

Modulating the surface expression of cold receptors

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Temperature sensing is essential in every organism in order to adapt and survive. However, the mechanisms by which temperature is perceived and detected remain unclear. Recent findings on thermally-activated ion channels have shed light on the puzzle and unravel molecular entities for temperature detection and transduction in mammals.

Somatic sensation (the ability to feel touch, temperature, and noxious stimuli) requires a set of ion channels to detect and respond to the peripheral environment. Members of the Transient Receptor

Comment on: Toro CA, et al. Agonist-dependent modulation of cell surface expression of the cold receptor TRPM8. *J Neurosci* 2015; 35:571–82; PMID:25589752; <http://dx.doi.org/10.1523/JNEUROSCI.3820-13.2015>

Keywords: cold sensing, membrane dynamics, regulated exocytosis, signal amplification, TRPM8

Abbreviations: TRP, transient receptor potential; TRPM8, Transient receptor potential cation channel subfamily M member 8; PM, plasma membrane; TRPV1, transient receptor potential cation channel subfamily V member 1; AMPA, α -Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

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Submitted: 01/26/2015

Revised: 02/02/2015

Accepted: 02/02/2015

<http://dx.doi.org/10.1080/23328940.2015.1017087>

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Potential (TRP) family of ion channels are important mediators of both cellular sensing and sensory integration, which involves the summing of diverse environmental cues and their subsequent conversion into electrical signals. It is now clear that vesicular trafficking plays a key role in TRP-dependent cell responses. However, the mechanisms associated with the dynamic control of TRP channel density at the plasma membrane (PM) and the consequence of these phenomena at the physiological level are still poorly understood. TRPM8 receptors are expressed in peripheral sensory neurons and are responsible for the detection of environmental cold. Our recent work¹ suggests that agonist-induced recruitment of TRPM8 to the PM sets off a positive-feedback loop in which calcium flux through the channels further increases their expression at the PM. This mechanism may contribute to a rapid and long-lasting amplification of peripheral sensory signals causing a transitory increase in the number of active channels supporting sustained cold responses.

Organisms detect environmental changes in temperature through peripheral sensory nerves innervating the skin and internal organs. The other terminal of these nerves connects to the thalamus and the somatosensory cortex of the central nervous system, where perception of external temperature can occur (i.e. feeling of hot, warm, cool or cold). The cold-and-menthol receptor TRPM8 is responsible for the detection of environmental cold by peripheral nerves.² TRPM8 channels are able to integrate multiple channel-activating signals (e.g., cold, menthol, and voltage), and transduce the stimuli into membrane depolarization via calcium and sodium permeation.³ In previous work, we showed that TRPM8-transporting vesicles undergo distinct patterns of movement in, or very near the PM. The vesicles show a dynamic behavior that involves

vesicles dwelling times up to several seconds within defined regions of the PM which we interpreted as membrane corrals.⁴ We suggested then, that modulation of TRPM8 channels by vesicular translocation and delivery of channels to the cell surface might constitute a regulatory mechanism for channel function.⁴

In our most recent study¹ we used biochemical and imaging techniques to track TRPM8 channels near the plasma membrane and the changes induced by agonist stimulation, showing that TRPM8 expression at the PM is modulated by specific channel agonists. We report that the recruitment of TRPM8-containing vesicles is transitory and sensitive to TRPM8 activation, suggesting a positive feedback of the channel response at the cellular level. In addition, we show that agonist-induced recruitment of vesicles stimulates the exocytotic pathway at the same time that it partially inhibits endocytotic processes, leading to an overall 6-fold increase in expression of functional cold receptors at the cell surface. Our results support a model in which the activation of TRPM8 channels located at the PM induces a short-lived recruitment of a TRPM8-containing vesicular pool to the cell surface (Fig. 1). Using skin-nerve preparations, incubated with botulinum toxin, we demonstrate that pool-recruitment is necessary to sustain intrinsic properties of cold receptor responses. Our results suggest that tightly-regulated trafficking is essential to maintain normal cold sensitivity.

It is well known that calcium ions play a key role not only in promoting vesicular fusion, but also in coupling the modulation between exocytosis and compensatory endocytosis during post-fusion stages. Moreover, local intracellular calcium changes are able to modulate both membrane recruitment and calcium-dependent desensitization of channels. Our results show that calcium influx through TRPM8

