Opening the TRPML Gates

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The intracellular TRPML channels have multiple biological roles, but the physiological stimuli that open them remained unknown. In a previous issue of *Chemistry & Biology*, Grimm et al. report a high-throughput chemical screen that identified a plethora of selective activators of TRPML3 that should open the way to fully characterize these channels and their physiological roles.

TRP is a remarkable family of cation channels that mediate numerous cellular functions (Nilius et al., 2007). Most TRP channels are located in the plasma membrane and mediate Ca^{2+} influx in response to, among other things, receptor stimulation, temperature changes, membrane stretch, and noxious compounds that can access the channels at the plasma membrane (Nilius et al., 2007). The TRPML subfamily of the TRP channels is unique in that these channels are mostly expressed in intracellular organelles, and the physiological stimuli that open them are not known at present.

The TRPML subfamily consists of three members: the founding member TRPML1, TRPML2 and TRPML3. TRPML1 was identified as the protein mutated in the lysosomal storage disease mucolipidosis type IV (Bargal et al., 2000). TRPML2 was found by database searches and TRPML3 was identified as the protein mutated in mice with the varitint-waddler phenotype (Di Palma et al., 2002). The varitint-waddler phenotype is characterized by auditory, vestibular, and melanocyte phenotypes, which are caused by the gain-of-function mutation A419P in the predicted fifth transmembrane domain of TRPML3 (Grimm et al., 2007; Kim et al., 2007). TRPML1 is expressed in late endosomes and lysosomes (Treusch et al., 2004), TRPML2 is found mainly in lysosomes (Venkatachalam et al., 2006), and TRPML3 shuttles between multiple intracellular compartments and the plasma membrane (Kim et al., 2009). The wild-type channel behavior in mammalian cells is known only for TRPML3 that can be activated by preincubation in low Na^+ medium (Kim et al., 2008), while, when expressed in insect cells, TRPML2 displays some spontaneous channel activity (Lev et al., 2010). When the proline mutation in the predicted fifth transmembrane domain was inserted into TRPML2(A396P) and TRPML1(V432P), the resulting channels showed spontaneous activity (Grimm et al., 2007). However, this mutation is likely to affect channel behavior, since TRPML3(A419P) has altered ionic selectivity and Ca^{2+} permeability (Kim et al., 2008), along with altered trafficking and posttranslational modification (Kim et al., 2007; van Aken et al., 2008).

The intracellular localization of the TRPML channels, the lack of knowledge of their physiological stimuli, and the inactivity of the available clones of wild-type TRPML1 and TRPML2 pose significant difficulties in characterizing the properties on these channels and in delineating the physiological function of the native channels. Grimm et al. (2010) overcome these difficulties by conducting a high-throughput screen for small molecule activators of TRPML3. The screen yielded 53 specific TRPML3 activators with EC_{50} of <5 μM that can be classified into 9 chemical scaffolds and 19 singletons. The most active compound was the cell impermeant singleton dubbed SN-2. Considering that most TRPML3 is found in intracellular organelles, SN-2 may not be the ideal compound to study the cellular role of TRPML3. Other compounds, like the cell permeant SF-11, may be more suitable for such studies.

Interestingly, two of the TRPML3 activators also activated TRPML1 (SF-21) and TRPML2 (SF-22). These offer the opportunity for the first time to characterize the channel properties of the wild-type TRPML1 and TRPML2 expressed in mammalian cells and perhaps to study the effect of their opening on cell function. Of particular interest will be the effect of channel activity on cellular trafficking, as both TRPML1 (Treusch et al., 2004) and TRPML3 (Kim et al., 2009) were shown to affect membrane trafficking and autophagy. In addition, SF-21 and SF-22 can be lead compounds to develop specific chemical activators and inhibitors of TRPML1 and TRPML2, respectively.

Grimm et al. (2010) used the TRPML3 activators to make the important observation that they did not evoke TRPML3 activity in cochlear hair cells, and only SN-2 at high concentration activated TRPML3 in melanocytes. The potential explanations offered for this observation are that TRPML3 in the plasma membrane of native cells is regulated to prevent its opening by the TRPML3 activators; TRPML3 may exist in complex with other TRPML channels, and TRPML3 in the complex is not responsive to the activators; or that the level of TRPML3 at the plasma membrane of native cells is very low. The latter appears to be the most likely explanation, since TRPML3 can function as a channel when expressed in TRPML1$^{-/-}$ cells (Kim et al., 2009) and knockdown of TRPML1 had no effect of the SN-2-mediated activation of TRPML3 in melanocytes (Grimm et al., 2010). In addition, it is of note that hair cells obtained from the varitint-waddler mouse show robust TRPML3 current (van Aken et al., 2008), indicating than when present at the plasma membrane of hair cells, TRPML3 is active as a channel.

A key future question is how the different activators open TRPML3. Answering this question will provide important information on this and other TRPML channels and possibly provide insight into the function of other TRP channels. A clue for how some of the compounds are gating TRPML3 is provided by the observed synergism between low extracytosolic Na^+ ($Na^+_{e\text{-}cyto}$) and the compounds to activate TRPML3.

