

CFTR: An ion channel with a transporter-type energy-coupling mechanism

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Once, as a medical student, I learned from a pathology textbook that CFTR is a chloride ion channel—a unique one that I should keep in mind because its failure causes the life-threatening human genetic disease cystic fibrosis. It was several years later, after I began studying CFTR, that I saw the other side of its uniqueness. It is the only known ion channel in an enormous transporter family, namely the ATP-binding cassette (ABC) protein family. Classical wisdom has been that the logic of transporters and ion channels is vastly different in thermodynamic essence. There is hence an intriguing question: how closely is CFTR function linked to ABC transporter mechanisms? An article in this issue of the Journal by Jih et al. greatly advances our understanding of this topic by demonstrating that CFTR unidirectionally cycles through chloride-conducting (open) and non-conducting (closed) states using the free energy of ATP hydrolysis. Readers interested in this topic are also referred to an elegant work published recently by Csanády et al. (2010).

ABC transporters are thermodynamic engines in nature. They are designed to transfer the free energy of ATP hydrolysis to the uphill movement of substrates across a membrane in which a chemical potential difference is created and maintained. Recent crystallographic studies of ABC proteins (Oldham et al., 2008; Rees et al., 2009) nicely show that this free energy transfer process can be achieved by a conformational coupling mechanism (Tanford, 1983): conformational changes in the cytoplasmic nucleotide-binding domains (NBDs), resulting from ATP binding and hydrolysis, are coupled to the rearrangements in the transmembrane helices, leading to a change in accessibility of the substrate-binding site from one side of the membrane to the other. In this mechanism, the essentially irreversible ATP hydrolysis step, which drives the alternating exposure of substrates, effectively ensures a one-direction translocation of substrates against the chemical gradient.

On the other hand, the CFTR channel, although equipped with all molecular components of ABC family members, catalyzes downhill diffusion of chloride ions. For such a passive transport system, there is no obvious need for external energy input (e.g., ATP hydrolysis).

It is therefore unclear if the operation of CFTR, like other ABC transporters, requires strict coupling between the substrate translocation pathway and the ATP catalytic cycle. In fact, the opening and closing of ion-conducting pathways for channels are usually found to represent conformational changes at equilibrium, which may favor open states by the binding of ligands (ligand-gated channels), imposition of membrane potentials (voltage-gated channels), etc. Indeed, CFTR has also been proposed to be a ligand-gated channel, with ATP binding favoring channel opening. Here, no energy coupling is involved; ATP hydrolysis simply provides an easy means for the channel to get rid of the ligand (Aleksandrov et al., 2007).

In all models in which gating is at equilibrium, fundamentals of thermodynamics dictate that microscopic reversibility must be strictly obeyed; channel closing must in every detail be the reverse of the opening process. To examine this, Csanády et al. (2010) analyzed the statistical distribution of CFTR open times. They immediately noticed a peaked distribution with an early rising phase (Fig. 1 A), a finding that demands an irreversible step upon channel closure (Colquhoun and Hawkes, 1982). Conceptually, this is because the open channel O_1 must first proceed through a distinct open state O_2 before shutting can occur ($O_2 \rightarrow C$), so very brief openings are rarely observed (Fig. 1 B). Here, the principle of microscopic reversibility is violated, as the channel takes different routes for opening ($C \rightarrow O_1$) and closing ($O_1 \rightarrow O_2 \rightarrow C$); the direct consequence is the exclusion of any equilibrium scheme. The irreversible step, unsurprisingly, is coupled to ATP hydrolysis ($O_1 \rightarrow O_2$ in Fig. 1 B), as the open-time distribution of a catalysis-deficient CFTR mutant exhibits an exponential decay, which is consistent with equilibrium gating.

Now, Jih et al. (2012) demonstrate nonequilibrium gating of CFTR in a visually satisfactory way. By mutating residue R352 in the transmembrane domain to cysteine, they created a mutant CFTR channel (R352C-CFTR) that harbors experimentally distinguishable O_1 and O_2 states not seen in wild-type (WT) channel gating.

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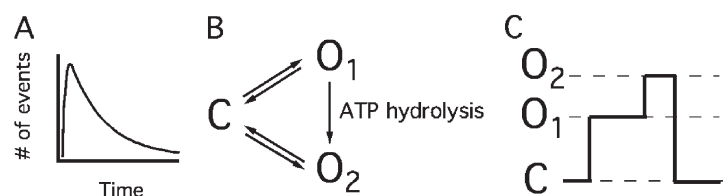


Figure 1. Nonequilibrium gating of CFTR. (A) A sample open-time histogram featuring an irreversible step during channel closure. (B) Opening and closing of CFTR in a cyclic scheme. (C) A single-channel event of R352C-CFTR with three conductance states, acquired with my high-resolution word editor.

