We speculate that, as for many aspects of NDPK function (G proteins, ion channel and dynamin regulation, cell migration, Ras scaffold assembly, the fast turnover of NDPK (1000 per second) could supply the missing link between the biochemistry and the electrophysiology.

REGULATION OF CFTR ACTIVITY BY THE 5′-AMP-ACTIVATED PROTEIN KINASE

It has been established that phosphorylation of the R domain of CFTR is essential for the opening of the ion channel.[56] However, CFTR function is basally inhibited by the 5′-AMP-activated protein kinase (AMPK). It has been shown by yeast two hybrid and functional studies with interfering peptides that the COOH-terminal regulatory domain of the catalytic $\alpha 1$ subunit of the AMPK interacts with residues 1420–1457 of CFTR,[70] and that AMPK predominantly phosphorylates CFTR at Ser-768 in the R domain of the CFTR protein.[71] The AMPK is a principal metabolic sensor that responds to conditions of nutritional demand and to the corresponding changes in the AMP/ATP ratio. AMPK exerts this function by inhibiting the ATP-consuming pathways in the cells,[72] and by regulating other energy demanding metabolic processes, such as glucose-dependent gene expression and cellular glucose uptake.[73] AMPK is a ubiquitously distributed (albeit with

variable subunit composition) as a highly conserved heterotrimeric multi-substrate Serine/Threonine (Ser/Thr) kinase. It comprises a catalytic subunit (α1 or α2) and a regulatory subunit (β1 or β2), and a single AMP/ATP-binding subunit (γ1, γ2, or γ3). The multiple isoforms show differing tissue distributions. A low ATP to AMP ratio causes AMP to displace ATP from the γ-subunit of AMPK, initiating a conformational change in the protein. That change allows the phosphorylation at Thr^{172} residue of AMPK by one of the upstream kinases followed by the activation of a catalytic α-subunit.[74] Each of the γ-subunits of AMPK has three AMP-binding sites, two of which are capable of reversibly binding either AMP or Mg^{2+}-ATP and thus regulating the kinase activity of the AMPK protein, whereas the third AMP-binding site of AMPK binds AMP molecule irreversibly.[75] Under physiological conditions, whereby the concentration of the Mg^{2+}-ATP is higher than that of the ADP, and much higher than that of the AMP, regulation of AMPK activity is also facilitated by a significantly higher affinity of AMPK for binding ADP than Mg^{2+}-ATP. Although a rise in the local concentration of ADP and the corresponding decreasing ATP levels do not lead to the allosteric activation of AMPK, such conditions further enhance AMPK activity by preventing dephosphorylation of AMPK by phosphatases.[75] Working with the Hallows group, we reported that just one isoform of AMPK (alpha one and not two) and one isoform of NDPK (A not B) interacted with each other in the context of CFTR function. The data are supportive of a complex link between the activities of AMPK and NDPK-A which co-localize[23] (see below). This is compatible with our early data on chloride as a signal to phosphohistidine generation and perhaps there exists a latent functional inter-regulation between NDPK, that is part of an epithelial, chloride-sensitive, membrane-delimited phosphorylation system working with membrane-bound AMPK[76] in association with CFTR.

It is now evident that CFTR interacting proteins (CIPs) play an important role in regulating CFTR function.[13] However, most CIPs have been identified under baseline conditions and as such are inhibitory to CFTR function.[77–79] One such protein belongs to a family of t-SNARE proteins that are often found on endosomes. A model was proposed invoking the idea that at baseline, cell surface CFTR is held inactive (in complex with proteins inhibitory to CFTR function—for example, syntaxin 1A, syntaxin 8; AMPKα[77–79]) but after PKA is activated, additional component plasticity is induced which is permissive for channel opening.[22] In this regard it was demonstrated that annexin A2 (anx 2) and S100A10 can form a functionally important complex with CFTR in airway and gut epithelial cells in a cAMP, PKA, and protein phosphatase (calcineurin A) dependent manner.[18] Interestingly, disruption of this complex significantly attenuates CFTR function in wild-type cells. In CF cells bearing the common mutant form of CFTR, this PKA/CnA-driven anx 2-S100A10 complex with mutant CFTR fails to form.[80] The recent links between cell calcium and PKA and CFTR function have been reviewed elsewhere by Hanrahan, Kunzelmann and Mehta[20,81] (and cited by Forman-Kay and co-workers[12]) and will not be discussed further here, but we note that calcineurin coupled to the calcium-binding properties of the annexin family and calmodulin itself now firmly place this regulatory cation with the CFTR family of hub proteins.

