

Generally Physiological

Of volume regulation, arterial constriction, and apoptotic breakdown



This month's installment of *Generally Physiological* concerns the identification of a membrane protein that plays a crucial role in the regulatory volume decrease that counteracts osmotic cell swelling, angiotensin II regulation of TRPV4 activity in arterial myocytes, and an unexpected role for pannexin 1 in regulating cell fragmentation during apoptosis.

A SWELL1 protein involved in volume regulation

A decrease in extracellular osmolarity elicits rapid influx of water into animal cells, causing them to swell and activating an anion-selective current ($I_{Cl, \text{swell}}$) that carries mainly chloride ions under physiological conditions. The molecular identity of the underlying volume-regulated anion channel (VRAC), which plays a crucial role in the regulatory volume decrease that counteracts swelling, has remained elusive. Using HEK293 cells stably expressing the halide-sensitive yellow fluorescent protein to monitor iodide influx after a hypotonic challenge, Qiu et al. (2014) performed a genome-wide RNAi screen and identified *LRCC8A* (leucine-rich repeat-containing protein 8A) as encoding a protein required for hypotonicity-induced anion flux, which they named SWELL1. SWELL1, which is predicted to contain four transmembrane helices, was broadly expressed in mouse tissues and was localized to the plasma membrane. Analysis of Myc-tagged constructs indicated that the N and C termini were intracellular, whereas residues located in the putative TM1–TM2 and TM3–TM4 loops were extracellular. Whereas SWELL1 knockdown failed to affect

basal volume or the initial increase in volume in response to hypotonic solution, it slowed the subsequent recovery. Moreover, whole-cell patch-clamp analysis indicated that SWELL1 knockdown suppressed endogenous

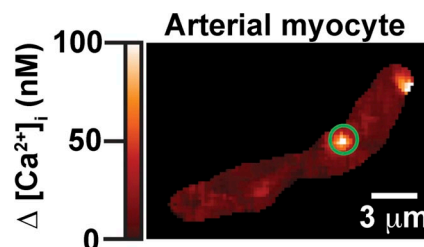
A membrane protein involved in volume regulation, angiotensin II regulation of TRPV4 activity, and an unexpected role for PANX1

$I_{Cl, \text{swell}}$ in various cell types, an effect that was rescued by RNAi-resistant SWELL1. Furthermore, substitution of a threonine residue at position 44 affected VRAC anion selectivity. The authors thus conclude that SWELL1 represents an essential component that either forms or is close to the pore of the long-sought VRAC.

A dynamic TRPV4 complex to oppose vasoconstriction

Calcium is generally viewed as promoting muscle contraction, as occurs in arterial myocytes with calcium influx through L-type $\text{Ca}_v1.2$ channels, which leads to a global increase in intracellular calcium concentration ($[\text{Ca}^{2+}]_i$). In marked contrast, calcium influx through transient receptor potential vanilloid 4 (TRPV4) channels is thought to mediate arterial myocyte relaxation through a process that involves its triggering calcium release from the sarcoplasmic reticulum, with the ensuing calcium spark activating BK channels to hyperpolarize the membrane, close $\text{Ca}_v1.2$ channels, and lower $[\text{Ca}^{2+}]_i$.

The magnitude and location of TRPV4 calcium signals are thus crucial to their effects on arterial function. In this issue, Mercado et al. combined electrophysiological analyses with confocal, TIRF, and super-resolution ground state depletion microscopy to investigate the spatial organization, calcium signaling properties, and regulation by the vasoconstrictor angiotensin II of TRPV4 channels. Angiotensin II induced stronger constrictions in arteries from mice lacking TRPV4 than in those from wild-type mice, indicating that TRPV4 opposes angiotensin II-induced vasoconstriction. Intriguingly, however, angiotensin II stimulated the activity of “sparklets” associated with calcium influx through TRPV4 channels; a combination of pharmacological and optogenetic analyses implicated PKC in this increase in TRPV4 activity.



TIRF image of an arterial myocyte showing sites of TRPV4 sparklets. From Mercado et al. (2014).