Their current recordings vividly show that closed channels first open into the smaller conductance state O_1 and then the larger O_2 state before closure ($C \rightarrow O_1 \rightarrow O_2 \rightarrow C$ in Fig. 1 C). The reverse gating events of $C \rightarrow O_2 \rightarrow O_1 \rightarrow C$, however, are extremely rare. Microscopic reversibility—which teaches us that if the easiest pathway from one side of the mountain to the other is along the base, the easiest way back should not involve a detour to the summit—is violated in broad daylight! CFTR is therefore deemed to operate far from equilibrium, which mandates an irreversible step during gating. The authors then took the story one step further by introducing a catalysis-abolishing mutation E1371S into R352C-CFTR; these hydrolysis-deficient channels now open and close reversibly ($C \rightarrow O_1 \rightarrow C$ and $C \rightarrow O_2 \rightarrow C$), indicating that ATP hydrolysis underlies a unidirectional transition from O_1 to O_2 (Fig. 1 B).

Collectively with Csanády's earlier works, this experiment firmly establishes that gating of the CFTR pore in the transmembrane domains is coupled to the ATPase cycle in the cytoplasmic NBDs—the spirit of ABC transporter's energy coupling mechanism is preserved in CFTR. Despite this conclusion, it is very important to point out here that the passive transport nature of chloride ion flow implies that external energy input might not be essential for CFTR function. Indeed, unlike ABC transporters, which absolutely require ATPase activity to move substrates, CFTR mutants incapable of catalyzing ATP hydrolysis (e.g., K1250A, D13710N, and E1371Q) exhibit gating transitions with open probabilities comparable to WT (Powe et al., 2002; Vergani et al., 2003). Moreover, albeit at a lower efficacy, nonhydrolyzable ligands, such as AMP-PNP (Aleksandrov et al., 2000; Vergani et al., 2003) and pyrophosphate (Tsai et al., 2009) also support CFTR function. In fact, even in ATP-dependent gating of WT-CFTR, there is a small portion of gating events that involve no ATP hydrolysis, as shown in the two studies mentioned here. CFTR is thus only one step away from escaping the shadow of transporters to join the club of classical ligand-gated channels.

The story should not simply end here. By using the R352C mutation as a tool, now Jih et al. (2012) have the ability to directly quantify the rate constants connecting C, O_1 , and O_2 states, and thus better characterize previously undissectible molecular events, such as ATP hydrolysis ($O_1 \rightarrow O_2$), at the single molecule level. This opens up unprecedented opportunities to deepen our

understanding to the fundamental mechanism of CFTR and potentially other ABC transporters. For instance, mutational effects on ATP hydrolysis can now be compared more easily, and this would not only allow an evaluation of how CFTR's two ATP-binding sites communicate with each other but also permit in-depth investigation of how transmembrane domains allosterically affect ATPase activity of NBDs. In this case, caution has to be taken too. Nonhydrolyzable ATP analogues and orthovanadate or BeF_3 should be used to carefully examine how well the experimentally observable $O_1 \rightarrow O_2$ transition represents the invisible ATP hydrolysis event.

A critical issue that has not been addressed by Jih et al. (2012) is to what degree the R352C mutation distorts normal molecular behaviors of CFTR. This is a tough problem, but it again reminds us of the benefits of taking complementary scientific approaches. In Csanády et al. (2010), WT-CFTR dwell-time distributions were fit with maximum likelihood (ML) optimization, and kinetic parameters for gating models were obtained. This allows a qualitative comparison of WT and R352C gating behavior. It should be noted that the new tool from Jih et al. (2012) also creates opportunities for the ML method. This statistical method has the exceptional power of discriminating competing models and extracting kinetic parameters, but its usefulness and accuracy decline rapidly as kinetic steps with unknown parameters are increased. Now, the rate constants derived from recording the R352C mutant could provide constraints for the ML method, which can then be applied to examine CFTR kinetic models in an even more satisfactory detail.