REGULATION OF CTFR FUNCTION BY VARIOUS ISOFORMS OF NUCLEOSIDE DIPHOSPHATE KINASE (NDPK)

NDPK-A (NME1, nm23-H1) and NDPK-B (NME2, nm23-H2) lie within group I of this histidine kinase family as two closely family members that share 88% sequence similarity. They are thought to exist in heterohexameric forms but our data suggest that they do not always create mixed moieties. Despite their close homology, the intracellular functions of NDPK-A and NDPK-B are markedly different and they do not co-localize to the same macromolecular complexes. For example, others have shown that NDPK-B, but not the NDPK-A, can bind and trans-phosphorylate the β-subunit of G protein, thus increasing the activity of the α-subunit of G protein.[82,83] This ligand-independent pathway promotes baseline cAMP synthesis. NDPK-B has been implicated in the regulation of the G protein-coupled receptor TPβ, and in the regulation of the activity of the calcium-dependent potassium channel KCa3.1.[84,85] The latter is also proposed to bind to CFTR.[12] The combined observations point to the distinct role of the NDPK-B isoform (which binds to NBD1 at residues 351–727), and histidine phosphorylation, in membrane-local signaling events that differ from the above interactions between NDPK-A, CFTR, and AMPK. The role of CK2 remains unclear other than its proposed links to NDPK-A.

As with NDPK-B, the catalytic activity of NDPK-A also requires the 'self' or autophosphorylation event of the catalytic His-118 residue that lies in a water-excluding cleft common to both protein kinases. It has been suggested that the residue Ser-120 of NDPK-A could also be involved in the control of this self-phosphorylation process, perhaps via local electrostatic charge repulsion, as the negatively charged residue substitutions at Ser-120 inhibit autophosphorylation in NDPK-A and disrupt the AMPK-dependent inhibition of CFTR.[86] That AMPK and NDPK might combine to retard the function of CFTR, which has an indirect link to faulty fatty acid metabolism found in CF disease. NDPK-A phosphohistidine generation regulates the key fatty acid regulator, the cytosolic enzyme ATP-citrate lyase,[87] which then cleaves acetyl CoA from citrate exported from the mitochondria as a key step during cytosolic lipid synthesis. In parallel, AMPK regulates acetyl CoA carboxylase,[87] a key enzyme generating the malonyl CoA that balances fatty acid biosynthesis *versus* fatty acid uptake by mitochondria. There are two possible mechanisms that could explain the role of the NDPK-A and AMPK in such regulation whether it be towards CFTR or in fatty acid metabolism. In the first 'substrate channeling'

model, nucleotides are directly supplied to AMPK-alpha-1 isoform by NDPK-A which controls local interconversion of various phosphorylated nucleotides. This would enable the AMPK to acquire local 'force fed' ATP and inhibit the CFTR Cl^- channel even in conditions when global ATP is depleted. The second mechanism stipulates that AMPK reciprocally modulates NDPK-A which acts as an intermediary between AMPK and CFTR in addition to regulating other signal mediators that modulate CFTR activity.[86] Importantly, this NDPK-A/AMPK/CFTR interaction exists independently of the NDPK-B isomer,[88] implying that CFTR may have different partners in different parts of the cell membrane with different functions. One possibility is that new membrane formation during membrane recycling needed for embryogenesis might be abnormally controlled after CFTR mutation. This idea is explored using lessons from fly genetics and tracheal lumen formation later in this review.

We have recently demonstrated that a fraction of NDPK-B, following cell stimulation translocates from cytosol to the membrane to form a functional complex with CFTR in the apical membrane of epithelial cells.[88] This is cAMP/PKA-dependent process. We provided a range of biological and biophysical data consistent with the idea that NDPK-B interacts with NBD1 in CFTR via a motif involving amino acid residues 36–56 on the surface of NDPK-B. Thus NDPK-B, a key basal cAMP generator, is ideally placed to alter the function of NBD domains of CFTR, the dimerization of which drives ATP hydrolysis and thus the opening and closing of the channel. Since a concerted action of phosphorylation and ATP binding and hydrolysis is necessary to activate CFTR channel, it is proposed by these authors that cAMP binding may be another controlling event that takes place during this NDPK-B/CFTR interaction because cAMP enhances the binding of NDPK-B to CFTR in far Western experiments. The details of such a bridging interaction are still not understood but NDPK-B is a crucial element of the *in vivo* transport across the epithelial membranes via the regulation of the activity of both CFTR and related ORCC channels, once again stimulated by cAMP/PKA. Further research is necessary to elucidate the interplay of those activities in the CFTR under different physiological conditions. Since functionally, NDPK maintains pools of nucleotides for cellular processes central to energy utilization, association of NDPK-B particularly with CFTR's first NBD is interesting and suggests NDPK-B could play an important role in the provision and regulation of local nucleotide pools and/or the energy required for NBD function. This may be relevant to the proposal by Dormer *et al* that CFTR also has a cAMP-binding site in its structure and the work of others suggesting homology between domains of CFTR and G protein structure.

