Generally Physiological

Of ATPase activity, cellular energy distribution, and linking depolarization and division

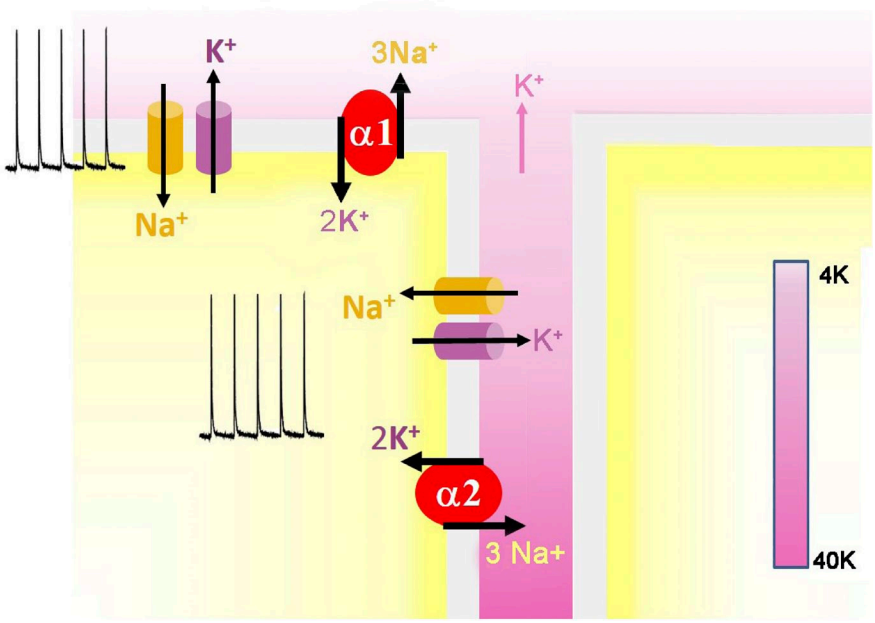
This month's installment of *Generally Physiological* considers how differences in Na-pump K^+ affinity and localization enable their efficient operation over the range of $[K^+]_o$ in resting and active muscle, how a mitochondrial reticulum could enable the rapid distribution of energy throughout skeletal muscle, and how signaling from K-Ras may link membrane depolarization with cell proliferation.

Matching activity with location

The fractional occupancy of substrate sites by Na^+ and K^+ provides a primary mechanism for the rapid regulation of Na,K-ATPase activity. The apparent affinity of the ubiquitously expressed α1 isoform of the Na,K-ATPase catalytic subunit (α1) for K^+ ($K_{1/2,K}$) is close to 1 mM, so that under physiological conditions of 4–5 mM of extracellular K^+, acute regulation of its catalytic activity depends mainly on changes in the intracellular Na^+ concentration. α1 is the major isoform in most cell types; in adult skeletal muscle, however, the Na,K-ATPase α2 isoform, which is the only isoform present in the transverse tubules, predominates. Whereas α1 (localized to the sarcolemmal surface) mediates most of the basal Na^+/K^+ transport required to maintain the gradients of these ions, α2 operates substantially below its maximum capacity in resting muscle, but is crucial to maintaining contraction and resisting fatigue in working muscle. Noting that the K^+ concentration in the restricted extracellular space of the transverse tubule lumen increases with muscle activity, in this issue DiFranco et al. measured activation of Na,K-ATPase current by external K^+ in voltage-clamped single mouse muscle fibers to determine the K^+ affinity of the α2 isoform. They determined that it was substantially lower than that of α: rather than saturating at resting K^+ concentration, current increased with increasing K^+ at concentrations up to 40 mM. Thus, unlike α1, the activity of α2 was susceptible to acute stimulation by K^+ over the range of concentrations found in the transverse tubules of active muscle. The authors thus propose that the existence of two Na,K-ATPase α isoforms with distinct locations and differing K^+ affinities enables their preferential operation over the different ranges of extracellular K^+ concentrations characteristic of resting and active muscle, with α1 playing the major role at rest and α2 taking over as K^+ accumulates in the transverse tubules of working muscles.

A cellular energy conduit

Muscle cell function depends on the distribution of the potential energy generated through mitochondrial oxidative phosphorylation to the intracellular locales at which it is required. Metabolite-facilitated diffusion has been proposed to provide a mechanism for mediating intracellular energy distribution; however, knockout studies in mice suggest that the proposed facilitated diffusion pathways are dispensable for near-normal



The α1 isoform of the Na,K-ATPase, located on the plasma membrane, maintains ion gradients in resting cells. Location of the α2 isoform to the transverse tubules enables its activation if K^+ builds up in the transverse tubule lumen of actively contracting muscle. See DiFranco et al. (2015).

