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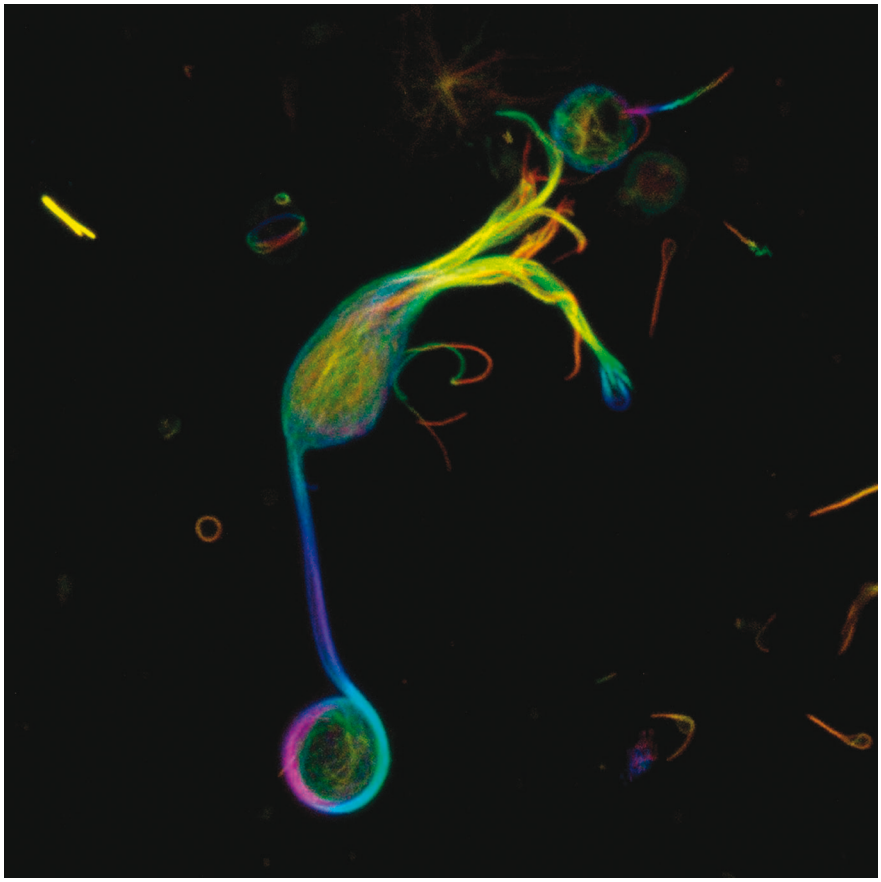
Generally Physiological

Of bipolar cell synapses, light-activated K^+ channels, and substrate binding to DAT

This month's installment of *Generally Physiological* considers a previously undescribed structure found in the synaptic terminals of retinal bipolar cells, a photoactivatable K^+ channel, how the dopamine transporter distinguishes substrates from inhibitors, and recent *JGP* Video Summaries.

Maintaining the mitochondrial supply

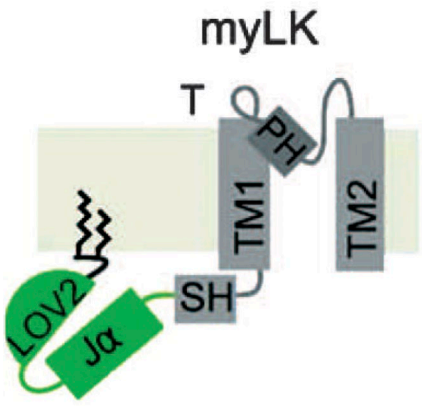
The bipolar cells of the retina are small interneurons that transmit information from the photoreceptors to the retinal ganglion cells, which convey information to the brain. Like various other cells involved in the early stages of sensory processing, bipolar cells are tonically active and are characterized by synaptic ribbons, specialized structures that enable the sustained release of large numbers of synaptic vesicles; however, the detailed spatial organization of bipolar synaptic terminals has been poorly understood. In this issue, Graffe et al. used a combination of three-dimensional fluorescence microscopy and three-dimensional electron microscopy to visualize the subcellular architecture of the giant presynaptic terminals of goldfish retinal bipolar cells and, unexpectedly, discovered a thick band of microtubules that extended from the axon into the synaptic terminal and then circled its periphery. The microtubule structure, which was subject to posttranslational modification, did not appear to associate with synaptic ribbons; it did, however, appear to localize with mitochondria. Moreover, pharmacological inhibition of the microtubule-associated motor protein kinesin led to the formation of mitochondria-filled varicosities in bipolar cell axons. The authors thus propose that this previously unidentified microtubule structure provides a mechanism for the transport of mitochondria crucial to maintain energy supplies into these large and highly active bipolar cell terminals.