CFTR AND CALMODULIN SIGNALING

The tracheal rings of CF babies are incomplete at birth and this may contribute to airway dysfunction. NDPK is an important player during tracheogenesis, along with cell calcium and the exocytotic cell machinery linked to endosome cycling. Forman-Kay and co-workers have shown that cAMP and calcium regulation of CFTR coalesce through the acquisition of calmodulin binding to the R domain region and Kunzelmann, Hanrahan, and Mehta have independently proposed a hidden role of cell calcium in CFTR function.[12,20,81] Tracheogenesis is a complex lumen generating process that has been unraveled using the power of developmental genetics in the fly.[14] It is not common knowledge that flies have tracheas that feed the lumen of a branched tubular set of primitive airways that carry oxygen to mitochondria-rich terminal tips in the fly body. The embryonic formation of a tracheal lumen first aligns and then hollows out adjacent epithelial cells that initially feel for each other using filopodial membrane extensions.[89,90] This blind attachment of membrane 'fingers' between cells may be likened to having magnets attached to the tips of each of the fingers of your two hands that are free to move in space. If the magnets are such that opposite poles are present on each hand, then the fingers will naturally gravitate towards each other. Now we have to imagine that the magnets then draw the long sides of the fingers together and consequently align each hand in a straight line. It was recently shown that adjacent tracheal cells put out finger like extensions from each side that fuse with the tips of their neighboring equivalents during embryogenesis.[90,91] Next, microtubules attach to the fused tips mediating the local arrival of rab 11 and lysosome-related organelles together with Munc13–4/stac calcium-binding proteins. This creates calcium spikes at the ER associated with fusion events that make a lumen by cellular hollowing. It is clear from parallel experiments in worms that tip migration and movement in other parts of development is dependent on NDPK function and Ras signaling through a scaffold protein called KSR. Given that NDPK is dysfunctional in CF cells in that it is overexpressed, mislocalized, and unable to generate phosphohistidine, a parsimonious explanation for tracheal ring malformation in CF babies might relate to an abnormal NDPK, CFTR interaction after CFTR mutation. These ideas coupled to a link between the two calcium-binding domains on Munc13-4/stac and their interaction with the t-SNARE role of syntaxin during late endosome fusion at the time of early fly tracheogenesis might be related to known interactions of the N-terminus of CFTR with syntaxins. Interestingly, a novel Munc13-4/S100A10/Annexin A2 complex has been shown to support acute Weibel-Palade body (WPBs) exocytosis by tethering WPBs to the plasma membrane via Annexin A2-S100A10 in endothelial cells.[92] Thus a wealth of disparate data from embryogenesis where cell tip migration, cell corpse disposal, autophagy, and endosome fusion events have been recently linked to new membrane synthesis through fat metabolism are beginning to point to aspects of CF disease that are otherwise difficult to explain. A key hurdle is that those who research CFTR as a cause of CF do not share a research agenda with

developmental biologists interested in tracheal formation, and in particular it is becoming clear that the embryo can reuse exocytotic events needed for mucus release during fetal lumen formation. For example, about 10% of CF babies have interrupted gut lumens at birth of such severity that they die without surgery and intensive care. We need to speak in a common language if those working on CFTR, membrane blocks in autophagy, endosome recycling, and apical membrane function are ever to meet in a common research arena with those interested in phosphohistidine generation. For example, we suggest that new research might aim to link up the following ideas: (a) CK2 is log-orders overactive in CF cells bearing the common CFTR mutation;[93] (b) CK2 phosphorylates key residues on calmodulin located between two of its EF hands;[94] (c) NDPK controls dynamin as the terminal step in endocytosis and is important in cell homing during embryogenesis;[95,96] (d) AMPK and NDPK control adjacent supply points for the cytosolic availability of acetyl units needed for new membrane synthesis;[97] (e) CK2 phosphorylation controls both the localization and function of sodium channels that become dysfunctional after CFTR mutation.[98–100] These are some of the known unknowns in CFTR and NDPK research that our model seeks to clarify. But how good is our model? In other papers in this series, using the worm NDPK model, for example, developed in Budapest, by Takács-Vellai and colleagues, a variable birth phenotype is observed when just one NDPK gene is deleted in early embryonic life. In a related manner, not all patients will exhibit tracheal defects in childhood and only 10% will develop defective gut lumens at birth needing surgery to avoid lethality. An important test of validity for any model of any disease is whether it can predict the existence of obscure complications. In our work, partly confirmed by others on other family members, we find that the first two members of the annexin protein family of calcium-binding proteins are dysfunctional in CF[21,22,101] alongside our reported NDPK defects. Combining our data with the known functions of annexin A2 in membrane hub assembly and signaling of exocytosis as proposed by the Gerke group,[92] for example, our model predicts platelet dysfunction in CF. The authors were unaware of certain platelet CF literature at the time of using the model of 'annexin-NDPK-opathy' as a prediction tool. We were gratified to note that others have reported platelet pathologies and one paper[102] has even proposed mean platelet volume as a biomarker for CF disease exacerbation. Thus, we believe our approach linking poor CF phosphohistidine generation to an excess non-functional NDPK protein levels has predictive power for currently unexplained aspects of the disease phenotype. In the title, we posed a question about the presence of NDPK A and B in the vicinity of CFTR. If NDPK-B is judged by its established roles in local energy boosting towards other proteins, then the next step must be to determine what is being boosted in the vicinity of CFTR. Whether that is CFTR itself or some other member of the multi-protein complex remains to be determined. For NDPK-A, our own data and that of independent scientists such as K Hallows[86] concur on a bi-directional regulator of NDPK in the context of AMPK's inhibitory role towards CFTR channel function. The other roles of NDPK-A and B in CF pathogenesis need to be determined but we note that NDPK-A has been proposed as a regulator of RAC in relation to the function of epithelial NADPH oxidases[103–105] and that NDPK-B is a core regulator of both ligand-independent G protein function and calcium activated potassium channel activity.

