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COMMENTARY

Tweaking the catalytic efficiency of the CFTR ion channel

Nael A. McCarty[1]

The superfamily of ATP-binding cassette (ABC) transporters comprises the largest known family of transmembrane (TM) transporters, and representatives of this superfamily are found in all domains of life. The vast majority of these protein systems function to catalyze the active transport of substrates—varying widely in terms of chemical composition and molecular weight—unidirectionally, either into cells (importers) or out of cells (exporters). These protein systems are expressed in modular form. The basic functional unit includes two TM domains (TMDs) comprised of several TM helices and two nucleotide-binding domains (NBDs) that dimerize in the presence of ATP. Binding of nucleotide induces a conformational change that is transmitted into the TMDs by cytoplasmic connecting loops resulting in a shift in equilibrium between states exposing the substrate binding pocket to the cytoplasmic or extracellular space. In many prokaryotic ABC transporters, the TMD proteins and the NBD proteins are expressed on separate genes (Ford and Beis, 2019; Thomas et al., 2020). In some cases, the functional unit includes an extra TMD, and in some prokaryotic importer systems the substrate is sequestered and presented to the membrane-localized TMDs by a soluble or membrane-associated substrate-binding domain (van der Heide and Poolman, 2002). Almost all ABC transporters transport their substrate(s) via a conscribed sequence of events that includes access from only one membrane face, closure of the access pathway to establish an occluded state, and opening of access to the other membrane face. These conformational changes are regulated in strict stoichiometry by the binding and hydrolysis of ATP. However, alone in the ABC transporter superfamily (as far as we know at present, anyway), one protein system has evolved to break this strict stoichiometry to enable the transport of millions of substrate molecules per ATP hydrolyzed (Fig. 1), via processes explored in an article in this issue from the lab of László Csanády (Simon and Csanády, 2023).

The cystic fibrosis transmembrane conductance regulator (CFTR) is an ABC transporter to which multiple functions have been ascribed, both in membranes of intracellular organelles and the plasma membrane. Variably proposed to function in the regulation of other ion channels and in the non-conductive transport of macromolecules such as glutathione, CFTR has been shown with certainty to be a chloride and bicarbonate ion channel that provides a pathway for regulated anion flux in many tissues of epithelial origin. Loss of function of CFTR represents the primary defect in cystic fibrosis (CF), an orphan life-shortening genetic disease impacting people of all ethnic origins.

While most ABC transporter systems transport multiatomic substrates, CFTR in most epithelia mediates the transport of the lowly chloride ion, the most abundant inorganic anion in human bodily fluids. Unlike the well-known Na^+/K^+-ATPase, which moves three sodium ions and two potassium ions per molecule of ATP hydrolyzed, CFTR's catalytic efficiency is enhanced by at least three modifications to this gene/protein that enable it to use the electrochemical driving force for chloride to maximize chloride flux (Infield et al., 2021). These include: (1) slowing ATP hydrolysis to allow the NBDs to remain dimerized, the state associated with the open channel; (2) degradation of the structures at the cytoplasmic end of the pore-forming pathway that contribute to formation of the occluded state in other ABC transporters; and (3) stabilization of the open conformation by intraprotein residue–residue interactions that hold the channel open in the state with maximum unitary conductance for typically >0.5 s (in the wildtype human channel). The position of extracellular loop #1 (ECL1) was shown previously to be critical to the process of pore-gating in human CFTR, where mutations at multiple residues introduced instability of the open state (Infield et al., 2016). Of course, CFTR also has features that stabilize the closed state, including both other residue–residue interactions and the addition of a regulatory (R) domain that interferes with the productive transmission of mechanical energy from the NBDs to the TMDs unless the R domain is phosphorylated by protein kinase A (Infield et al., 2023). The fact that CFTR can be studied by high-resolution electrophysiological approaches, including the use of single-channel patch clamp, makes it possible to study the functional readouts of these rapid conformational changes in excruciating detail, both in the temporal and amplitude domains, enabling conclusions that may

[1]Emory University School of Medicine, Atlanta, GA, USA.