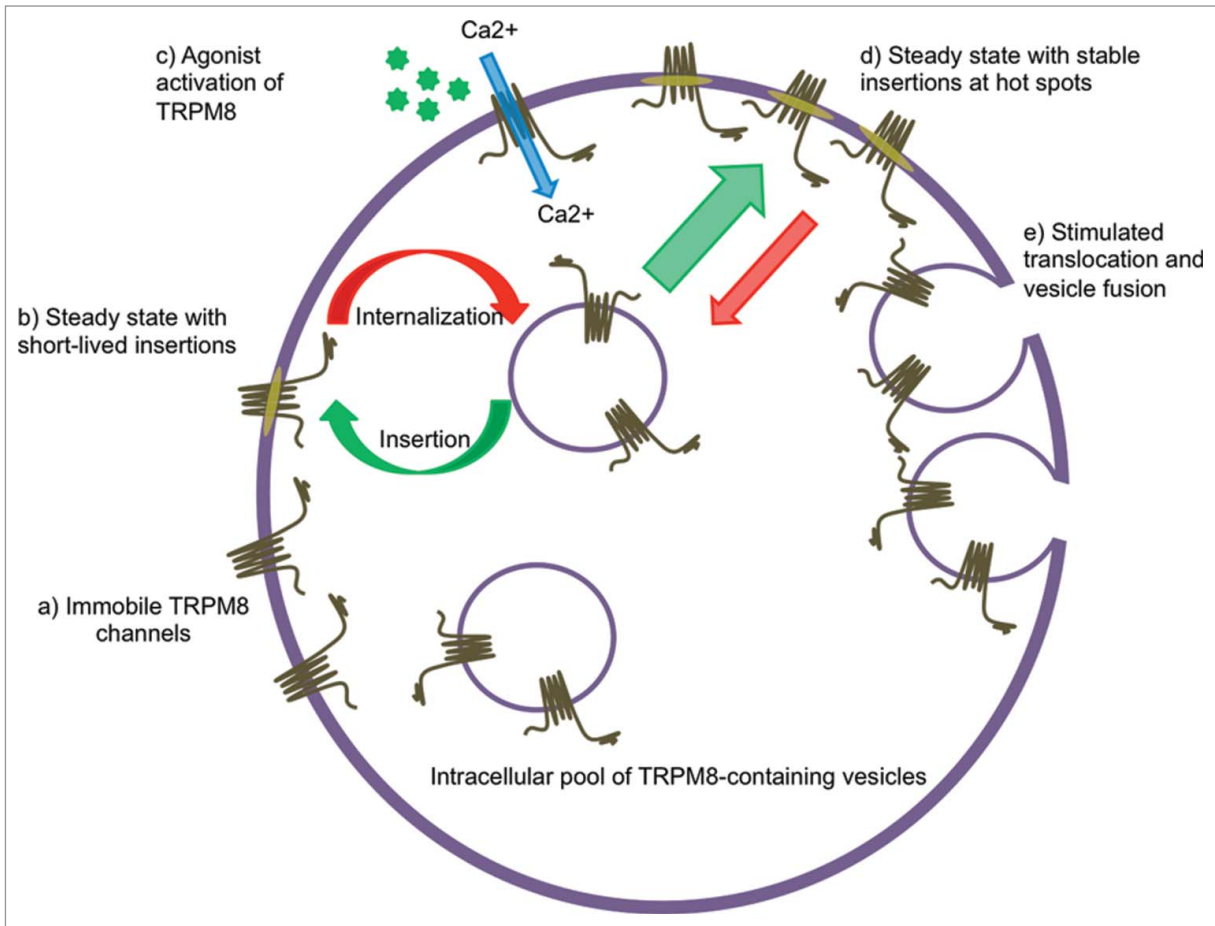


Figure 1. TRPM8 surface expression and model for signal amplification. TRPM8 channels at the plasma membrane are dynamic and relatively constant in number under normal conditions (**A**, **B**). While a small population of channels remain immobilized at the surface (**A**), most channels exist at a steady-state level where channels are continually recycled into and out of the membrane (**B**). In presence of chemical agonists, activated TRPM8 channels generate a localized calcium signal which results in a rapid recruitment and anchoring of TRPM8-containing vesicles to hot spots domains at the surface (**C**), effectively increasing the number of channels at the plasma membrane (**D**, **E**). This positive feedback loop may help the cell reach the depolarization threshold necessary to originate a sensitized cellular response or, alternatively, provide fresh non-desensitized channels helping to maintain a steady cellular response.

channels is necessary and sufficient for the recruitment of TRPM8-containing vesicles to the PM, resulting in positive-feedback between channel activation and expression. Interestingly, the capsaicin receptor, TRPV1, seems to use a similar control mechanism. In that case, external calcium most likely permeating through TRPV1, appear to regulate the lateral diffusion of TRPV1 channels allowing for “channel anchoring” to hot spots in the PM⁵.

We observed that agonist-dependent recruitment of TRPM8 is strong but transitory, providing a window for low threshold activation of the nerve.

A parallel mechanism of regulation has been described, whereby there TRPM8-

channels undergo a profound calcium-dependent desensitization when recorded at whole cell level.⁶ These mechanisms (i.e., recruitment and desensitization) may converge *in vivo* as part of a fine tuned mechanism to control neuronal firing. Moreover, the mechanism we just describe will let fresh non-desensitized channels move to the surface, allowing a sustained response before desensitization of the nerve terminal. Based on our short (30 sec.) stimulation period, our current hypothesis is that under stimulation (i.e., temperature and/or agonist binding), TRPM8 channels located at the PM of nerve endings are able to generate small and localized calcium signals that permit a fast recruitment of a ‘stand-by’ TRPM8-

containing vesicular pool. Calcium then would both promote fusion and help in the stabilization of the vesicles, ultimately altering the number of channels together with the time they spend at the surface in contact with the extracellular environment (Fig. 1).

A positive feedback loop through this mechanism may help a sensory cell to reach its depolarization threshold in order to generate rapid neuronal discharge. Desensitization of the channels then may provide the necessary halt to control the positive feedback. Therefore, post-fusion calcium entry via TRPM8 channels would play an important role in regulated exocytosis controlling cellular excitability. Similar mechanism in

a longer-lasting time scale has been observed before for AMPA receptors, where their intracellular traffic becomes a key mechanism in the control of fast synaptic transmission, therefore providing a 'new' set of non-desensitized receptors to the postsynaptic density, regulating the recovery from postsynaptic depression.⁷

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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