In conclusion, internationally recognised CF experts recently made the following observations on CFTR: "*What is known about the molecular mechanism underlying its ATP-dependent gating suggests that gating is strictly coupled to an ATP hydrolysis cycle. This behaviour is typical of active transporters. But is it just a vestige of CFTR's evolution from its ABC transporter ancestors? Or could some of the many proteins that interact with CFTR slow its ATPase activity, thereby prolonging channel-open dwell times and so providing a sensitive means of regulating channel activity, analogous to modulation of the GTPase activity of G proteins? Or could CFTR still be an active transporter of some as yet unknown substrate, and the concomitant electrodiffusive flow of Cl- be a paraphenomenon – albeit a physiologically vital one – of that transport process?.*"[106] In addition, recent work using the C. elegans model has provided a direct link between NDK1, worm annexin (Nex 1) and Pseudomonas aeroginosa. (ref [111]) which is a primary pathogen in CF. As such, we believe that our model has direct relevance to CF pathogenesis and both NDPK and annexin are dysfunctional. Our work, taken in the context of what others have suggested,[107,108] provides the mechanistic insight into how such actions might occur (Figure 1).[109–110]

ACKNOWLEDGMENTS

We apologize that we were unable, due to space constraints, to include in our review other protein kinases such as SYK that act as a further layer of regulation for CFTR. We thank Sam Miezi and Linda M Muimo for help with the imaging illustration.

DISCLOSURE/CONFLICT OF INTEREST

The authors declare no conflict of interest.

1. Boissan M, Dabernat S, Peuchant E, *et al.* The mammalian Nm23/NDPK family: from metastasis control to cilia movement. Molec Cell Biochem 2009;329:51–62.
2. Desvignes T, Pontarotti P, Fauvel C, *et al.* Nme protein family evolutionary history, a vertebrate perspective. BMC Evol Biol 2009; 9.
3. Lacombe ML, Milon L, Munier A, *et al.* The human Nm23/nucleoside diphosphate kinases. J Bioenerg Biomembr 2000;32:247–258.
4. Lascu I, Gonin P.The catalytic mechanism of nucleoside diphosphate kinases. J Bioenerg Biomembr 2000;32:237–246.
5. Attwood Paul V.Histidine kinases from bacteria to humans. Biochem Soc Trans 2013;41:1023.
6. Kaetzel DM, Zhang QB, Yang MM, *et al.* Potential roles of 3 '-5 ' exonuclease activity of NM23-H1 in DNA repair and malignant progression. J Bioenerg Biomembr 2006;38:163–167.
7. Zwick M, Esposito C, Hellstern M, *et al.* How phosphorylation and ATPase activity regulate anion flux though the cystic fibrosis transmembrane conductance regulator?(CFTR). J Biol Chem 2016;291: 14483–14498.
8. Stoltz DA, Meyerholz DK, Welsh MJ.Origins of cystic fibrosis lung disease. N Engl J Med 2015;372:351–362.
9. Kerem B, Rommens JM, Buchanan JA, *et al.* Identification of the cystic fibrosis gene: genetic analysis. Science 1989;245:1073.