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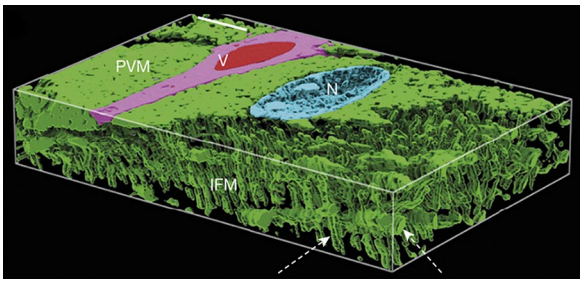
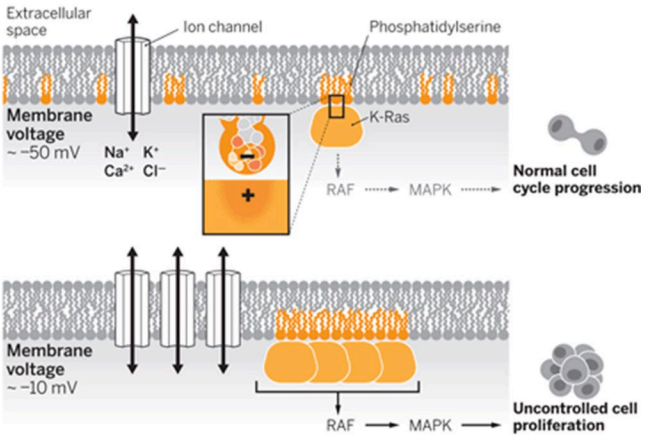


3-D imaging reveals a highly connected network of muscle mitochondria (green). Blue, nucleus (N); magenta, capillary (V); red, red blood cell. PVM, paravascular mitochondria; IFM, intrafibrillar mitochondrial network. (Reprinted by permission from Macmillan Publishers, Ltd. B. Glancy et al. *Nature*. http://dx.doi.org/10.1038/nature14614, copyright 2015.)

skeletal muscle function. Using high resolution 3-D microscopy to visualize muscle cell structure, Glancy et al. (2015) found that the mitochondria formed a highly interconnected network throughout the cell, suggesting that they might not only be crucial to cellular energy production, but also to its distribution. Immunostaining revealed that mitochondrial complex IV (involved in the generation of proton-motive force) was more abundant at the cell periphery, consistent with its preferential localization in a paravascular pool of mitochondria, whereas complex V (which uses proton-motive force to produce ATP from ADP) was more abundant in the intrafibrillar region. Remarkably, localized depolarization of the mitochondrial membrane potential ($\Delta\Psi$) in a small interior region of the cell by means of a photo-activated uncoupler led to a rapid decrease in $\Delta\Psi$ throughout the cell, indicating that the mitochondria are tightly electrically coupled and function as a syncytium with regard to $\Delta\Psi$. The authors thus propose that the mitochondria function as a cellular "power grid" to provide a conductive pathway for the rapid transport of potential energy from the paravascular mitochondria in the periphery to enable ATP production near ATPases deep inside the cell that support contraction and transport.

Potential proliferation with K-Ras

Although depolarization of the plasma membrane is perhaps best known for mediating excitatory processes, it may also promote cell division. The specific link between membrane potential and mitosis, however, has been obscure. Zhou et al. (2015) found that membrane depolarization specifically causes the reorganization of the nanoscale anionic inner leaflet phospholipids phosphatidylserine (PS) and phosphatidylinositol 4,5-bisphosphate (PIP_2), promoting the reversible formation of nanoclusters of PS and PIP_2, without affecting the lateral distribution of phosphatidic acid or phosphatidylinositol 3,4,5-trisphosphate. The membrane-associated GTPase K-Ras plays a role in cell differentiation, proliferation, and survival; indeed, mutations causing constitutive activation of K-Ras have been implicated in many cancers. Noting that activation of the mitogen-activated protein kinase (MAPK) signaling cascade by K-Ras depends on its assembly into nanoclusters, and that electrostatic interactions between residues in the K-Ras C-terminal tail and charged membrane lipids are key to this aggregation, the authors explored the effects of membrane potential on K-Ras localization and MAPK signaling. They found that depolarization promoted K-Ras association with PS, K-Ras nanoclustering, and MAPK signaling, whereas repolarization reversed these effects. The authors thus propose that membrane potential regulates gain in K-Ras signaling by controlling its assembly in plasma membrane nanoclusters and speculate on the implications of this regulation. Accardi (2015) provides thoughtful commentary, noting that ion channel dysregulation has been postulated to play a role in oncogenesis and the implications of identifying a pathway between membrane potential to cell proliferation for developing strategies to silence oncogenic signaling.



In nonproliferating cells (top), K-Ras localizes to small PS nanoclusters, stimulating low levels of MAPK signaling. Depolarization secondary to ion channel overexpression (bottom) increases clustering of PS and K-Ras, stimulating MAPK signaling and thereby cell proliferation. (From Accardi. 2015. *Science*. http://dx.doi.org/10.1126/science.aad0874. Reprinted with permission from AAAS.)

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