Generally Physiological

Of tracking channel activity, modulating AMPA receptor function, permeating potassium ions, and the plant response to osmotic stress

This month's installment of *Generally Physiological* considers modification of tarantula toxins to track channel activation state, how the prototypical TARP stargazin regulates AMPA receptor signaling, how potassium ions make it through the selectivity filter, and identification of a calcium-permeable channel that may allow *Arabidopsis* to sense hyperosmotic stress.

Assessing channel activity

The firing pattern of any excitable cell depends on the activity of its

et al. synthesized labeled forms of the tarantula toxin guangxitoxin-1E (GxTX), which selectively binds Kv2 channels, that, like the parent toxin, produced a positive shift in Kv2.1 activation voltage. A fluorescently labeled form of GxTX colocalized with Kv2.1 channels in CHO-K1 cells and neurons and reported channel activation: after determining that GxTX binding was state dependent (with lower affinity for activated than resting channels), the authors showed that depolarization decreased

cells expressing Kv2.1 channels bound in a voltage-dependent manner to a GxTX variant conjugated to microbeads, providing a potential approach to identifying previously unknown activity-dependent ligands for voltage-gated ion channels. The authors conclude that modified voltage-sensor toxins can be used as highly selective optical monitors of specific channel types in living cells.

Promoting clamshell closure

Although transmembrane AMPA receptor regulatory proteins (TARPS) were identified through their role in trafficking of the AMPA-type glutamate receptor (AMPAR, the receptor that mediates the majority of fast excitatory synaptic transmission in the central nervous system), they also modulate AMPAR gating kinetics and pharmacology. For instance, TARPS slow AMPAR deactivation and desensitization and increase glutamate potency as well as increase the efficacy
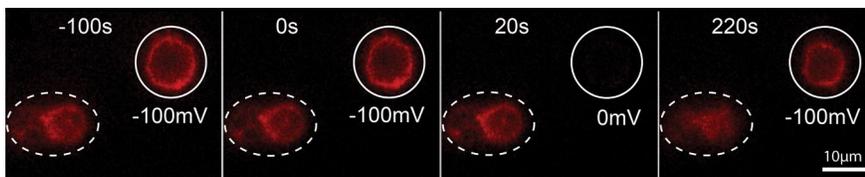
Association of fluorescent GxTX with Kv2.1-expressing CHO cells decreases when voltage is stepped from 100 to 0 mV. Clamped cell is surrounded by a solid circle; unclamped cell is surrounded by a dashed circle. (Reprinted by permission from Chemoselective tarantula toxins report voltage activation of wild-type ion channels in live cells. Tilley, D.C., K.S. Eum, S. Fletcher-Taylor, D.C. Austin, C. Dupré, L.A. Patrón, R.L. Garcia, K. Lam, V. Yarov-Yarovoy, B.E. Cohen, and J.T. Sack. *Proc. Natl. Acad. Sci. USA.* 2014. http://dx.doi.org/10.1073/pnas.1406876111)

specific complement of ion channels; thus, modulation of a particular channel type provides a potential mechanism for controlling aberrant activity. Many methods exist to measure cellular electrical activity; identifying which populations of channels are active under specific physiological or pathophysiological conditions, however, has presented more of a challenge. Tilley et al. (2014) tackled this challenge by developing molecular reporters that show state-dependent binding to the Kv2.1 potassium channel voltage sensor as prototypes of such activity-reporting probes. Noting that spider venoms contain numerous toxins that target the voltage sensors of ion channels, Tilley
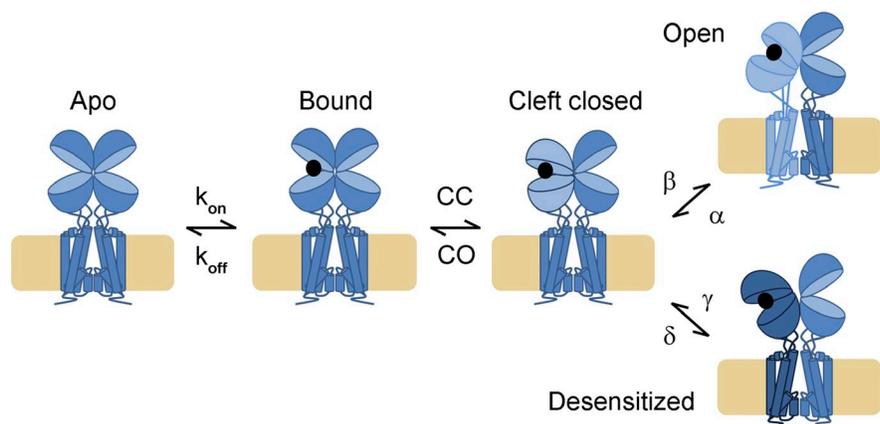
Model illustrating ligand binding within the cleft of the AMPAR ligand-binding domain leading first to "clamshell" closure and then to either the open or desensitized state. CC, cleft closure; CO, cleft open. From MacLean et al. (2014).

