# Generally Physiological

## Of lipid transfer, resistance to $F^-$, and stimulus-dependent channel conformations

This month's installment of *Generally Physiological* considers the nonvesicular transfer of lipids between membranes, channels and transporters that enable microorganisms to resist the toxic effects of $F^-$, and alternative, stimulus-dependent open channel conformations of a pannexin channel.

### Transferring lipids to target membranes

The endoplasmic reticulum (ER) comes into close proximity with the plasma membrane and the membranes of other organelles, forming contact sites that enable signaling between cellular compartments and the nonvesicular exchange of lipids between the apposed membranes. Proteins in the extended synaptotagmin (E-SYT) family, which act as tethers between the ER and the plasma membrane, contain an N-terminal ER membrane anchor domain, C2 membrane-targeting domains, and a region identified by bioinformatics analyses as a potential lipid-binding module (the SMP domain). Schauder et al. (2014) determined the 2.44-Å-resolution crystal structure of a fragment of human E-SYT2 (residues 163–634) that included the SMP domain and two of its three C2 domains (C2A and C2B) and found that the SMP domain dimerized to form a cylinder. A 10-Å-wide channel lined with hydrophobic residues spanned the 90-Å-long cylinder, connecting to solvent at both ends and through a longitudinal seam. A combination of electron density mapping and mass spectrometric analysis indicated that E-SYT2 binds lipids; each monomer can bind two lipid molecules, with the fatty acid moiety lying in the hydrophobic SMP channel and the polar head group protruding through the seam. The authors thus conclude that E-SYTs, and perhaps other SMP domain–containing proteins present at ER membrane contact sites, play a role in lipid transport, proposing "bridge" or "shuttle" models, whereby SMP dimers, perhaps in conjunction with other lipid transfer proteins, could transfer lipids from sites of synthesis in the ER to target membranes.

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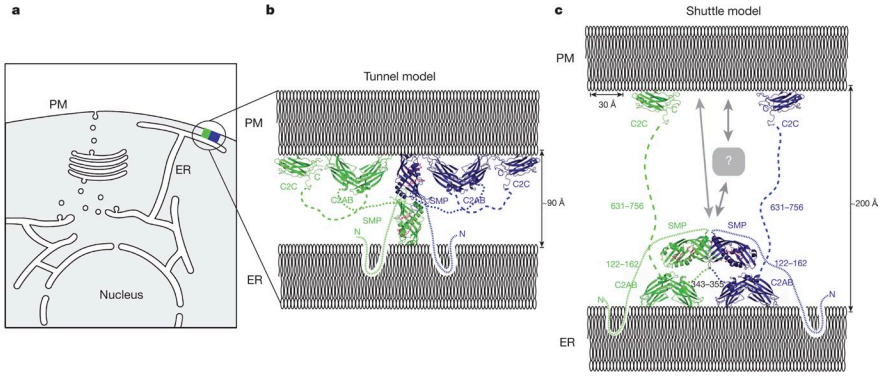
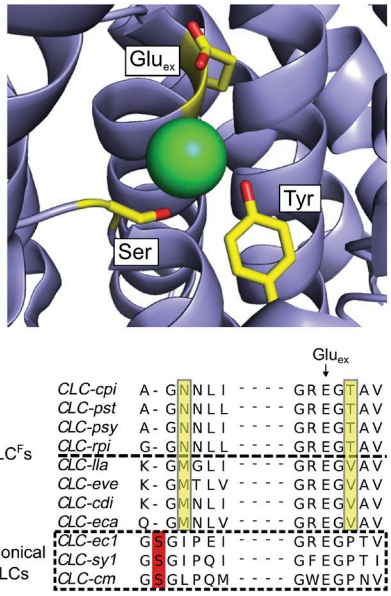
### Fighting against fluoride

Fluoride ($F^-$) is ubiquitous in soil and in water, posing an existential threat to unicellular microorganisms through its inhibition of crucial enzymes. Two distinct families of $F^-$ exporters that help combat $Fl^-$ toxicity have recently been identified, the bacterial $CLC^F$-type $F^-/H^-$ antiporters (a subset of the CLC superfamily of anion transport proteins) and the Fluc family of $F^-$ channels. Noting that $Cl^-$ is far more abundant in the environment than $F^-$, Brammer et al. (2014) investigated the basis of $CLC^F$ selectivity for $F^-$ over $Cl^-$. Sequence analysis showed that the $CLC^F$s lacked a serine implicated in coordinating $Cl^-$ in canonical CLCs; moreover, two phylogenetically distinct $CLC^F$ subclades showed distinct amino acid signatures, with one subclade