10. Riordan JR, Rommens JM, Kerem BS, *et al.* Identification of the cystic-fibrosis gene - cloning and characterization of complementary-DNA. Science 1989;245:1066–1072.
11. Rommens JM, Iannuzzi MC, Kerem BS, *et al.* Identification of the cystic-fibrosis gene - chromosome walking and jumping. Science 1989;245:1059–1065.
12. Bozoky Z, Ahmadi S, Milman T, *et al.* Synergy of cAMP and calcium signaling pathways in CFTR regulation. Proc Natl Acad Sci USA 2017;114:E2086–E2095.
13. Guggino WB, Stanton BA.New insights into cystic fibrosis: molecular switches that regulate CFTR. Nat Rev Molec Cell Biol 2006;7:426–436.
14. Aydogan V, Belting HG, Affolter M.Mapping the molecular steps of secretory-lysosome-driven tracheal tube fusion. Nat Cell Biol 2016;18: 720–722.
15. Muimo R, Banner SJ, Marshall LJ, *et al.* Nucleoside diphosphate kinase and Cl(-)-sensitive protein phosphorylation in apical membranes from ovine airway epithelium. Am J Respir Cell Mol Biol 1998;18: 270–278.
16. Muimo R, Crawford RM, Mehta A.Nucleoside diphosphate kinase A as a controller of AMP-kinase in airway epithelia. J Bioenerg Biomembr 2006;38:181–187.
17. Muimo R, Hornickova Z, Riemen CE, *et al.* Histidine phosphorylation of annexin I in airway epithelia. J Biol Chem 2000;275: 36632–36636.
18. Borthwick LA, McGaw J, Conner G, *et al.* The formation of the cAMP/protein kinase A-dependent annexin 2-S100A10 complex with cystic fibrosis conductance regulator protein (CFTR) regulates CFTR channel function. Mol Biol Cell 2007;18:3388–3397.
19. Treharne KJ, Marshall LJ, Mehta A.A novel chloride-dependent gtp-utilizing protein-kinase in plasma-membranes from human respiratory epithelium. Am J Physiol 1994;267:L592–L601.
20. Kunzelmann K, Mehta A.CFTR: a hub for kinases and crosstalk of cAMP and Ca2+. FEBS J 2013;280:4417–4429.
21. Borthwick LA, Riemen C, Goddard C, *et al.* Defective formation of PKA/CnA-dependent annexin 2-S100A10/CFTR complex in Delta F508 cystic fibrosis cells. Cell Signal 2008;20:1073–1083.
22. Muimo R.Regulation of CFTR function by annexin A2-S100A10 complex in health and disease. Gen Physiol Biophys 2009;28(Spec No Focus):F14–19.
23. Treharne KJ, Best OG, Mehta A.The phosphorylation status of membrane-bound nucleoside diphosphate kinase in epithelia and the role of AMP. Molec Cell Biochem 2009;329:107–114.
24. Dalli J, Rosignoli G, Hayhoe RPG, *et al.* CFTR Inhibition provokes an inflammatory response associated with an imbalance of the annexin A1 pathway. Am J Pathol 2010;177:176–186.
25. Bompadre SG, Hwang TC.Cystic fibrosis transmembrane conductance regulator: a chloride channel gated by ATP binding and hydrolysis. Acta Physiol Sin 2007;59:431–442.
26. Dean M, Hamon Y, Chimini G.The human ATP-binding cassette (ABC) transporter superfamily. J Lipid Res 2001;42:1007–1017.
27. Jones PM, George AM.The ABC transporter structure and mechanism: perspectives on recent research. Cell Molec Life Sci 2004;61:682–699.
28. Tosoni K, Stobbart M, Cassidy DM, *et al.* CFTR mutations altering CFTR fragmentation. Biochem J 2013;449:295–305.
29. Zou X, Hwang TC.ATP hydrolysis-coupled gating of CFTR chloride channels: structure and function. Biochemistry 2001;40:5579–5586.
30. Gadsby DC, Dousmanis AG, Nairn AC.ATP hydrolysis cycles and the gating of CFTR Cl- channels. Acta Physiol Scand Suppl 1998;643: 247–256.
31. Linsdell P.Functional architecture of the CFTR chloride channel. Molec Membr Biol 2014;31:1–16.
32. Moran O.On the structural organization of the intracellular domains of CFTR. Int J Biochem Cell Biol 2014;52:7–14.
33. Sheppard DN, Welsh MJ.Structure and function of the CFTR chloride channel. Physiol Rev 1999;79:S23.
34. Bozoky Z, Krzeminski M, Muhandiram R, *et al.* Regulatory R region of the CFTR chloride channel is a dynamic integrator of phospho-dependent intra- and intermolecular interactions. Proc Natl Acad Sci USA 2013;110:E4427–4436.
35. Ostedgaard LS, Baldursson O, Welsh MJ.Regulation of the cystic fibrosis transmembrane conductance regulator Cl- channel by its R domain. J Biol Chem 2001;276:7689–7692.
36. Meng X, Clews J, Kargas V, *et al.* The cystic fibrosis transmembrane conductance regulator (CFTR) and its stability. Cell Molec Life Sci 2017;74:23–38.
37. Farinha CM, Swiatecka-Urban A, Brautigan DL, *et al.* Regulatory crosstalk by protein kinases on CFTR trafficking and activity. Front Chem 2016;4:1.
38. Rosenberg MF, O'Ryan LP, Hughes G, *et al.* The cystic fibrosis transmembrane conductance regulator (CFTR): three-dimensional structure and localization of a channel gate. J Biol Chem 2011;286: 42647–42654.
39. Liu FY, Zhang Z, Csanady L, *et al.* Molecular structure of the human CFTR ion channel. Cell 2017;169:85.
40. Zhang Z, Chen J.Atomic structure of the cystic fibrosis transmembrane conductance regulator. Cell 2016;167:1586.
41. Zhang Z, Liu FY, Chen J.Conformational changes of CFTR upon phosphorylation and ATP binding. Cell 2017;170:483.
42. Sun F, Hug MJ, Bradbury NA, *et al.* Protein kinase A associates with cystic fibrosis transmembrane conductance regulator via an interaction with ezrin. J Biol Chem 2000;275:14360–14366.
43. Linsdell P.Mechanism of chloride permeation in the cystic fibrosis transmembrane conductance regulator chloride channel. Exp Physiol 2006;91:123–129.
44. McCarty NA.Permeation through the CFTR chloride channel. J Exp Biol 2000;203(Pt 13):1947–1962.
45. Gadsby DC, Vergani P, Csanady L.The ABC protein turned chloride channel whose failure causes cystic fibrosis. Nature 2006;440: 477–483.
46. Randak C, Welsh MJ.An intrinsic adenylate kinase activity regulates gating of the ABC transporter CFTR. Cell 2003;115:837–850.
47. Tabcharani JA, Chang XB, Riordan JR, *et al.* Phosphorylation-regulated Cl- channel in CHO cells stably expressing the cystic fibrosis gene. Nature 1991;352:628–631.
48. Hwang TC, Kirk KL.The CFTR ion channel: gating, regulation, and anion permeation. Cold Spring Harbor Perspect Med 2013;3: a009498.
49. Moran O.The gating of the CFTR channel. Cell Molec Life Sci 2017;74: 85–92.
50. Meng X, Wang Y, Wang X, *et al.* Two small molecules restore stability to a sub-population of the cystic fibrosis transmembrane conductance regulator with the predominant disease-causing mutation. J Biol Chem 2017;292:3706–3719.
51. Hwang TC, Sheppard DN.Gating of the CFTR Cl- channel by ATP-driven nucleotide-binding domain dimerisation. J Physiol 2009;587(Pt 10): 2151–2161.
52. Csanady L, Vergani P, Gadsby DC.Strict coupling between CFTR's catalytic cycle and gating of its Cl- ion pore revealed by distributions of open channel burst durations. Proc Natl Acad Sci USA 2010;107: 1241–1246.
53. Zeltwanger S, Wang F, Wang GT, *et al.* Gating of cystic fibrosis transmembrane conductance regulator chloride channels by adenosine triphosphate hydrolysis. Quantitative analysis of a cyclic gating scheme. J Gen Physiol 1999;113:541–554.
54. Aleksandrov L, Mengos A, Chang X, *et al.* Differential interactions of nucleotides at the two nucleotide binding domains of the cystic fibrosis transmembrane conductance regulator. J Biol Chem 2001;276:12918–12923.
55. Weinreich F, Riordan JR, Nagel G.Dual effects of ADP and adenylylimidodiphosphate on CFTR channel kinetics show binding to two different nucleotide binding sites. J Gen Physiol 1999;114: 55–70.
56. Randak CO, Welsh MJ.Role of CFTR's intrinsic adenylate kinase activity in gating of the Cl(-) channel. J Bioenerg Biomembr 2007;39: 473–479.
57. Dong Q, Ernst SE, Ostedgaard LS, *et al.* Mutating the conserved Q-loop Glutamine 1291 selectively disrupts adenylate kinase-dependent channel gating of the ATP-binding cassette (ABC) adenylate kinase cystic fibrosis transmembrane conductance regulator (CFTR) and reduces channel function in primary human airway epithelia. J Biol Chem 2015;290:14140–14153.
58. Randak CO, Dong Q, Ver Heul AR, *et al.* ATP and AMP mutually influence their interaction with the ATP-binding cassette (ABC) adenylate kinase cystic fibrosis transmembrane conductance