Correspondence to Nael A. McCarty: namccar@emory.edu.

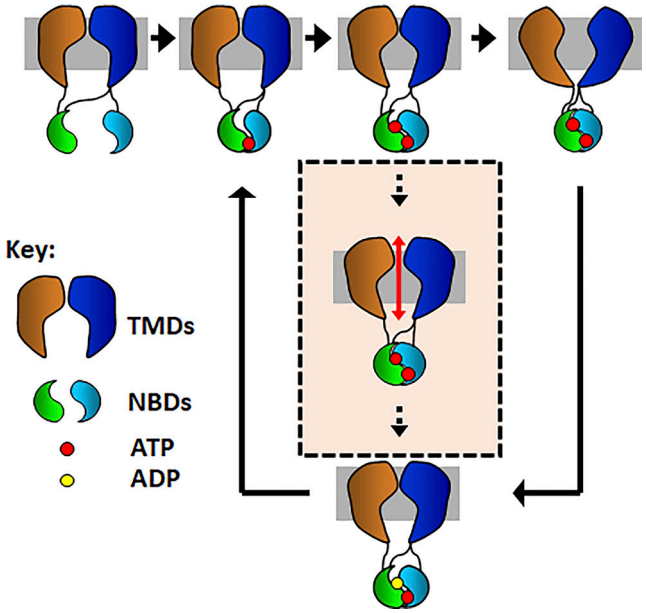

Figure 1. **Hypothesis for emergence of channel function in CFTR.** Modification of ATP-dependent transport activity in ABC transporters led to channel behavior, co-opting the conformational changes necessary for unidirectional substrate transport in common ABC transporter systems. CFTR evolved features that break the alternating access cycle (solid-line arrows), enabling it to be open at both ends (box). The R domain is excluded, for simplicity. Figure from Infield et al. (2021).

be extended to other closely related ABC transporters that lack conductive properties.

The article from Simon and Csanády (2023) describes the results of a study to explore the importance of residue–residue contacts at the extracellular end of CFTR in stabilizing the open channel state. Specifically, they seek to understand the role of a specific residue in evolution of function within CFTR, largely by taking advantage of a combination of structural analysis and sequence analysis across evolutionarily distanced orthologs: human and zebrafish. While several hundred full or partial CFTR orthologs are included in the GenBank/UniProt database, with the lamprey ortholog serving as the most evolutionarily distant from the human (Cui et al., 2019), structural data are only available as far back as the zebrafish.

Simon and Csanády hypothesized that a specific hydrogen bond pair in the outer mouth of the human CFTR channel (hCFTR), but missing in the equivalent domain of the zebrafish CFTR channel (zCFTR), might help explain the relative instability of the zCFTR open state. The authors relied upon careful study of the electron density maps from cryo-EM data for both hCFTR and zCFTR in both the inward-facing and outward-facing conformations that reflect the closed and almost-open channel states, respectively (Liu et al., 2017; Zhang and Chen, 2016; Zhang et al., 2017; Zhang et al., 2018b). The authors tested their hypothesis by employing mutant cycle analysis of variants expressed in *Xenopus laevis* oocytes and studied by both single-channel and macropatch electrophysiological methods.

CFTR channels open to a bursting state that includes brief closures to a "flickery" intraburst closed state; bursts are separated by the much longer interburst closed states that represent ATP hydrolysis and at least partial de-dimerization of the NBDs. The catalytic efficiency of even hCFTR is poor, represented by an open probability of ~0.4 under physiological conditions (Fuller et al., 2005). However, zCFTR is far worse, with an open probability of only ~0.03 (Zhang et al., 2018a). Mutation of R117 in ECL1 to histidine in the human channel (hR117H) greatly reduces open probability (Sheppard et al., 1993; Yu et al., 2016), and causes a relatively mild form of CF (Dean et al., 1990; Wilschanski et al., 1995). The arginine at position equivalent to R117 in hCFTR is highly conserved across evolution (Simon and Csanády, 2021). Indeed, hR117A cannot be locked into a stable open state even with disruption of ATP hydrolysis, confirming its important role in the proper positioning of the extracellular loops in supporting effective channel function (Cui et al., 2014).

