Developmental Cell
Previews

Neuronal Osmotransduction: Push-Activating TRPV1 with Microtubules

Marta Andrés[1] and Martin C. Göpfert[1,*]
[1]Department of Cellular Neurobiology, University of Göttingen and DFG Collaborative Research Centre SFB 889-A1, 37077 Göttingen, Germany
*Correspondence: mgoepfe@gwdg.de
http://dx.doi.org/10.1016/j.devcel.2014.08.012

Regulating the osmotic pressure of our body fluids relies on osmosensory neurons that depolarize when their volume decreases. Recently in *Neuron*, Prager-Khoutorsky et al. (2014) report that this depolarization arises from direct interactions between the transient receptor potential channel TRPV1 and microtubules, which seem to directly push open the channel.

Mammals employ sophisticated regulatory mechanisms to control the systemic osmolality of their body fluids (Pedersen et al., 2011). Central elements in this regulation are osmosensory neurons (ONs) in the supraoptic nucleus of the hypothalamus that depolarize when the osmotic pressure of the extracellular fluids increases (Bourque, 2008). This depolarization promotes water reabsorption in the kidney by triggering the release of the antidiuretic hormone vasopressin from the ONs (Trudel and Bourque, 2010). Experimentally, the cellular response of ONs to hypertonicity can be mimicked by applying suction through a patch pipette, demonstrating that ON depolarization is evoked mechanically by the osmotically induced shrinkage of the cell (Ciura et al., 2011). Such mechanical excitation points to parallels with the well-studied osmotransduction mechanism of the bacterium *Escherichia coli*, which monitors increases of its volume with mechanosensitive ion channels that are directly pulled open by the resulting stretch of the cell membrane (Kung et al., 2010) (Figure 1A). Unlike these stretch-activated channels (Nilius and Honoré, 2012), however, the respective channels of ONs open when the cell volume drops and the cell membrane slackens, which means that they cannot be pulled open by membrane tension. A possible alternative is that cytoskeletal elements may contribute to activation of these channels. This possibility was suggested when the Bourque group discovered that osmotransduction in ONs is mediated by the TRPV1 channel (Sharif Naeini et al., 2006), whose carboxy terminus was found to bear two sites that bind β-tubulin (Goswami, 2012).

In a technical tour de force, the Bourque group (Prager-Khoutorsky et al., 2014) now systematically explores the role of tubulin in ON function, revealing that the direct interaction with microtubules is essential for the mechanical activation of TRPV1. Using superresolution microscopy, Prager-Khoutorsky and colleagues uncovered a particularly elaborate microtubule network in the somata of rat ONs that is essential for osmotransduction and demonstrated that microtubule-disrupting agents abolish the mechanically evoked ON depolarization and associated changes of the cell membrane's conductance. In contrast, these cellular responses were facilitated when the cells were treated with microtubule-stabilizing agents. Coimmunoprecipitation analysis confirmed that TRPV1 interacts physically with tubulin in ONs under in vivo conditions, and, using proximity ligation assays, multiple TRPV1-tubulin interaction sites were identified in ONs that were located beneath the cell membrane. Perturbing these interactions with synthetic peptides against the TRPV1 tubulin binding sites abolished the mechanically evoked depolarization. Intriguingly, only the mechanical activation of TRPV1 was disrupted, and TRPV1 could still be activated by the peptide hormone angiotensin II, previously identified by the authors as an ON-specific TRPV1 agonist that acts via G protein-mediated signaling pathways (Sharif Naeini et al., 2006). Perturbing the TRPV1-tubulin interactions thus leaves TRPV1 itself unaffected but specifically disrupts its mechanical activation.

Having established that the physical interaction with microtubules is essential for the mechanical activation of TRPV1, the authors reasoned that changes in the number of these interactions might mediate the mechanically evoked depolarization. Proximity assays, however, revealed that this number remains rather constant when the cell volume drops, suggesting that it is the interactions proper, rather than their numbers, that are required for mechanical activation of TRPV1. As pointed out by the authors, a likely scenario is that the shrinkage of the cells compresses the microtubule network, making the microtubules push against TRPV1 and thereby directly opening this channel (Figure 1B). The fast onset of depolarization, which, in some instances, commenced only 4 ms after suction was applied to the ONs, provides experimental support for direct activation because it leaves very little time for a second-messenger signaling cascade.