regulator (CFTR) at separate binding sites. J Biol Chem 2013;288: 27692–27701.
59. Treharne KJ, Xu Z, Chen JH, *et al.* Inhibition of protein kinase CK2 closes the CFTR Cl- channel, but has no effect on the cystic fibrosis mutant Delta F508-CFTR. Cell Physiol Biochem 2009;24:347–360.
60. Mathews CJ, Tabcharani JA, Hanrahan JW.The CFTR chloride channel: nucleotide interactions and temperature-dependent gating. The Journal of membrane biology 1998;163:55–66.
61. Csanady L, Nairn AC, Gadsby DC.Thermodynamics of CFTR channel gating: a spreading conformational change initiates an irreversible gating cycle. J Gen Physiol 2006;128:523–533.
62. Callebaut I, Hoffmann B, Lehn P, *et al.* Molecular modelling and molecular dynamics of CFTR. Cell Molec Life Sci 2017;74:3–22.
63. Ostedgaard LS, Meyerholz DK, Chen JH, *et al.* The Delta F508 mutation causes CFTR misprocessing and cystic fibrosis-like disease in pigs. Sci Transl Med 2011;3.
64. Venerando A, Franchin C, Cant N, *et al.* Detection of phospho- sites generated by protein kinase CK2 in CFTR: mechanistic aspects of Thr1471 phosphorylation. PloS ONE 2013;8.
65. Castellani S, Conese M.Not all is CFTR - neutrophils and cholesterol in cystic fibrosis. EBioMedicine 2017;24:28–29.
66. Kaur H, Lakatos-Karoly A, Vogel R, *et al.* Coupled ATPase-adenylate kinase activity in ABC transporters. Nat Commun 2016;7:13864.
67. Ramjeesingh M, Li CH, Garami E, *et al.* Walker mutations reveal loose relationship between catalytic and channel-gating activities of purified CFTR (cystic fibrosis transmembrane conductance regulator). Biochemistry 1999;38:1463–1468.
68. Jih KY, Hwang TC.Vx-770 potentiates CFTR function by promoting decoupling between the gating cycle and ATP hydrolysis cycle. Proc Natl Acad Sci USA 2013;110:4404–4409.
69. Sorum B, Czege D, Csanady L.Timing of CFTR pore opening and structure of its transition state. Cell 2015;163:724–733.
70. Hallows KR, Raghuram V, Kemp BE, *et al.* Inhibition of cystic fibrosis transmembrane conductance regulator by novel interaction with the metabolic sensor AMP-activated protein kinase. J Clin Invest 2000;105:1711–1721.
71. King Jr. JD , Fitch AC, Lee JK, *et al.* AMP-activated protein kinase phosphorylation of the R domain inhibits PKA stimulation of CFTR. Am J Physiol Cell Physiol 2009;297:C94–101.
72. Hardie DG, Carling D.The AMP-activated protein kinase–fuel gauge of the mammalian cell? Eur J Biochem 1997;246:259–273.
73. Leclerc I, Kahn A, Doiron B.The 5'-AMP-activated protein kinase inhibits the transcriptional stimulation by glucose in liver cells, acting through the glucose response complex. FEBS Lett 1998;431:180–184.
74. Oakhill JS, Scott JW, Kemp BE.Structure and function of AMP-activated protein kinase. Acta Physiol 2009;196:3–14.
75. Xiao B, Sanders MJ, Carmena D, *et al.* Structural basis of AMPK regulation by small molecule activators. Nat Commun 2013;4:3017.
76. Semianrio-Vidal L, van Hesuden C, Mugesh G, *et al.* Ebselen is a potent non-competitive inhibitor of extracellular nucleoside diphosphokinase. Purinergic Signall 2010;6:383–391.
77. Cormet-Boyaka E, Di A, Chang SY, *et al.* CFTR chloride channels are regulated by a SNAP-23/syntaxin 1A complex. Proc Natl Acad Sci USA 2002;99:12477–12482.
78. Bilan F, Thoreau V, Nacfer M, *et al.* Syntaxin 8 impairs trafficking of cystic fibrosis transmembrane conductance regulator (CFTR) and inhibits its channel activity. J Cell Sci 2004;117:1923–1935.
79. Hallows KR, McCane JE, Kemp BE, *et al.* Regulation of channel gating by AMP-activated protein kinase modulates cystic fibrosis transmembrane conductance regulator activity in lung submucosal cells. J Biol Chem 2003;278:998–1004.
80. Borthwick LA, Riemen C.B, Goddard C., Colledge W.H., Mehta A., Gerke V., Muimo RDefective formation of PKA/CnA-dependent annexin 2-S100A10/CFTR complex in ΔF508 cystic fibrosis cells. Cell Signal 2008;20:1073–1083.
81. Billet A, Hanrahan JWThe secret life of CFTR as a calcium-activated chloride channel. J Physiol 2013;591:5273–5278.
82. Cuello F, Schulze RA, Heemeyer F, *et al.* Activation of heterotrimeric G proteins by a high energy phosphate transfer via nucleoside diphosphate kinase (NDPK) B and Gβ subunits: complex formation of NDPK B with Gβγ dimers and phosphorylation of His-266 in Gβ. J Biol Chem 2003;278:7220–7226.
