CFTR gating: Invisible transitions made visible

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By tracking conformational transitions of individual ion channel proteins, single-channel recordings allow the observer to deduce gating mechanisms, as well as estimate rate constants of microscopic gating steps. For most ion channels, gating mechanisms are complex, comprising multiple stable open (O) and closed (C) conformations among which conformational transitions may follow a network of possible kinetic pathways. The ensemble of all states and the connectivities among them are called a gating scheme. The strictest limitation to deciphering complex gating schemes is the fact that only transitions between closed and open states can be detected, whereas transitions among closed states, or among open states, remain invisible in single-channel current traces. It is true that such silent transitions are betrayed by the shapes of the dwell-time distributions, which carry information both on the numbers of closed and open states and on the magnitudes of the rate constants of “invisible” transitions (Colquhoun and Hawkes, 1981). But in practice, extracting such information is not at all straightforward: whereas fitting a mechanism to the data by maximum likelihood is a convenient way for estimating rate constants once the underlying gating scheme is known, deciphering the mechanism itself is a much harder task. Typically, several alternative schemes provide satisfactory fits, and comparison of log-likelihood scores facilitates the choice among models only under specific circumstances (when one model is a subset of the other). Thus, clearly, rendering an invisible transition as directly observable in a single-channel current trace is extremely informative for addressing mechanisms. In the previous issue of The Journal of General Physiology, Zhang and Hwang exploit mutants of CFTR, the chloride channel mutated in cystic fibrosis patients, to confirm the aforementioned hypothesis by demonstrating violation of microscopic reversibility for CFTR gating. In heavily filtered (10 Hz) single-channel recordings of WT CFTR reconstituted in lipid bilayers, the presence of the organic buffer MOPS in the cytosolic compartment caused the appearance of a unique subconductance pattern: channels preferentially opened to a lower conductance level (termed O₁) but briefly

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transitioned to a higher conductance level (termed $O_2$) before closing (Fig. 1A). Furthermore, mutations that disrupt ATP hydrolysis in CFTR and other ABC proteins eliminate this obvious time asymmetry (Gunderson and Kopito, 1995). This conductance pattern, observed at low bandwidth, corresponds to a differential pattern of rapid flickery block by the deprotonated form of the buffer ($\text{MOPS}^-$) and reflects a change in the binding affinity of the blocker at some point within each open-channel event (Ishihara and Welsh, 1997). Based on the observation that the $O_1\rightarrow O_2$ conductance transition is not observed when ATP hydrolysis is prevented, it seemed natural to suggest that the subconductance transition coincides with ATP hydrolysis. Thus, the $O_1$ and $O_2$ conductance states were ascribed to pre- and post-hydrolytic states of the NBDs, respectively (Fig. 1B).

Further support for a nonequilibrium gating cycle was provided by the shapes of CFTR’s dwell-time distributions: both the distribution of closed (interburst) dwell times (when observed at subsaturating ATP concentrations; Zetlwanter et al., 1999) and open (burst) dwell times (Csanády et al., 2010) were found to display peaks. In the latter study, a mutation that disrupts ATP hydrolysis was shown to convert the open-time distribution to a monotonically decaying distribution. These peaked distributions for WT CFTR were again consistent with a cyclic mechanism in which channels normally shuttle through (at least) two sequential closed states (ATP free $\rightarrow$ ATP bound) before opening and then through (at least) two sequential open states (pre-hydrolytic $\rightarrow$ post-hydrolytic; Fig. 1B) before closing.