In addition to putting forward TRPV1 as a mechanosensitive channel, the findings of Prager-Khoutorsky and colleagues establish a functional role for microtubules in mechanosensory transduction and delineate a new paradigm for mechanosensitive ion channel gating (Figure 1B). Given that TRPV1 is a heat-activated channel that is implicated in the sensation of pain, its mechanical activation in ONs is rather surprising. Previous work by the Bourque group, however, had shown that ONs express an unconventional TRPV1 isoform with a truncated amino terminus (Sharif Naeini et al., 2006), which might explain the unconventional mechanism of TRPV1

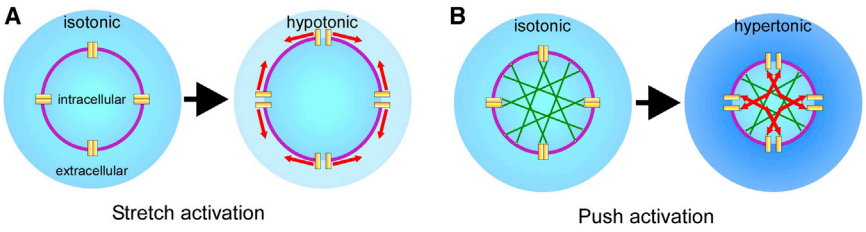

Figure 1. Gating Paradigms of Mechanosensitive Ion Channels in Response to Osmotic Stimulation

(A) Stretch activation. If the osmotic pressure of the extracellular decreases, changing from isotonicity (left) to hypotonicity (right), the cell expands and the cell membrane (purple circle) stretches. This membrane stretch directly pulls open (red arrows, right) mechanosensitive ion channels (depicted in yellow) in the cell membrane. (B) Push activation, as proposed by Prager-Khoutorsky et al. (2014). If the osmotic pressure of the extracellular medium increases and thus changes from isotonicity (left) to hypertonicity (right), the cell shrinks, compressing the microtubule cytoskeleton. This compression results in elastic forces that the microtubules exert on mechanosensitive ion channels (red arrows, right), thereby pushing the channels open.

activation in these cells. Precedence for a direct physical interaction between microtubules and mechanosensitive ion channels comes from the *Drosophila* TRPN1 channel, which is mechanically activated and binds to microtubules with its amino terminus (Zanini and Göpfert, 2013). Although the functional relevance of the microtubules for TRPN1 gating has not yet been established, this example nicely illustrates that the microtubule-based push activation of channels that is proposed by Prager-Khoutorsky and colleagues (2014) might be a more widespread mechanism in mechanosensory stimulus transduction.

Other than TRPV1, several other TRP channels have been implicated in osmotic stimulus transduction. TRPV4, for example, mediates the responses to hypotonically induced volume increases in various cell types. Unlike the stretch-activated channels of *Escherichia coli*, however, the mechanical activation of TRPV4 is not direct and involves intermediate signaling mechanisms (Garcia-Elias et al., 2014). Such indirect activation cannot yet be fully excluded for TRPV1 in ONs. Even though a latency of 4 ms hardly leaves time for transduction via intermediate signaling cascades, it nevertheless appears to be long for direct mechanical channel activation that should occur virtually instantaneously after the mechanical stimulus is applied. Experiments utilizing fast-force actuation protocols might show how fast the depolarization of ONs can set in, and detailed biophysical analyses may help to clarify how the microtubules deform during cell shrinkage, as well as how this mechanical deformation translates into the TRPV1 activation. Should such studies confirm a direct activation of TRPV1, it still remains possible that the microtubule push on TRPV1 focally increases the membrane tension in the vicinity of TPRV1, thereby leading to stretch-induced activation rather than push-induced activation of the channel. Prager-Khoutorsky and colleagues (2014) provide solid grounds to explore these possibilities, and their proposed push-activation mechanism (Figure 1B) undoubtedly presents an intriguingly simple and elegant explanation of how mechanosensitive ion channels can be activated by hypertonicity-evoked cell shrinkage. The stretch-activation of ion channels by membrane tension (Figure 1A) constitutes a prime paradigm of mechanosensory transduction channel gating in response to membrane tightening (Figure 1A) (Nilius and Honoré, 2012). The Bourque group now has put forth a complementary gating paradigm that relies on cytoskeletal elements and involves a push activation of the channels that takes place when the cell membrane slackens.

REFERENCES

Bourque, C.W. (2008). Nat. Rev. Neurosci. *9*, 519–531.

Ciura, S., Liedtke, W., and Bourque, C.W. (2011). J. Neurosci. *31*, 14669–14676.

Garcia-Elias, A., Mrkonjić, S., Jung, C., Pardo-Pastor, C., Vicente, R., and Valverde, M.A. (2014). Handbook Exp. Pharmacol. *222*, 293–319.

Goswami, C. (2012). J. Neurochem. *123*, 1–13.

Kung, C., Martinac, B., and Sukharev, S. (2010). Annu. Rev. Microbiol. *64*, 313–329.

Nilius, B., and Honoré, E. (2012). Trends Neurosci. *35*, 477–486.

Pedersen, S.F., Kapus, A., and Hoffmann, E.K. (2011). J. Am. Soc. Nephrol. *22*, 1587–1597.

Prager-Khoutorsky, M., Khoutorsky, A., and Bourque, C.W. (2014). Neuron *83*, in press. Published online August 7, 2014.

Sharif Naeini, R., Witty, M.F., Séguéla, P., and Bourque, C.W. (2006). Nat. Neurosci. *9*, 93–98.

Trudel, E., and Bourque, C.W. (2010). Nat. Neurosci. *13*, 467–474.

Zanini, D., and Göpfert, M.C. (2013). Curr. Biol. *23*, R349–R351.