83. Hippe H-J, Lutz S, Cuello F, *et al.* Activation of heterotrimeric G proteins by a high energy phosphate transfer via nucleoside diphosphate kinase (NDPK) B and Gβ subunits: specific activation of Gsα by an NDPK B·Gβγ complex in H10 cells. J Biol Chem 2003;278: 7227–7233.
84. Rochdi MD, Laroche G, Dupre E, *et al.* Nm23-H2 interacts with a G protein-coupled receptor to regulate its endocytosis through an Rac1-dependent mechanism. J Biol Chem 2004;279:18981–18989.
85. Srivastava S, Li Z, Ko K, *et al.* Histidine phosphorylation of the potassium channel KCa3.1 by nucleoside diphosphate kinase B is required for activation of KCa3.1 and CD4 T cells. Mol Cell 2006;24:665–675.
86. King Jr. JD , Lee J, Riemen CE, *et al.* Role of binding and nucleoside diphosphate kinase A in the regulation of the cystic fibrosis transmembrane conductance regulator by AMP-activated protein kinase. J Biol Chem 2012;287:33389–33400.
87. Hardie DG.AMP-activated protein kinase: an energy sensor that regulates all aspects of cell function. Genes Dev 2011;25:1895–1908.
88. Borthwick LA, Kerbiriou M, Taylor CJ, *et al.* Role of interaction and nucleoside diphosphate kinase B in regulation of the cystic fibrosis transmembrane conductance regulator function by cAMP-dependent protein kinase A. PLoS ONE 2016;11:e0149097.
89. Hsu T.NME genes in epithelial morphogenesis. Naunyn-Schmiedeberg's Arch Pharmacol 2011;384:363–372.
90. Hsouna A, Lawal HO, Izevbaye L, *et al.* Drosophila dopamine synthesis pathway genes regulate tracheal morphogenesis. Dev Biol 2007;308: 30–43.
91. Kerman BE, Cheshire AM, Andrew DJFrom fate to function: the Drosophila trachea and salivary gland as models for tubulogenesis. Differ Res Biol Divers 2006;74:326–348.
92. Chehab T, Santos NC, Holthenrich A, *et al.* A novel Munc13-4/S100A10/Annexin A2 complex promotes Weibel-Palade body exocytosis in endothelial cells. Molec Biol Cell 2017;28:1688–1700.
93. Pagano MA, Marin O, Cozza G, *et al.* Cystic fibrosis transmembrane regulator fragments with the Phe(508) deletion exert a dual allosteric control over the master kinase CK2. Biochem J 2010;426: 19–29.
94. Arrigoni G, Marin O, Pagano MA, *et al.* Phosphorylation of calmodulin fragments by protein kinase CK2. Mechanistic aspects and structural consequences. Biochemistry 2004;43:12788–12798.
95. Fancsalszky L, Monostori E, Farkas Z, *et al.* NDK-1, the homolog of NM23-H1/H2 regulates cell migration and apoptotic engulfment in *C. elegans*. Plos ONE 2014;9.
96. Takacs-Vellai K, Vellai T, Farkas Z, *et al.* Nucleoside diphosphate kinases (NDPKs) in animal development. Cell Molec Life Sci 2015;72: 1447–1462.
97. Pietrocola F, Galluzzi L, Bravo-San Pedro JM, *et al.* Acetyl coenzyme A: a central metabolite and second messenger. Cell Metab 2015;21: 805–821.
98. Brechet A, Fache M-P, Brachet A, *et al.* Protein kinase CK2 contributes to the organization of sodium channels in axonal membranes by regulating their interactions with ankyrin G. J Cell Biol 2008;183: 1101–1114.
99. Bachhuber T, Almaca J, Aldehni F, *et al.* Regulation of the epithelial Na+ channel by the protein kinase CK2. J Biol Chem 2008;283: 13225–13232.
100. Rasband MN.Na+ channels get anchored ... with a little help. J Cell Biol 2008;183:975–977.
101. Bensalem N, Ventura AP, Vallee B, *et al.* Down-regulation of the anti-inflammatory protein annexin A1 in cystic fibrosis knock-out mice and patients. Molec Cell Proteom 2005;4:1591–1601.
102. Maloney JP, Narasimhan J, Biller J.Decreased TGF-beta(1) and VEGF release in cystic fibrosis platelets: further evidence for platelet defects in cystic fibrosis. Lung 2016;194:791–798.
103. Vázquez-Medina JP, Dodia C, Weng L, *et al.* The phospholipase A(2) activity of peroxiredoxin 6 modulates NADPH oxidase 2 activation via lysophosphatidic acid receptor signaling in the pulmonary endothelium and alveolar macrophages. FASEB J 2016;30: 2885–2898.
104. George A, Pushkaran S, Konstantinidis DG, *et al.* Erythrocyte NADPH oxidase activity modulated by Rac GTPases, PKC, and plasma cytokines contributes to oxidative stress in sickle cell disease. Blood 2013;121:2099–2107.