Finally, there are two more issues in Jih et al. (2012) that warrant discussion. First, as also mentioned in their article, a preferred order of gating transitions ($C \rightarrow O_1 \rightarrow O_2 \rightarrow C$) was reported more than 10 years ago for WT CFTR channels. In this case, the O_1 and O_2 states are differentiated by their sensitivity to block by MOPS (Gunderson and Kopito, 1995; Ishihara and Welsh, 1997; Hennager et al., 2001). These experiments are technically challenging though, as the difference between the O_1 and O_2 states is less obvious and requires some unusual filtering conditions to appear in the single-channel record. Second, with some maneuvers, including the introduction of mutations into transmembrane domains and MTS reagent modification, Jih et al. (2012) show that CFTR might consume many ATP molecules during an opening burst. In other words, the

stoichiometry of ATPase and gating cycles might be more than 1:1. For many readers, it is all-too-easy to dismiss this finding as physiologically irrelevant, as this phenomenon is rarely seen in WT channels, and as a deviation from 1:1 stoichiometry would certainly translate to a decreased efficiency of energy usage for ABC transporters, which require a full conformational cycle in the transmembrane domains to complete the translocation of a substrate cargo. Nonetheless, from my point of view, we should see these results as a warning that the interactions between NBDs and TMDs in ABC proteins could have more complicated dynamics than previously thought and deserve further careful and open-minded investigations in the future.

REFERENCES

- Aleksandrov, A.A., X. Chang, L. Aleksandrov, and J.R. Riordan. 2000. The non-hydrolytic pathway of cystic fibrosis transmembrane conductance regulator ion channel gating. *J. Physiol.* 528:259–265. <http://dx.doi.org/10.1111/j.1469-7793.2000.00259.x>
- Aleksandrov, A.A., L.A. Aleksandrov, and J.R. Riordan. 2007. CFTR (ABCC7) is a hydrolyzable-ligand-gated channel. *Pflugers Arch.* 453:693–702. <http://dx.doi.org/10.1007/s00424-006-0140-z>
- Colquhoun, D., and A.G. Hawkes. 1982. On the stochastic properties of bursts of single ion channel openings and of clusters of bursts. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 300:1–59. <http://dx.doi.org/10.1098/rstb.1982.0156>
- Csanády, L., P. Vergani, and D.C. Gadsby. 2010. 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.* 107:1241–1246. <http://dx.doi.org/10.1073/pnas.0911061107>
- Gunderson, K.L., and R.R. Kopito. 1995. Conformational states of CFTR associated with channel gating: the role ATP binding and hydrolysis. *Cell.* 82:231–239. [http://dx.doi.org/10.1016/0092-8674\(95\)90310-0](http://dx.doi.org/10.1016/0092-8674(95)90310-0)
- Hennager, D.J., M. Ikuma, T. Hoshi, and M.J. Welsh. 2001. A conditional probability analysis of cystic fibrosis transmembrane conductance regulator gating indicates that ATP has multiple effects during the gating cycle. *Proc. Natl. Acad. Sci. USA.* 98:3594–3599. <http://dx.doi.org/10.1073/pnas.051633298>
- Ishihara, H., and M.J. Welsh. 1997. Block by MOPS reveals a conformation change in the CFTR pore produced by ATP hydrolysis. *Am. J. Physiol.* 273:C1278–C1289.
- Jih, K.-Y., Y. Sohma, and T.-C. Hwang. 2012. Nonintegral stoichiometry in CFTR gating revealed by a pore-lining mutation. *J. Gen. Physiol.* 140:347–359.
- Oldham, M.L., A.L. Davidson, and J. Chen. 2008. Structural insights into ABC transporter mechanism. *Curr. Opin. Struct. Biol.* 18:726–733. <http://dx.doi.org/10.1016/j.sbi.2008.09.007>
- Powe, A.C., Jr., L. Al-Nakkash, M. Li, and T.C. Hwang. 2002. Mutation of Walker-A lysine 464 in cystic fibrosis transmembrane conductance regulator reveals functional interaction between its nucleotide-binding domains. *J. Physiol.* 539:333–346. <http://dx.doi.org/10.1113/jphysiol.2001.013162>
- Rees, D.C., E. Johnson, and O. Lewinson. 2009. ABC transporters: the power to change. *Nat. Rev. Mol. Cell Biol.* 10:218–227. <http://dx.doi.org/10.1038/nrm2646>
- Tanford, C. 1983. Mechanism of free energy coupling in active transport. *Annu. Rev. Biochem.* 52:379–409. <http://dx.doi.org/10.1146/annurev.bi.52.070183.002115>
- Tsai, M.F., H. Shimizu, Y. Sohma, M. Li, and T.C. Hwang. 2009. State-dependent modulation of CFTR gating by pyrophosphate. *J. Gen. Physiol.* 133:405–419. <http://dx.doi.org/10.1085/jgp.200810186>
- Vergani, P., A.C. Nairn, and D.C. Gadsby. 2003. On the mechanism of MgATP-dependent gating of CFTR Cl[−] channels. *J. Gen. Physiol.* 121:17–36. <http://dx.doi.org/10.1085/jgp.20028673>