Simon and Csanády recognized that R117 and E1124 in the hCFTR ortholog appear to interact to stabilize the open (bursting) state. In contrast, zR118, the residue equivalent to hR117, does not appear to contribute to an equivalent hydrogen bond, and the R118H mutation did not affect function of zCFTR. Instead, Simon and Csanády identified an interaction between zS109 and zN120 that only exists in the outward-facing state of zCFTR, which contributes to the flickery intraburst closed state. This interaction is not found in the zCFTR inward-facing state, nor in any of the hCFTR structures. The equivalent serine is conserved in hCFTR (hS108), but at the position equivalent to zN120 hCFTR bears an isoleucine (hI119). Sequence comparisons showed that a polar residue is found in ancient orthologs at this site (N or Q), but this is replaced by a hydrophobic residue in later orthologs. We note that the hR117–E1124 interaction shows a similar pattern, where the R is strongly conserved but the position equivalent to E1124 shows a hydrophobic residue in ancient orthologs but is either E or D in later orthologs. Interestingly, mutation of hE1126 to R, very near hE1124 in the sixth extracellular loop, also introduced instability of the open burst structure in a prior study (Cui et al., 2014), suggesting that a true network of residue–residue interactions contribute to channel stability in hCFTR. Furthermore, Simon and Csanády also found that hR117 and hS108 interact, and mutations at hS108 have a similar effect as mutations at hR117. This suggests that R117, S108, and E1124 may share interactions that contribute to stability of the open state in hCFTR. Overall, it appears that as CFTR function has evolved in both ancient and modern species, the solution to the need for mechanisms to optimize chloride flux by stabilizing the open pore conformation was achieved by convergent means, relying upon the establishment of a dynamic network of residue–residue interactions in the external mouth of the pore.

The study is limited in part by the focus on only the human and zebrafish CFTR orthologs; while only these are represented by high-resolution cryo-EM models available at present, there is much information available to investigators interested in molecular evolution of function in the CFTR lineage in terms of bioinformatics and sequence analysis. For example, neither the hR117–hE1124 pair nor the hS108–hI119 pair are conserved in the equivalent sites in the lamprey CFTR ortholog (Cui et al., 2019); of course, there might be other interactions in this ortholog that

serve the same purpose. The study is also subject to some uncertainty in the electron densities of the relevant residues, given the poor resolution of structures in these relatively floppy domains. Fortunately, the application of mutant cycle analysis by Simon and Csanády (2023) provides functional confirmation of the hypothesized interactions at a quantitative level. One also must consider that the extracellular tips of these two CFTR orthologs extend into vastly different environments: the bicarbonate-rich solution of the pancreatic duct and the thin layer of airway-surface liquid in humans, compared with, let's say, the freshwater fluid bathing the zebrafish gill. Hence, it would be a mistake to take an anthropocentric view of molecular evolution in CFTR to propose that hCFTR represents the final, optimized state of CFTR molecular evolution.

Nonetheless, it is encouraging to think that the application of quantitative, mechanistic approaches such as used by Simon and Csanády in this study, hopefully accompanied and facilitated by the publication of new structures of other CFTR orthologs, will lead to a better understanding of how this unique representative of a massively complex superfamily of proteins has been tweaked by the forces of molecular evolution. One might even predict that such approaches could lead to the design of even-more-evolved versions of human CFTR that could be used in genetic therapies, in place of the native human ortholog, to enable even better catalytic efficiency of chloride secretion in tissues impacted in CF.

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