105. Mizrahi A, Molshanski-Mor S, Weinbaum C, *et al*. Activation of the phagocyte NADPH oxidase by Rac guanine nucleotide exchange factors in conjunction with ATP and nucleoside diphosphate kinase. J Biol Chem 2005;280:3802–3811.
106. Vergani P, Gadsby DC, Csanády LCFTR, an ion channel evolved from ABC transporterIn:Roberts GCK, (ed)Encyclopedia of Biophysics. Springer Berlin Heidelberg: Berlin, Heidelberg, 2013, pp 254–265.
107. Schwiebert EM, Kizer N, Gruenert DC, *et al*. GTP-binding proteins inhibit cAMP activation of chloride channels in cystic fibrosis airway epithelial cells. Proc Natl Acad Sci USA 1992;89:10623–10627.
108. Farkas Z, Fancsalszky L, Saskoi E, *et al*. The dosage-dependent effect exerted by the NM23-H1/H2 homolog NDK-1 on distal tip cell migration in *C. elegans*. Lab Invest 2017.
109. Kuehnl A, Musiol A, Raabe CA, *et al*. Emerging functions as host cell factors - an encyclopedia of annexin-pathogen interactions. Biol Chem 2016;397:949–959.
110. Liu SX, Gao Y, Yu XL, *et al*. Annexin-1 mediates microglial activation and migration via the CK2 pathway during oxygen-glucose deprivation/reperfusion. Int J Molec Sci 2016;17:1770.
111. https://doi.org/10.1016/j.jprot.2016.04.025.