Noting that TRPV4 channels formed puncta of various sizes along the myocyte sarcolemma, whereas sparklet activity localized to specific regions, the authors investigated the role of the scaffolding protein AKAP150 in targeting PKC to TRPV4. Indeed, angiotensin II failed to increase TRPV4

sparklet activity in myocytes from mice lacking AKAP150; moreover, angiotensin II increased the fraction of AKAP150 puncta within 30 nm of TRPV4 puncta. The authors thus propose that angiotensin II increases TRPV4 activity through the activation of AKAP150-targeted PKC, and that AKAP150, PKC, and TRPV4 complexes form dynamic calcium signaling domains as part of a feedback system that opposes vasoconstriction in arterial myocytes.

Regulating apoptotic fragmentation

Apoptosis, a highly regulated process of programmed cell death that involves cell fragmentation into membrane-bound apoptotic bodies as well as the release of “find-me” signals to phagocytes, plays a crucial role during development and in maintaining tissue size (see Gregory, 2014). Noting that opening of the pannexin 1 (PANX1) channel during apoptosis enables not only the release of nucleotide find-me signals but also the influx of the fluorescent dye TO-PRO-3, Poon et al. (2014) monitored TO-PRO-3 uptake into apoptotic Jurkat cells in a screen for PANX1 modulators. Unexpectedly, they found that the quinolone antibiotic trovafloxacin inhibited PANX1 function in apoptotic cells, decreasing TO-PRO-3 uptake, ATP release, and apoptosis-induced PANX1 inward current. Moreover, trovafloxacin promoted the production of apoptotic bodies in cells undergoing apoptosis and led to the formation of string-like structures that the authors called apoptopodia.

A dominant-negative form of PANX1 also promoted formation of apoptotic bodies, whereas PANX1 overexpression decreased their formation. PANX1 also appeared to influence apoptosis in vivo: trovafloxacin enhanced fragmentation of thymocytes induced to undergo apoptosis, an effect that was phenocopied by loss of PANX1. The authors thus conclude that PANX1 plays a role in regulating cell fragmentation during apoptosis and speculate about the possible relevance of their findings to toxic side effects of trovafloxacin.

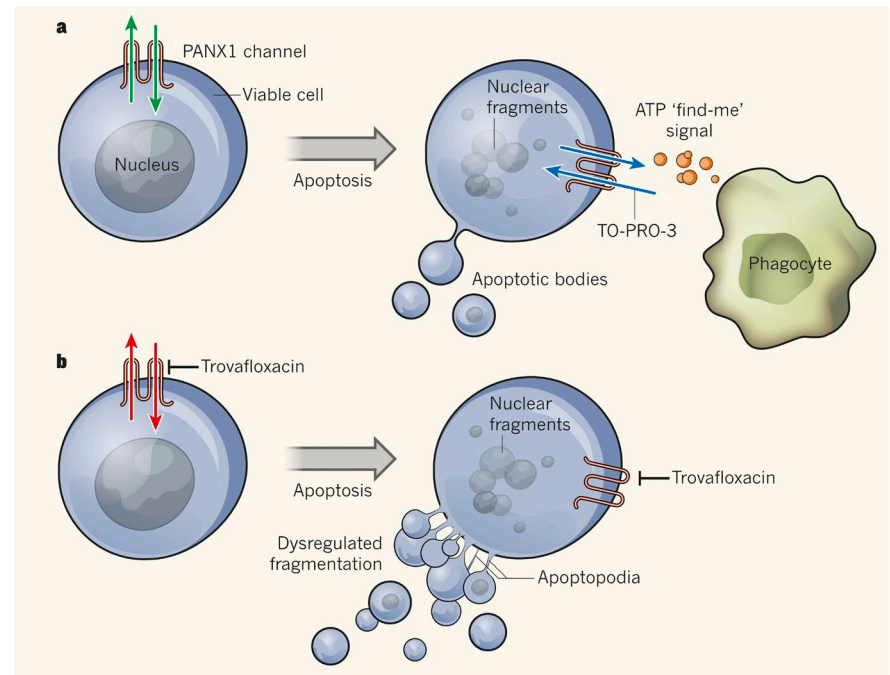
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(a) PANX1 channels release ATP “find-me” signals from cells undergoing apoptosis. (b) PANX1 blockade with trovafloxacin inhibits ATP release, increases the production of apoptotic bodies, and leads to formation of apoptopodia. (Reprinted by permission from Macmillan Publishers, Ltd. C.D. Gregory. 2014. *Nature*. <http://dx.doi.org/10.1038/nature13213>, copyright 2014.)