Goldfish retinal bipolar cell stained with the fluorogenic microtubule probe SiR-tubulin. See Graffe et al. (2015).

Building a light-activated K^+ channel

Potassium (K^+) channels are modular, such that the sensor domains that enable their response to physiological stimuli are distinct from the pore domain through which ions traverse the membrane. Cosentino et al. (2015) exploited this modular architecture, fusing the plant LOV2 (light, oxygen, or voltage 2)-Jα photosensory module to the viral Kcv channel (as a pore domain) and tinkering with the constructs thereby obtained to create a K^+ channel reversibly activated by blue light. When expressed in HEK293T cells, activation of blue light–induced K^+ channel 1 (BLINK1), which had a unitary conductance of ∼70 pS (consistent with the high conductance of Kcv) and failed to show activity in darkness, moved the reversal potential with E_K. BLINK1 could be repetitively activated and did not appear to undergo inactivation in light. Moreover, when

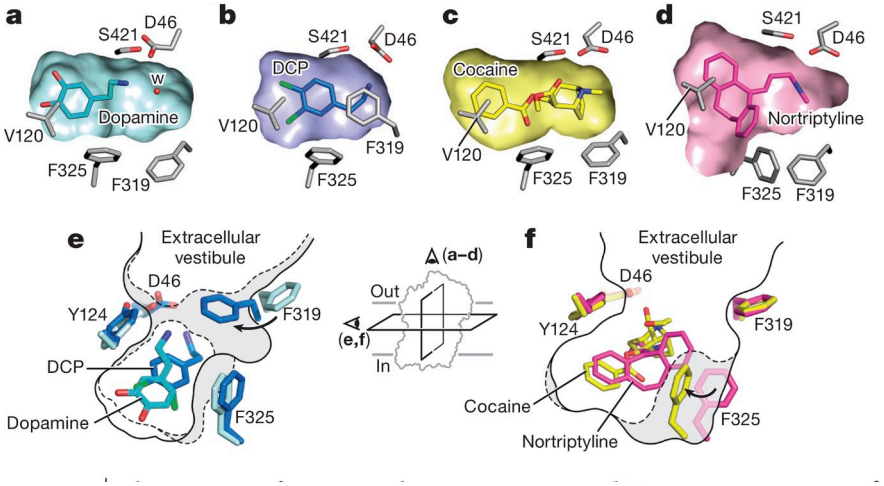


A prototype light-gated K^+ channel (myLK, shown as a monomer in the membrane) created by fusing LOV2-Jα to Kcv (comprising slide helix [SH], transmembrane domains 1 and 2 [TM1, TM2], pore-helix [PH] and turret [T]) and adding an N-terminal myristoylation/palmitoylation sequence. (From Cosentino et al. 2015. *Science*. http://dx.doi.org/10.1126/science.aaa2787. Reprinted with permission from AAAS.)

expressed in zebrafish embryos, BLINK1 photoactivation reversibly suppressed the escape response. The authors thus conclude that BLINK1 represents a promising optogenetic tool for mediating prolonged cellular hyperpolarization.

DAT plastic pocket

The dopamine transporter (DAT), a Na^+/Cl^--coupled symporter, clears synaptically released dopamine to terminate its activity at its receptors. Various pharmacologically active agents, including amphetamines, cocaine, and some antidepressants, act as DAT substrates or nontransported inhibitors to modulate mood and behavior. All of these ligands (including dopamine) are thought to interact with the DAT central binding site; however, distinctions in the binding of substrates or inhibitors have been unclear. Wang et al. (2015) have now determined the x-ray crystal structure of a *Drosophila* DAT construct that retains transport activity, the dDAT minimal functional construct ($dDAT_{mfc}$) bound to a series of small molecule ligands that included both DAT substrates and inhibitors. They determined that flexibility of the ligand-binding pocket enabled its interaction with ligands of various sizes and shapes, with substrates such as dopamine contracting the binding pocket, to drive $dDAT_{mfc}$ into the occluded state, and inhibitors acting as wedges to lock it into an outward-open conformation.



Schematic representing plasticity of the $dDAT_{mfc}$ ligand-binding pocket bound to the substrates dopamine or 4-dichlorophenethylamine (DCP) (e) or the inhibitors cocaine or nortriptyline (f). (Reprinted by permission from Macmillan Publishers, Ltd. K.H. Wang et al. *Nature*. http://dx.doi.org/10.1038/nature14431, copyright 2015.)

New video summaries

People who enjoy reading the Journal online may be familiar with the Video Summaries that occasionally accompany *JGP* articles. I'm delighted to draw your attention to our three most recent video summaries, published in association with articles by Berthier et al. (2015), Harraz et al. (2015), and Petrovič et al. (2015), and to invite you to view the videos, all of which concern aspects of muscle function, and interested authors to submit their own Video Summaries.

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