the association of fluorescent GxTX with Kv2.1-expressing cells. Moreover,

of the partial agonist kainite and convert CNQX from an antagonist

The Rockefeller University Press
J. Gen. Physiol. Vol. 144 No. 6   491–493
www.jgp.org/cgi/doi/10.1085/jgp.201411317

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A KcsA potassium channel

Selectivity filter

Ion-binding sites

B Soft knock-on mechanism

C Hard knock-on mechanism
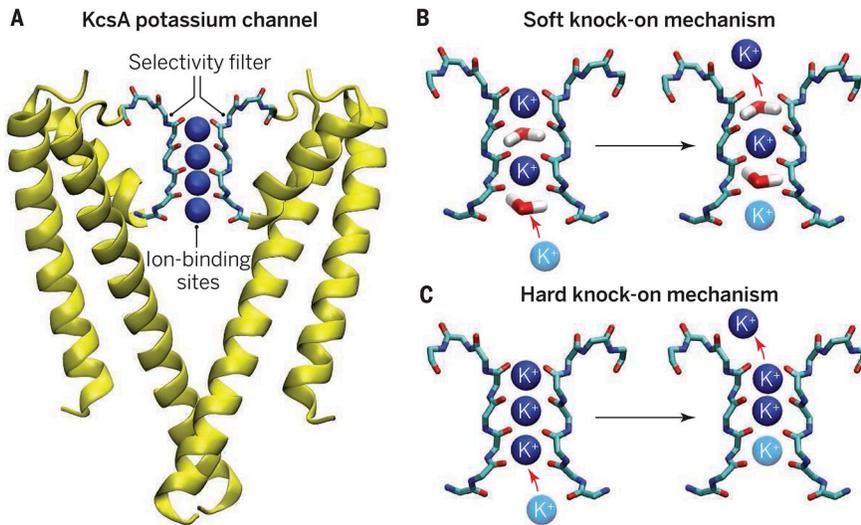
(A) KcsA potassium channel, indicating ion-binding sites in the selectivity filter. (B) Model depicting alternating K^+ ions and water molecules passing through the selectivity filter. (C) Model depicting K^+ ions in direct contact. (From Hummer. 2014. *Science.* http://dx.doi.org/10.1126/science.1260555. Reprinted with permission from AAAS.)

into a partial agonist. The AMPAR ligand-binding domain has been described as a "clamshell-like" structure, with ligand binding within the cleft promoting a more closed conformation of the clamshell, thereby leading to channel opening or desensitization. In this issue, MacLean et al. used a combination of electrophysiological and fluorescence-based approaches to investigate the structural basis whereby the prototypical TARP stargazin affects AMPAR gating. Stargazin rescued gating defects in mutant forms of the AMPAR in which closed cleft conformations are destabilized (consistent with its stabilizing the closed state) and slowed binding of the bulky antagonist NBQX (consistent with reduced accessibility of the binding site). Moreover, luminescence resonance energy transfer indicated that stargazin promoted cleft closure regardless of ligand binding. MacLean et al. (2014) thus propose that stargazin promotes closure of the clamshell to potentiate AMPAR signaling.

