

# Generally Physiological

## Of fish oil, rafts, and scrambling things up



This month's installment of *Generally Physiological* explores various interactions between lipids and calcium- and voltage-gated membrane proteins.

### Greasing BK activation?

Long-chain polyunsaturated omega-3 fatty acids, which are abundant in oily fish, have been reported to have various health benefits, including lowering blood pressure in individuals with hypertension. Hoshi et al. (2013) recently showed that the omega-3 fatty acid docosahexaenoic acid (DHA) activates the Slo1 large-conductance  $\text{Ca}^{2+}$ - and voltage-gated  $\text{K}^+$  (BK) channel, which is present in vascular smooth muscle cells and plays a role in regulating vascular tone. Moreover, they found that DHA injection lowers blood pressure in wild-type mice but not in mice lacking the pore-forming Slo1  $\alpha$  subunit. Slo1 coassembles with various auxiliary subunits (see Latorre and Contreras, 2013), and coexpression with the  $\beta 1$  (as in vascular BK complexes) or  $\beta 4$  (as in neuronal BK complexes)

subunits markedly enhances its activation by DHA.

An essential fatty acid in humans, DHA is not normally found in the fruit fly *Drosophila*; here, Hoshi et al. (2013) compared its effects on human and *Drosophila* Slo1 (hSlo1 and dSlo1, respectively) in the absence of auxiliary subunits to explore the mechanism whereby DHA activates Slo1. Whereas DHA clearly enhanced hSlo1 currents, it had little

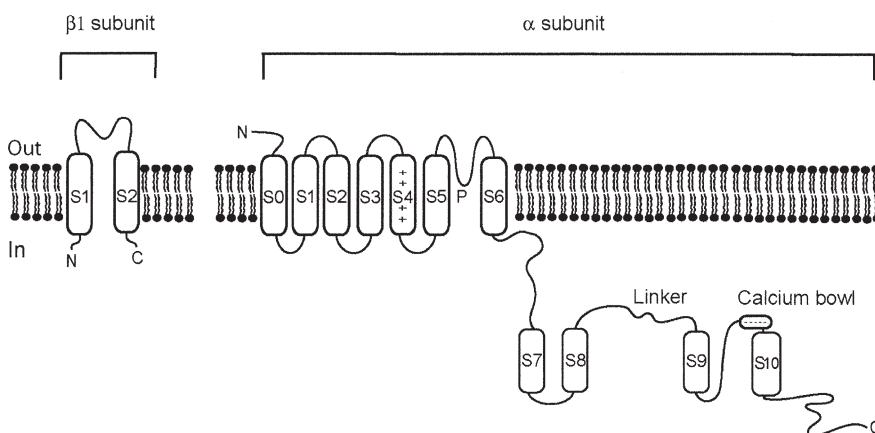
### Interactions between lipids and calcium- and voltage-gated membrane proteins

effect on dSlo1 currents, and analyses of chimeric hSlo1/dSlo1 constructs implicated the pore domain (S5-P-S6 segment) in hSlo1 sensitivity to DHA. Introduction of human-to-*Drosophila* point mutations into the pore domain revealed that replacing hSlo1 tyrosine residue 318 (Y318) in S6 with serine (as in the corresponding dSlo1 residue) abolished hSlo1 sensitivity to DHA but

did not affect the response to several other fatty acids. Y318 thus appears to be a crucial determinant of hSlo1 sensitivity to DHA, and may provide a useful tool for analyzing fatty acid modulation of Slo1 BK activity.

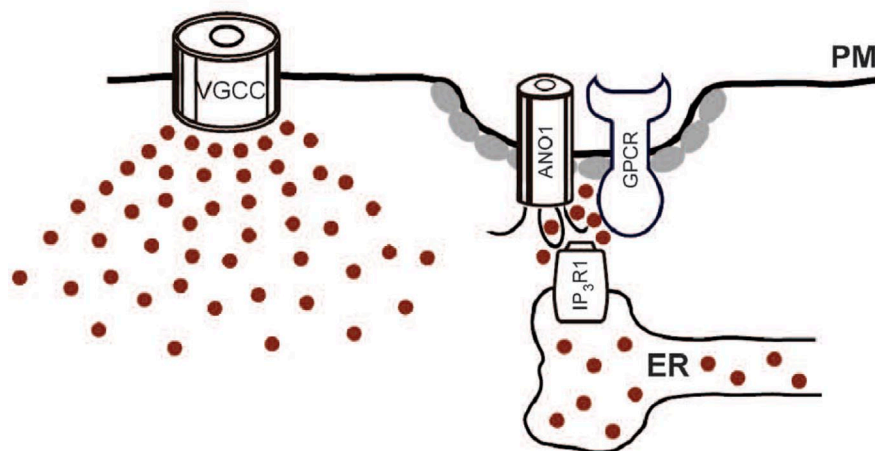
### Defining a TMEM16A signaling domain

Like Slo1 BK, the  $\text{Ca}^{2+}$ -activated  $\text{Cl}^-$  channel (CaCC) TMEM16A (also known as ANO1) is gated by both intracellular  $\text{Ca}^{2+}$  signals and depolarization. CaCC currents in nociceptive dorsal root ganglion (DRG) neurons are only rarely activated by calcium entering through voltage-gated calcium channels (VGCCs) but respond to calcium released from inositol 1,4,5-trisphosphate ( $\text{IP}_3$ )-sensitive stores in the endoplasmic reticulum (ER), providing a depolarizing signal to trigger action potentials after exposure to the inflammatory mediator bradykinin (BK). Using a combination of patch-clamp analysis and fluorescence imaging, Jin et al. (2013) confirmed that TMEM16A in small-diameter TRPV1-positive rat DRG neurons was preferentially activated by BK- or protease-activated receptor 2 (PAR-2)-mediated  $\text{Ca}^{2+}$  signals compared with those from VGCCs. Experiments using intracellular calcium chelators with different binding kinetics indicated that TMEM16A was close to ER  $\text{Ca}^{2+}$ -release sites, and fluorescence proximity ligation assay (PLA) showed that TMEM16A was in close proximity to the type 1  $\text{IP}_3$  receptor ( $\text{IP}_3\text{R1}$ ) but not VGCCs. Coimmunoprecipitation analysis suggested that TMEM16A associated with  $\text{IP}_3\text{R1}$ , PAR-2, and the  $\text{B}_2$  BK receptor ( $\text{B}_2\text{R}$ ); PAR-2 and  $\text{B}_2\text{R}$  also



