Journey of a cold sensor – VAMP7-dependent transport of TRPM8

Debapriya Ghosh a and Thomas Voets b

 aDepartment of Pharmacology, University of California, Davis, CA, USA; bDepartment of Cellular and Molecular Medicine, Laboratory of Ion Channel Research and TRP channel Research Platform Leuven (TRPLe), KU Leuven, Leuven, Belgium

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TRPM8, a member of the transient receptor potential (TRP) superfamily of cation channels, is expressed in a subset of somatosensory neurons, where it plays a central role in thermosensation, as the principal sensor of innocuous cold temperatures and cooling agents such as menthol or icilin. Pharmacological inhibition or genetic deletion of TRPM8 causes strong deficits in the sensation of innocuously cold temperatures, whereas hyperactivation causes pathological cold hypersensitivity. The (patho)physiological functions of TRPM8 depend on the total ionic current through active channels in the plasma membrane, which in turn is governed by its gating mechanisms, ion permeability and the total number of channels at the site of action. Earlier work has greatly advanced our knowledge about the gating and ion permeability properties of TRPM8, including its regulation by cellular signaling pathways. However, very little insight was available about the intracellular trafficking mechanism of TRPM8 to the plasma membrane (PM), as is true for most other TRP channels. Nevertheless, modulation of the number of active TRP channels at the cell surface represents an important regulatory mechanism under normal and pathophysiological conditions.

Earlier studies had provided evidence that TRPM8 may reside in lipid rafts at the PM, indicated that TRPM8 may exhibit various modes of near membrane trafficking, and suggested that channel stimulation may stimulate incorporation of additional channels at the PM via exocytosis. However, in these earlier studies, information regarding cellular structures and molecular determinants that governed the intracellular trafficking of TRPM8 to the cell surface were not elucidated.

In the article “VAMP7 regulates constitutive membrane incorporation of the cold-activated channel TRPM8” we have elucidated important aspects of the pathway carrying TRPM8 channels to the PM. In the first part of the work, we used Total-Internal-Reflection-Fluorescence-microscopy (TIRFM) to observe near membrane events in live cells. Using this technique, we showed that fluorescently tagged TRPM8 resides in very dynamic vesicles (speed 0.58 µm/s), which exhibit constitutive movement, mainly via microtubules. However, we were unable to measure an increased TRPM8 trafficking to the PM following agonist treatment, in contrast to a previous study. Next, we characterized the nature of these TRPM8-transporting vesicles, by determining which of a list of known markers of intracellular compartments are co-transported within these vesicles. To achieve this, we used dual color TIRFM to simultaneously monitor the movement of TRPM8-mCherry along with GFP-tagged marker proteins. Thus, TRPM8 was considered to dynamically colocalize with specific marker proteins when we observed a vesicular structure exhibiting both GFP and mCherry fluorescence moving alongside. Using this approach, we observed that TRPM8 is primarily found in LAMP1- and Rab7-carrying vesicles.

Although Rab7 and LAMP1 are classically known to as marker of endo-lysosomes, we observed that the TRPM8-containing vesicles were non-acidic in nature, and did not function as protein breakdown organelles.
Moreover, TIR-FRAP\textsuperscript{7} experiments revealed that these vesicles approach the PM from inside the cell, provoking us to hypothesize that these LAMP1 + TRPM8 vesicles may be fusing with PM. This assumption was based on previous studies that noted trafficking of LAMP1 to the PM, and it’s probable role in intracellular vesicle trafficking pathways.\textsuperscript{8} In addition, we also envisioned that the SNARE protein VAMP7,\textsuperscript{3} commonly associated with the fusion of LAMP1-containing vesicles, may act as the fusion machinery for TRPM8-transporting vesicles.

Indeed, we found a high degree of cotransport of TRPM8 and VAMP7. Moreover, using a pH-sensitive GFP (pHluorin),\textsuperscript{6} we were able to demonstrate that TRPM8- and VAMP7-containing vesicles fuse with the plasma membrane. These vesicles do not undergo a classical fuse-and-collapse type of fusion;\textsuperscript{7} instead, they are part of a vesicle-pool constantly interacting with the PM, in either a very transient or more persistent manner. Such an arrangement may be useful to produce a more rapid and dynamic regulation of the number of functional channels at specific sites in the membrane.

Using intracellular calcium imaging and patch-clamp recordings in HEK293 cells we were able to show that VAMP7 increases the number of functional TRPM8 channels at the plasma membrane, whereas a dominant negative VAMP7 had the opposite effect. Moreover, we found that sensory neurons isolated from VAMP7-deficient mice have reduced TRPM8-mediated responses to cold and menthol. Finally, behavior experiments revealed that VAMP7-deficient mice show significant deficits in detecting to innocuous cold, and substantially reduced cold hypersensitive following icilin injection, thereby corroborating the \textit{in vivo} relevance of our \textit{in vitro} finding.

This present study revealed for the first time the cellular constituents of intracellular TRPM8 transport to the PM. Such detailed understanding may have clinical significance for understanding the origin of increased TRPM8 activity in patients with cold allodynia or other TRPM8-related pain syndromes,\textsuperscript{9} and may represent a novel pathway that may be targeted to alleviate such hypersensitivity. Our study also highlighted that, although mostly associated with lysosomes, LAMP1 and VAMP7 may have other cellular functions. One may also ponder whether other TRP channels use this same pathway. The tools and approach documented in this work may be more generally employed to elucidate trafficking of other TRP channels.

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

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