Much structural and functional work on CFTR and related ABC proteins has outlined many of the mechanistic details of this unique gating cycle. In the presence of ATP, NBDs form head-to-tail dimers with two ATP molecules occluded in interfacing binding sites. Such ATP-bound dimers are extremely stable but dissociate after ATP hydrolysis (Moody et al., 2002). In a subset of asymmetric ABC proteins, including CFTR, only one composite binding site is catalytically active (the “canonical site”), whereas the other “degenerate” site is inactive, so that each cycle of NBD dimerization/disruption includes only one ATP hydrolysis event (Fig. 1B; Basso et al., 2003). This NBD catalytic cycle is coupled to TMD conformational changes. In ABC exporters, tight NBD dimerization flips the TMDs from inward to outward facing, and dimer disruption resets them to inward facing (Hollenstein et al., 2007). On the contrary, dimer formation in CFTR opens the pore and dimer disruption closes it (Vergani et al., 2005). Thus, WT CFTR open (burst) events have been proposed to involve occlusion of a single ATP molecule at the canonical site and to be terminated predominantly by hydrolysis of that ATP (Vergani et al., 2005; Csanády et al., 2010), consistent with the observations of Gunderson and Kopito (1995). However, later work proposed the possibility of more than one ATP occlusion event within a single open burst (Jih et al., 2012b), a hypothesis supported by the appearance of multiple $O_1\rightarrow O_2$ subconductance transitions within a single burst of a CFTR pore mutant (Jih et al., 2012a). Clearly, more work will be needed to fully understand the details of this unique gating cycle, and with such open questions at hand, studying the mechanism underlying the time-asymmetric changes in the conductance properties of CFTR has become increasingly important.

Despite numerous studies that have clarified the molecular mechanism of anion selectivity and conductance properties and outlined the rough structural organization of the open CFTR pore (Lindsell, 2017), no clues have emerged that would explain the $O_1-O_2$ subconductance pattern caused by MOPS$^-$ or similarly by 3-nitrobenzoate (Csanády and Töröcsik, 2014) until recently. The first hint about a region that might be involved in this phenomenon came from work in the Hwang laboratory (Jih et al., 2012a): neutralization of the positive charge of a pore-lining arginine, located in the intracel-

Figure 1. Time-asymmetric conductance pattern of CFTR correlates with its ATP hydrolytic activity. (A) Illustration of the $O_1-O_2$ phenotype. Schematic representation of single-channel current for a typical single open (burst) event, illustrating the two subconductance levels in their preferential order. (B) Cartoon representation of pre- (left) and posthydrolytic (right) open states of a CFTR channel. Arrows between the two states reflect the preferential order in which they are visited in each burst, as a consequence of a nonequilibrium gating cycle. TMDs, yellow; NBD1, red; NBD2, blue; ATP, green; ADP + phosphate, blue oval + red crescent; chloride ions, black spheres; water molecules, blue. The canonical ATP-binding site is depicted as the top site in the NBDs, and the noncanonical site is shown at the bottom. Red stars in the TMDs represent mutations that disturb the local charge distribution. In the posthydrolytic state, a suggested expansion of the internal vestibule results in the accommodation of a larger number of water molecules, which dampen the negative impact of the mutations on chloride throughput rate (stronger double arrows bracketing the chain of chloride ions).
lular vestibule at position 352 of TM helix 6 (TM6),
causd the appearance of the O1-O2 subconductance
pattern even in the absence of MOPS−. Given that
MOPS− is a large organic anion that blocks by binding
to the intracellular vestibule, this finding suggested that
the region in the vicinity of R352 might be involved in
the same open-pore conformational change that also
affects MOPS− binding affinity.