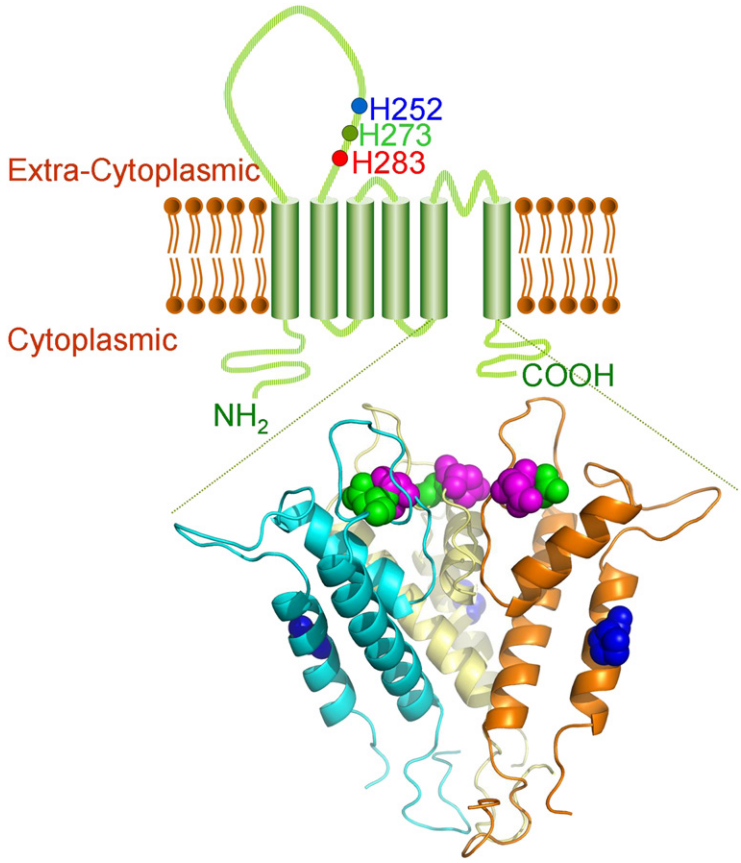

Figure 1. Predicted Topology of TRPML3 and Its Pore Structure
TRPML3 is predicted to have six transmembrane domains with cytoplasmic C and N termini and a large extracytosolic loop between transmembrane domains 1 and 2 that resides in the lumen of intracellular organelles or extracellularly when TRPML3 is in the plasma membrane. Shown are the position of His252, -273, and -283 that regulate TRPML3 activity by H^+ and Na^+ and the predicted structure of the TRPML3 pore. The position of the proline mutated in the varitint-waddler mouse (blue) and the pore aspartates (magenta, green) are also shown. The pore model was created by SWISS-MODEL server's alignment interface, using the KcsA potassium channel as template. A TRPML3 tetramer was created by aligning the TRPML3 model with the KcsA tetramer using PyMol, and the figure was rendered with the same program. We are indebted to Dr. Michael Dorwart, UT Southwestern Medical Center, for help with modeling the TRPML3 pore.

TRPML3 is highly expressed in stereocillia in the maturating organ of Corti, where it is exposed to high K^+, low Na^+ endolymph (Yamasaki et al., 2000). Exposing TRPML3 to low $Na^+_{e\text{-}cyto}$ is necessary to open the channel, and the activated channel spontaneously inactivates in the presence of $Na^+_{e\text{-}cyto}$ (Kim et al., 2008). Grimm et al. tested the relationship between low $Na^+_{e\text{-}cyto}$ and the activators SN-2 and SF-24 and found remarkable synergism between them in activation of TRPML3. Regulation of TRPML3 by $Na^+_{e\text{-}cyto}$ and $H^+_{e\text{-}cyto}$ is mediated by a string of three histidines in the large extracytosolic loop of TRPML3 (Figure 1). Mutations of His283 eliminates inhibition by $H^+_{e\text{-}cyto}$ and $Na^+_{e\text{-}cyto}$, whereas His252 and His273 facilitate the inhibition by increasing access of H^+ and Na^+ to His283 (Kim et al., 2008). It is thus possible that SN-2 and the compounds with the SF-24 scaffold act on the extracytosolic loop, perhaps the domain encompassing His 252, 273, and 283, to open TRPML3.

It is expected that the various TRPML3 activators indentified by Grimm et al. open the channel by multiple mechanisms, since they belong to different chemical scaffolds and some, like SF-11, activate the channel by acting from the cytoplasmic side. It will be of particular interest to reveal the mechanism by which the compounds belonging to different scaffolds and the singletons open TRPML3 and on what TRPML3 domain they act. An obvious target is the TRPML3 pore. Figure 1 shows the predicted architecture of the TRPML3 pore and the position of the A419P mutation in the fifth transmembrane domain (blue) and of two aspartates in the pore helix (green and magenta), which when mutated to alanines results in channel-dead TRPML3 (Kim et al., 2009). Accessing either of these sites from the cytosolic or extracytosolic sides by any of the compounds can result in channel opening. Finding the mechanism of action of the remarkably diverse chemical activators of TRPML3 has the promise of yielding ample information on the properties and the cellular function of TRPML3 and other members of the subfamily.

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