No alternating waters?
Studies involving x-ray crystallography, electrophysiological analyses,

and molecular dynamics simulations have led to a model of potassium permeation through potassium channels in which potassium ions alternate with water molecules as they traverse a selectivity filter containing four equidistant K^+-binding sites. Köpfer et al. (2014) challenge this view, however, concluding from atomistic molecular dynamics simulations of various potassium channels (including KcsA, MthK, and a $K_v1.2$–$K_v2.1$ chimera) that permeation involves direct contact of potassium ions passing through the selectivity filter, with repulsion between neighboring potassium ions key to high efficiency conduction. Hummer (2014) provides thoughtful commentary,

noting that the results of the present study appear to be inconsistent with prior electrophysiological data.

Sensing water deficiency
Plants respond to a lack of water with both short-term and long-term compensatory changes; however, the mechanisms whereby they sense hyperosmotic stress remain unclear. Noting that hyperosmolality triggers an increase in cytosolic free calcium concentration ($[Ca^{2+}]_i$), and that osmosensing Ca^{2+} channels have been identified in bacteria and animals, Yuan et al. (2014) undertook calcium imaging–based forward genetic screens of aequorin-expressing *Arabidopsis* mutants in the hopes of identifying such an osmosensing Ca^{2+} channel. 23 mutants that showed a decreased hyperosmolality-induced $[Ca^{2+}]_i$ increase were rescreened for an impaired response to osmotic stress; the authors named the most affected of these mutants reduced hyperosmolality-induced $[Ca^{2+}]_i$ increase 1 (*osca1*). Whereas basal $[Ca^{2+}]_i$ in *osca1* plants was similar to that in wild-type plants, the Ca^{2+} response to solutions containing sorbitol, or various other osmolytes, was attenuated, whereas that to H_2O_2 was not. Sorbitol-induced stomatal closure was reduced in *osca1* plants compared with
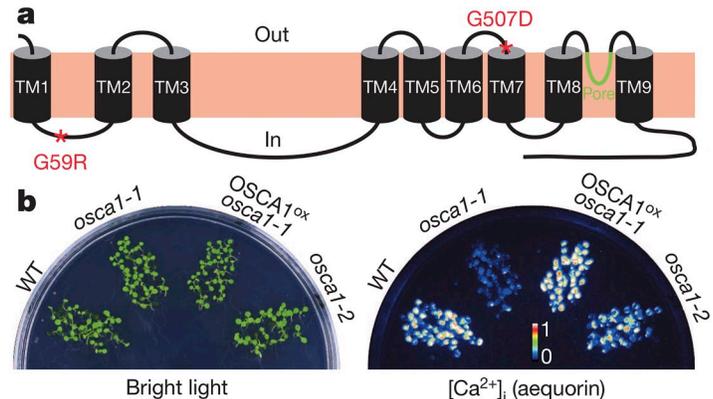
(a) Predicted membrane topology of OSCA1, indicating the G59R and G507D mutations identified in *osca1*. (b) Diminished hyperosmolality-induced $[Ca^{2+}]_i$ increase in *osca1* (*osca1-1* in figure; *osca1-2* is a second mutant showing a similar diminished hyperosmolality-induced $[Ca^{2+}]_i$ increase phenotype) is rescued by overexpression of OSCA1 (OSCA^ox *osca1-1*). (Reprinted by permission from Macmillan Publishers, Ltd. F. Yuan et al. *Nature.* http://dx.doi.org/10.1083/nature13593, copyright 2014.)

wild type, whereas wilting in response to osmotic stress and sorbitol-induced inhibition of root growth were exacerbated. Genetic analysis indicated that the *osca1* phenotype was caused by a recessive mutation in a single nuclear gene, and *OSCA1* was identified as a previously unknown gene encoding a 772–amino acid protein. OSCA1 was expressed in leaves, flowers, roots, and guard cells, and was located at the cell surface. When heterologously expressed in HEK293 cells, OSCA1 localized to the plasma membrane, mediated sorbitol-induced increases in $[Ca^{2+}]_i$, and functioned as a nonselective cation channel. OSCA1 thus appears to represent a hyperosmolarity-activated calcium-permeable channel involved in the plant response to osmotic stress that may function as a plant osmosensor.

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