Generally Physiological

Of GIRK channels, vitamin E transfer, and a vertebrate fluorescent protein

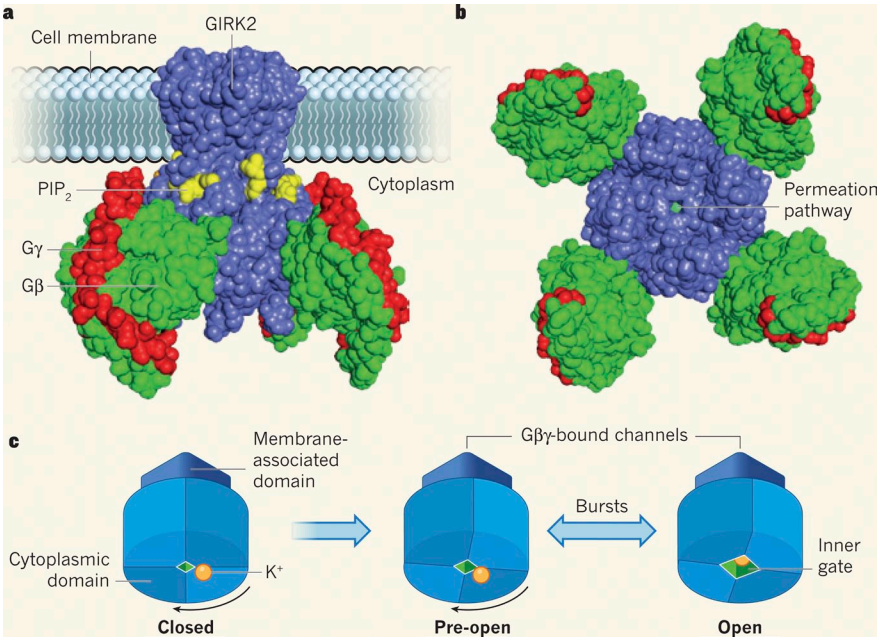
This month's installment of *Generally Physiological* concerns G protein–gated inward rectifier K^+ (GIRK) channel activation and role in the regulation of heart rate, intermembrane transfer of vitamin E, and identification of a ligand-inducible vertebrate fluorescent protein.

A new twist in GIRK activation

Neurotransmitters that signal through G protein–coupled receptors (GPCRs) can activate GIRK channels, eliciting K^+ efflux and consequently decreasing the excitability of the target cell. Ligand occupation of the GPCR promotes dissociation of the G protein into its Gα and Gβγ subunits; Gβγ is thereby freed to bind to and activate GIRK channels or other effectors (see Reuveny, 2013). However, the molecular details of GIRK channel activation have been unclear. Whorton and MacKinnon (2013) obtained the 3.5-Å resolution crystal structure of a homomeric GIRK channel (residues 52–380 of mouse GIRK2 [Kir3.2, found in neurons]) in a complex with Gβγ. They determined that the GIRK channel–Gβγ complex consisted of a GIRK2 tetramer, with one Gβγ dimer, as well as one molecule of the membrane phospholipid PI(4,5)P_2 (phosphatidyl-inositol-4,5-bisphosphate) and one Na^+ (both of which play a role in GIRK activation) bound per GIRK monomer (for a total of four Gβγ dimers, four PI(4,5)P_2 molecules, and four Na^+ ions per complex). A comparison of the structures of a GIRK channel bound to PIP_2, but not Gβγ, the GIRK channel–Gβγ complex, and a constitutively open GIRK mutant indicated that Gβγ, which bound at the interface between GIRK subunits, induced an ∼4° rotation of the cytoplasmic domains relative to the transmembrane domains along with a partial opening of the channel's inner helical gate. The authors thus propose that, consistent with the characteristic bursting kinetics observed with activation of single GIRK channels, Gβγ binding induces a "pre-open" conformation—intermediate between those of the closed and open channels—from which the channel can rapidly fluctuate into the fully open and conducting conformation.