In their new, elegant study, Zhang and Hwang (2017)
provide the first coherent set of information regarding
the structural underpinnings of this interesting phe-
nomenon. They show that the local arrangement of
surface charges in the vicinity of position 352 of the
internal vestibule indeed plays a crucial role in deter-
mining the conductance pattern of a burst. Whereas
the local charge distribution found in WT CFTR en-
sures that the permeation rate for chloride ions re-
mains identical throughout an open burst, specific
perturbations of this “native” charge distribution
(Fig. 1B, red stars) preferentially reduce chloride con-
duction rate in the O1 state, causing the appearance of
an O1-O2 subconductance pattern. The authors iden-
tify several different ways of disturbing the native
charge distribution—neutralization of the natively
positive charge at TM6 position 352 (R352C and
R352Q) or introduction of a negative charge into na-
tively neutral TM5 position 306 (N306D and N306E;
these two positions are located at similar depths in
the internal vestibule, at the spot where the latter tapers
down to a narrow tunnel [Zhang and Chen, 2016]—
all of which lead to the same result. But it is not simply
the magnitude of the total charge that matters because
the authors show that transplanting charges to differ-
ent positions, without affecting total charge, can also
cause the appearance of the O1-O2 phenotype (e.g.,
R352Q/M348R, R303C/S310R, and R303C/F311R).
Clearly, both the magnitude and the precise location
of the charges are important. Furthermore, it is re-
warding to see that the effects on conductance pattern
of the aforementioned mutations and of MOPS− are
not independent of each other. Whereas some of the
mutations further amplify the inherent difference in
MOPS− affinities of the O1 and O2 states (F311C), oth-
ers abolish this difference, eliminating the ability of
MOPS− to elicit the O1-O2 phenotype (e.g., R303C).

What is the mechanism behind the differential
chloride conduction rates of the O1 and O2 states
in the mutants? One effect that a positive surface
charge in the vestibule of an anion channel may
exert is to increase the local concentration of chlo-
ride ions, and the net positive surface charge in CF-
TR’s internal vestibule clearly does play such a role.
CFTR chloride conductance is a hyperbolic func-
tion of chloride concentration, and in WT channels,
conductance is half-maximal at ~50 mM chloride
(Lindsell et al., 1997). In contrast, for the N306D
mutant (in which net positive surface charge is re-
duced), conductance is shown to be smaller than
that of WT in low (~50 mM) but to approach that
of WT at very large (~1 M) chloride concentrations.
The conductance-concentration curve of the mutant,
consistent with a Kd of ~300 mM, indeed reports a
reduced apparent affinity of the pore for permeating
chloride, but this is shown to apply identically to the
O1 and the O2 state. Moreover, whereas a change in
local chloride concentration in the internal vestibule
would be expected to preferentially affect inward
currents at negative potentials (reflecting outward
chloride ion flow), the authors show that the O1-O2
conductance pattern of the N306D mutant is voltage
independent and can be observed even for outward
currents recorded at positive membrane potentials.
It thus seems unlikely that the increase in chloride
conductance between states O1 and O2 of this (and
other) mutants would reflect an increase in the
local chloride concentration in the internal vesti-
bule. Instead, the O1→O2 conformational change
of the mutated vestibules must affect the maximal
chloride throughput rate, which depends on the
rates of dehydration/binding and unbinding of per-
meating anions in the pore. The authors suggest an
expansion of the internal vestibule, which accompa-
nies the O1→O2 transition, as a possible explanation
(Fig. 1B): mutation-induced changes in the electro-
static potential that a permeating chloride ion expe-
riences in the O1 state might be dampened in the O2
state by a larger number of water molecules in the
vestibule (Fig. 1B). This could explain why muta-
tions preferentially reduce conductance in the O1
state relative to the O2 state, causing the appearance
of the O1-O2 conductance pattern.

Beyond the novel insights provided, a further merit
of this study by Zhang and Hwang (2017) is that it has
generated a valuable toolbox in the form of mutants
that allow visualization of the O1→O2 transition. These
tools open up new pathways for further studies aimed
at a better understanding of both the process of chlo-
ride ion permeation through the open pore and of the
ATP-dependent gating cycle. Along that first line,
it will be important to establish whether and how the
anion permeability sequence changes between the O1
and O2 states to decipher how the O1→O2 transition
affects the energetics of anion–protein interactions.
Along the second line, it will be important to under-
stand exactly what molecular rearrangements take
place at the NBDs concurrent with the TMD rearran-
gements that cause the (now observable!) O1→O2
transition. Last but not least, all of these future studies
will benefit tremendously from high-resolution struc-
tures of CFTR, an abundant source of information
which has finally become more than just a dream
(Zhang and Chen, 2016).
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