Generally Physiological

Of receptors, channels, and watching the red cell center lose hold

This month’s installment of Generally Physiological considers interactions of membrane proteins with their microenvironments, implication of an anion channel in thermosensation, and the process whereby maturing erythroblasts enucleate.

Mechanisms of GPCR modulation
Ligand binding to members of the G protein–coupled receptor (GPCR) family triggers conformational changes that enable these seven-transmembrane proteins to initiate downstream signaling pathways that mediate cellular responses to numerous stimuli. GPCR function is influenced by ions and by the lipid microenvironment; however, the mechanisms that underlie such modulatory effects have been unclear. Liu et al. (2012) obtained a 1.8-Å structure of a stabilized chimeric form of the human A2A adenosine receptor in complex with a high affinity antagonist, a high resolution structure that enabled the visualization of protein interactions with such potential modulators. The authors identified a network of 57 interior waters comprising three main clusters (an extracellular cluster, central cluster, and intracellular cluster) that formed a nearly continuous channel from the ligand-binding site to the G protein interaction site. The structure was indicative of the presence in the central water cluster of a sodium ion bound to a highly conserved aspartate residue, providing a structural basis for the allosteric effects of sodium on ligand binding. Moreover, GPCR interactions with cholesterol were apparent, consistent with a role for this component of the membrane bilayer in GPCR stabilization, as well as with ordered lipids.

Identifying PI(4,5)P2 sensitivity
GPCRs are, of course, not the only membrane proteins affected by the local microenvironment, nor is cholesterol the only component of the bilayer to influence membrane protein function. For instance, phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2), a low abundance phospholipid located in the cytoplasmic leaflet of the plasma membrane, has been implicated in modulating the activity of several membrane proteins, including various ion channels. Perhaps best known as a precursor for second messengers generated through its cleavage by phospholipase C (PLC), PI(4,5)P2 can also bind to channels directly and thereby modulate their gating. Kruse et al. (2012) coexpressed a series of potassium channels with a set of protein tools (a GPCR that mediates PLC activation, a voltage-sensitive phosphatase, and a fusion protein with lipid 4-phosphatase and 5-phosphatase activity) that could be stimulated to deplete PI(4,5)P2 to investigate channel regulation by PI(4,5)P2 (Hilgemann 2012). Although PI(4,5)P2 depletion decreased currents conducted by Kv2.1 channels, and by members of the Kv7 family of voltage-gated potassium (Kv) channels, it unexpectedly failed to affect the activity of several other Kv channels. Notably, these PI(4,5)P2-insensitive Kv channels included some previously thought to be modulated by PI(4,5)P2 (on the basis of analyses of excised patches), highlighting the crucial importance of preserving the cellular environment.
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in studies aimed at defining the physiological role of PI(4,5)P2 in regulating the activity of membrane proteins.

Heating things up with ANO1

Temperature-sensitive ion channels in peripheral sensory neurons in the trigeminal and dorsal root ganglia (DRG) respond to thermal stimuli, transducing changes in temperature into neuronal excitation. Members of the transient receptor potential (TRP) family, a family of cation channels that also mediates various other sensory modalities, activate across a range of temperatures, playing a prominent role in thermosensation (see Bandell and Patapoutian, 2012). TRPV1, which is found in small DRG neurons (consistent with a role in nociception), where it colocalized with nociceptor markers, including TRPV1; heat induced chloride currents in DRG neurons and, at physiological concentrations of chloride, stimulated DRG depolarization. ANO1 knockdown or knockout decreased heat-evoked chloride currents in isolated DRG neurons and behavioral responses to thermal pain in mice. The authors thus conclude that ANO1, like TRPV1, acts as a sensor for noxious heat.

Red cells caught in the act of enucleation

Although mammalian erythroblasts have long been known to undergo enucleation during the process of red cell maturation, the underlying mechanisms—and the roles of vesicle trafficking and different elements of the cytoskeleton—have been controversial, in part because of the rapidity with which enucleation takes place (see Palis, 2012). Konstantinidis et al. (2012) used imaging flow cytometry in combination with genetic and pharmacological manipulation to identify and investigate mouse erythroblasts caught in the process of undergoing enucleation. The authors developed a multistep model for enucleation in which microtubules participate in a preliminary process of erythroblast polarization, in which the nucleus moves off-center, followed by the Rac GTPase-dependent formation of a contractile actin/myosin ring, associated with lipid rafts, between the nascent reticulocyte and its soon-to-be-discarded nucleus.