Probing cardiac GIRK function

GIRK channels have long been known to contribute to parasympathetic regulation of cardiac function, in which acetylcholine released from the vagus binds to muscarinic receptors to activate cardiac G protein–activated, inwardly rectifying K^+ current (I_{KACh}) in the sino-atrial node (SAN; the dominant cardiac pacemaker region) and reduce the heart rate. However, muscarinic signaling influences multiple ion channels implicated in cardiac function, and the precise role of I_{KACh} remains incompletely understood. I_{KACh} is mediated by a GIRK1-GIRK4 heterotetramer, leading Mesirca et al. (2013) to use mice lacking GIRK4 (*Girk4*$^{-/-}$ mice), which is required for I_{KACh}, to investigate the functional consequences of I_{KACh} loss. GIRK4 immunoreactivity, which was present in the SANs and right atria of hearts from wild-type mice, was missing from those of the *Girk4*$^{-/-}$ mice, as was *Girk4* mRNA; moreover, as described previously, GIRK1 failed to localize to the membrane in *Girk4*$^{-/-}$ mice. Patch-clamp analysis revealed that I_{KACh} was absent from SAN cells of *Girk4*$^{-/-}$ mice, whereas I_f (mediated by HCN channels) and



GIRK2 activation by Gβγ. Side (A) and bottom (B) views of the GIRK2-Gβγ-PI(4,5)P_2. (C) Model of GIRK rearrangement from the closed to the preopen and open states. (Reprinted by permission from Macmillan Publishers, Ltd. *Nature*. Reuveny, 498:182–183, copyright 2013.)

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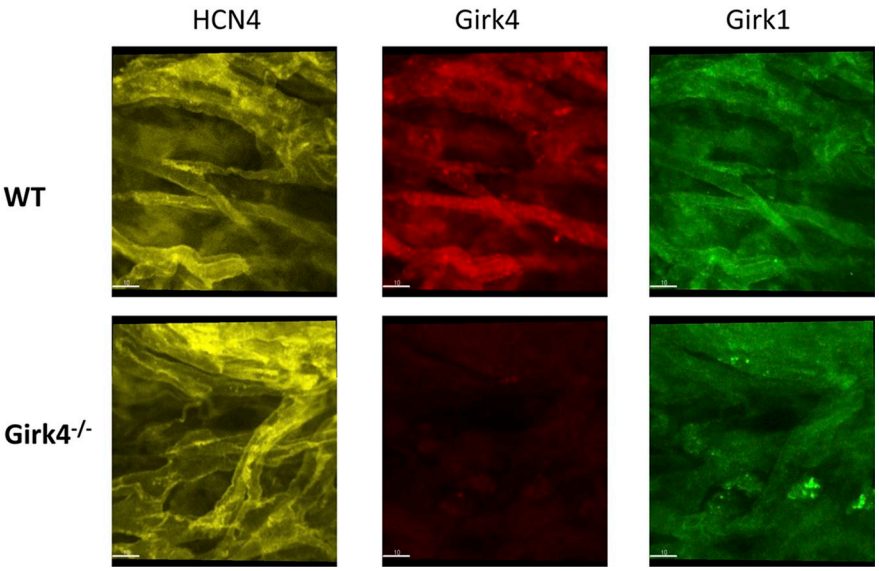


GIRK4 immunoreactivity colocalizes with immunoreactivity for HCN4 (as a marker for pacemaker cells) and GIRK1 in cells from the SAN of wild-type mice; GIRK4 immunoreactivity is lost and GIRK1 fails to localize at the membrane in cells from *Girk4*$^{-/-}$ mice. (From Mesirca et al., 2013.)

$I_{Ca,L}$ (mediated by L-type calcium channels) were unaffected. Acetylcholine elicited a dose-dependent decrease in the spontaneous firing rate of isolated SAN cells and heart rate in isolated perfused hearts, both of which were markedly attenuated in the absence of I_{KACh}. *Girk4*$^{-/-}$ mice showed a moderate increase in heart rate compared with wild-type mice and a marked decrease in the rate of return to basal heart rate after stress (forced swim), exercise (running on a treadmill), or administration of a β-adrenergic agonist (to simulate sympathetic activation). The authors thus conclude that I_{KACh} plays a critical role in both parasympathetic regulation of SAN pacemaker activity and the return to resting heart rate after activation of the sympathetic fight-or-flight response.