**(a) E-SYT2 at a contact site between the ER and the plasma membrane. The C2C domain binds to the plasma membrane, and the N-terminal domain region provides an anchor to the ER. (b) If the two membranes are ~90 Å apart, SMP dimers may provide a lipid transfer tunnel. (c) If the two membranes are closer than 200 Å, the SMP dimer may act as a shuttle. (Reprinted by permission from Macmillan Publishers, Ltd. C.M. Schauder. 2014. *Nature*. http://dx.doi.org/10.1038/nature13269, copyright 2014.)**




**(top) Conserved Glu, Ser, and Tyr residues implicated in coordinating $Cl^-$ in canonical CLCs. (bottom) Sequence alignment of $CLC^F$ homologues, highlighting residues that differ between the two subclades (yellow). Sequences in same region of canonical CLCs are shown below, with the $Cl^-$-coordinating Ser residue highlighted in red. (From Brammer et al., 2014.)**

characterized by an asparagine and a tyrosine (N-T subclade) near the ion-binding region and the other bearing a methionine and a valine (M-V subclade) in the same positions. Intriguingly, the M-V subclade showed increased $F^-$ selectivity compared with the N-T subclade with regard to both transport kinetics and equilibrium binding, and residue-swapping experiments confirmed the importance of these key amino acids to the differences between the two groups. In a second paper from the same research group in this issue, Ji et al. show that, in acidic environments, *E. coli* lacking Fluc channels accumulate $F^-$ through ion trapping. The membrane-permeant HF, a weak acid, crosses the membrane to deliver $F^-$ to the cytoplasm, and the $F^-$-permeable Fluc channels protect against such accumulation by enabling the escape of $F^-$ from the bacterium—with its highly negative membrane potential—down its own electrochemical gradient.

### Promoting distinct Panx conformations?

Although various lines of evidence indicate that Pannexin1 (Panx1) forms a large-conductance channel (450 pS) that mediates ATP release, this view has been challenged by research indicating that it forms a low-conductance (~70 pS), anion-selective channel. Noting that these different channel properties were observed under distinct experimental conditions, Wang et al. (2014) explored the hypothesis that they depended on stimulus-specific open channel states. Whereas exposing *Xenopus* oocytes that heterologously expressed Panx1 to high $[K^+]_o$ stimulated ATP release regardless of membrane potential, depolarization in the absence of high $[K^+]_o$ failed to do so. Furthermore, patch clamp analysis of Panx1-expressing oocytes indicated that single-channel properties differed under different experimental conditions: a channel with multiple subconductance states and a maximal single-channel conductance of ~520 pS was apparent at −100 mV in 150 mM $[K^+]_o$, whereas in 1 mM $K^+$, a channel with a unitary conductance of ~44 pS (and no subconductance states) was observed only at positive potentials. A C-terminal cysteine reactive to thiol reagents when the channel opens in response to depolarization was apparently inaccessible in the $K^+$-gated configuration, and electron microscopic analysis indicated that $K^+$ promoted the formation of channel with a larger pore than was otherwise apparent. The authors thus conclude that the Panx1 channel may assume different conformations that are associated with distinct biophysical properties in response to different forms of stimulation.

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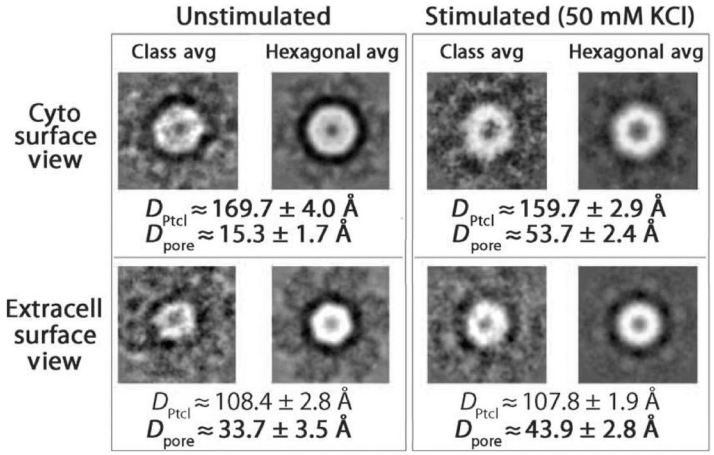


**Electron microscopic analysis reveals an increase in the pore diameter of Panx1 oligomers exposed to 50 mM KCl. (From Wang et al. 2014. *Sci. Signal.* http://dx.doi.org/10.1126/scisignal.2005431. Reprinted with permission from AAAS.)**