**BK channels have auxiliary subunits, such as the  $\beta 1$  subunit found in vascular BK complexes, in addition to the pore-forming Slo 1  $\alpha$  subunit (from Qian et al., 2002).**

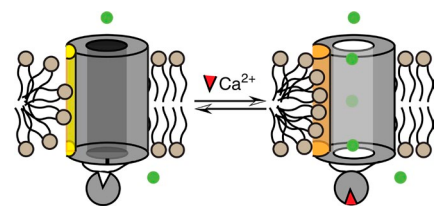
coimmunoprecipitated with the lipid raft marker caveolin-1, and density gradient fractionation substantiated the notion that TMEM16A, PAR-2, and B<sub>2</sub>R localize to lipid rafts. Cholesterol depletion, which disrupts lipid rafts, decreased IP<sub>3</sub>R1 coimmunoprecipitation with TMEM16A, IP<sub>3</sub>R1-TMEM16A association detected by PLA, and TMEM16 activation by B<sub>2</sub>R or PAR-2, whereas it increased coupling of TMEM16A to VGCCs. Thus, localization of TMEM16A and B<sub>2</sub>R or PAR-2 (or both) to a cholesterol- and caveolin-1-enriched membrane microdomain near a region of the ER containing IP<sub>3</sub>R1 may enable its selective activation by local inflammatory calcium signals rather than global calcium influx through VGCCs.



**Localization of TMEM16A and a GPCR in a membrane microdomain near IP<sub>3</sub>R1 on the ER enables the selective activation of TMEM16A by inflammatory signals. (From Jin et al. 2013. *Sci. Signal.* 6:ra73. Reprinted with permission from AAAS.)**

**A dual-function channel–scramblase**  
Whereas TMEM16A appears to act as a bona fide CaCC, the function(s) of other members of the TMEM16 family remains unclear. Intriguingly, some members of the TMEM16 family have been implicated in Ca<sup>2+</sup>-dependent scrambling, a process in which phospholipids are bidirectionally transported from one leaflet

of the plasma membrane to the other so that phospholipids typically found in the inner leaflet are exposed on the cell surface (and vice versa). Whether these proteins act as scramblases themselves or as channels that regulate scramblase activity, however, has been controversial. Malvezzi et al. (2013) have now identified a member of the TMEM16 family that, remarkably, appears to act as both a channel and a scramblase. The authors cloned a TMEM16 homologue found in *Aspergillus fumigatus* (afTMEM16) and reconstituted the purified protein in liposomes, where it formed poorly selective channels gated by both voltage and calcium. Experiments in which liposome phospholipids were tagged with



**Calcium (red triangle) binds to a single site on afTMEM16 to enable ion permeation and lipid scrambling through distinct pathways. (Reprinted by permission from Macmillan Publishers, Ltd. *Nature*. M. Malvezzi, M. Chalal, R. Janjusevic, A. Picollo, H. Terashima, A.K. Menon, A. Accardi. 2013. *Nat. Commun.* 4:2367, copyright 2013.)**

fluorophore that could be selectively quenched on the outer leaflet by exposure to the membrane-impermeant dithionite revealed that afTMEM16 also acted as a Ca<sup>2+</sup>-dependent scramblase, whereas two other TMEM16F homologues failed to mediate scrambling. afTMEM16's channel and scramblase activities showed similar selectivity for divalent

cations, and a mutant that abrogated the Ca<sup>2+</sup> sensitivity of scramblase activity (D511A/E514A) also abolished channel activation by Ca<sup>2+</sup>, suggesting that the same Ca<sup>2+</sup>-binding site mediates regulation of both functions. Whereas the lipid environment markedly influenced afTMEM16's channel activity, it had little effect on lipid scrambling; moreover, scramblase activity was present when ion movement was blocked by an impermeant ion. Thus, the authors conclude that afTMEM16 acts as a dual-function ion channel and scramblase, in which a single Ca<sup>2+</sup>-binding site regulates ion and lipid movement through two distinct transmembrane pathways.

Elizabeth M. Adler  
Executive Editor, *JGP*  
eadler@rockefeller.edu

## REFERENCES

- Hoshi, T., et al. 2013. *J. Gen. Physiol.* 142:507–522. <http://dx.doi.org/10.1085/jgp.201311061>
- Jin, X., et al. 2013. *Sci. Signal.* 6:ra73. <http://dx.doi.org/10.1126/scisignal.2004184>
- Latorre, R., and G. Contreras. 2013. *J. Gen. Physiol.* 142:487–491. <http://dx.doi.org/10.1085/jgp.201311100>
- Malvezzi, M., et al. 2013. *Nat Commun.* 4:2367. <http://dx.doi.org/10.1038/ncomms3367>
- Qian, X., et al. 2002. *J. Gen. Physiol.* 120:829–843. <http://dx.doi.org/10.1085/jgp.20028692>