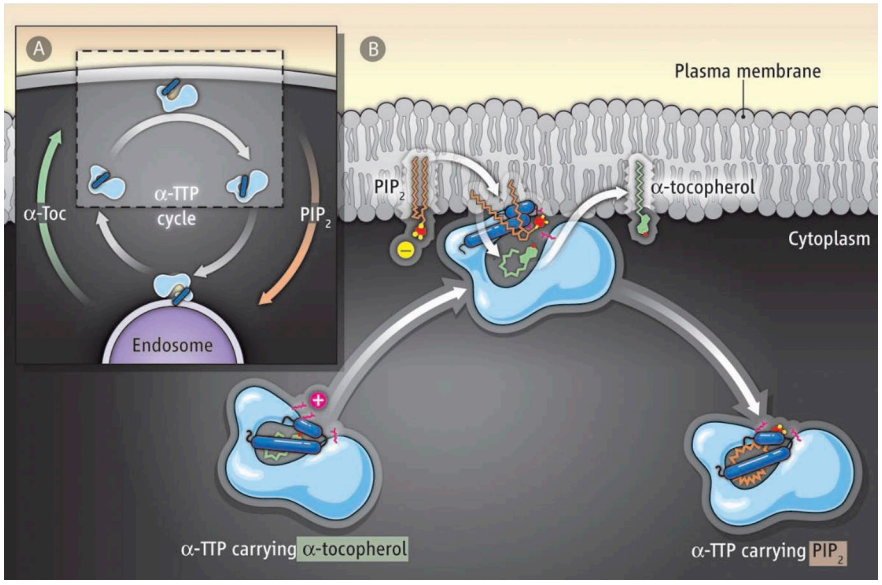
Exchanging PIPs for vitamin E

After its absorption in the small intestine, α-tocopherol (the most abundant form in mammals of the lipid-soluble antioxidant vitamin E) is transported to the liver, from which its release into the plasma is regulated by α-tocopherol transfer protein (α-TTP). Mutations in α-TTP, which transports α-tocopherol from endosomes to the plasma membrane, are associated with the neurodegenerative disorder ataxia with vitamin E deficiency (see Mesmin and Antonny, 2013). Noting that three of nine disease-associated α-TTP missense mutations (R59W, R192H, and R221W) involve arginines in a positively charged cleft on the α-TTP surface (a region distinct from the α-tocopherol–binding site), Kono et al. (2013) assessed lipid-binding and intermembrane transfer activities of R59W α-TTP. Whereas R59W α-TTP was able to bind α-tocopherol and transfer it between liposomes in vitro, it failed to stimulate its transport in cells. Moreover, unlike wild-type α-TTP, the R59W mutant failed to bind $PI(3,4)P_2$ or $PI(4,5)P_2$. Crystallographic analysis of the ternary complex revealed that α-TTP bound concurrently to α-tocopherol and PIP_2 (either $PI(3,4)P_2$ or $PI(4,5)P_2$); α-tocopherol was deep in the α-TTP core, whereas the PIP_2 negatively charged inositol phosphate head group interacted with the disease-associated arginine residues in the positively charged cleft. A comparison of the structures of α-TTP with or without bound α-tocopherol (in the absence of PIP_2) indicated that α-tocopherol was bound in a hydrophobic pocket with a "lid" that was open in its absence, with the PIP_2-bound complex assuming an intermediate conformation. In vitro analyses indicated that incorporation of PIP_2 into "target" liposomes increased α-tocopherol transfer by wild-type α-TTP but not by the R59W mutant. Thus, the authors suggest that α-TTP exchanges PIP_2 for α-tocopherol, with PIP_2 binding to α-TTP to open the lid and facilitate α-tocopherol transfer to the target membrane.

GIRK channels, intermembrane transfer of vitamin E, and a vertebrate fluorescent protein

A vertebrate fluorescent protein

Kumagai et al. (2013) identified and cloned a fluorescent protein from unagi, the Japanese fluorescent eel (*Anguilla japonica*). This protein, which they called UnaG, represents the first known vertebrate fluorescent protein, as well as the first ligand-inducible fluorescent protein identified. On the basis of an earlier report indicating that unagi muscle fibers fluoresce, Kumagai et al. (2013) determined that small-diameter muscle fibers of juvenile eels were strongly fluorescent and cloned UnaG, a 139–amino acid protein in the fatty acid–binding protein (FABP) family. Whereas UnaG showed robust (and oxygen-independent) green fluorescence when heterologously expressed in HeLa cells or embryonic mouse brain, it failed to do so when expressed in bacteria, suggesting that its fluorescence might depend on a vertebrate cofactor. Indeed, further analysis identified the membrane-permeable heme metabolite bilirubin as a high affinity, high specificity UnaG fluorogenic chromophore. The identification of fluorescent proteins has revolutionized optical approaches



Model for α-TTP delivery of α-tocopherol to the plasma membrane in exchange for PIP_2. (From A. Mesmin and B. Antonny. 2013. *Science*. 340:1051–1052. Reprinted with permission from AAAS.)

to cell biology. By virtue of its unusual properties, UnaG holds the promise of being the founding member of a new class of ligand-inducible fluorescent proteins that can be used under anaerobic conditions, as well as providing the potential for insight into bilirubin function and a fluorometric assay for bilirubin that is simpler and more sensitive than those currently in use.

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