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# A Cool Channel in Cold Transduction

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Transient receptor potential melastatin 8 (TRPM8), a calcium-permeable cation channel activated by cold, cooling compounds and voltage, is the main molecular entity responsible for detection of cold temperatures in the somatosensory system. Here, we review the biophysical properties, physiological role, and near-membrane trafficking of this exciting polymodal ion channel.

From insects to mammals, the transient receptor potential (TRP) channels play important roles in sensory transduction. TRP channels give origin to a super family consisting of seven subfamilies with scarce homology between them. The seven subfamilies are the classical TRP subfamily TRPC, the melastatin-related subfamily TRPM, the vanilloid-sensitive TRP subfamily TRPV, the ankyrin subfamily TRPA, the polycystin subfamily TRPP, the mucolipin subfamily TRPML, and the TRPN subfamily, after the non-mechanoreceptor potential C (nonpC) homologue (78, 97, 123). This review discusses the case of receptor potential melastatin 8 (TRPM8) channel, an ion channel that is the predominant thermoreceptor for cellular and behavioral responses to cold temperatures.

Cold is detected by specific cutaneous thermoreceptor neurons of the somatosensory system, which include unmyelinated primary afferent C-fibers and thinly myelinated Aδ-fibers (12, 44, 47, 49, 53). The transduction of cold stimuli into propagated electrical impulses takes place in the free endings of the thermoreceptor fibers, which correspond to axonal endings of cold-sensitive neurons from trigeminal (TG) and dorsal root ganglion (DRG). At resting temperature of the skin (~34°C), receptors detecting and encoding innocuous cold exhibit spontaneous electrical activity that increases in response to temperature reductions as small as 1°C or less (18). This response is inhibited by heating and sensitized by menthol (20, 46). Cold-thermoreceptor neurons express a wide variety of ion channels, including transduction channels as well as voltage-dependent channels, which give shape to their net excitability. A widely accepted model today maintains that the nonselective $Ca^{2+}$-permeable cationic channel TRPM8 is the main molecular transducer entity responsible for the sensitivity to innocuous cold in the somatosensory system.

## TRPM8 is a Polymodal Receptor

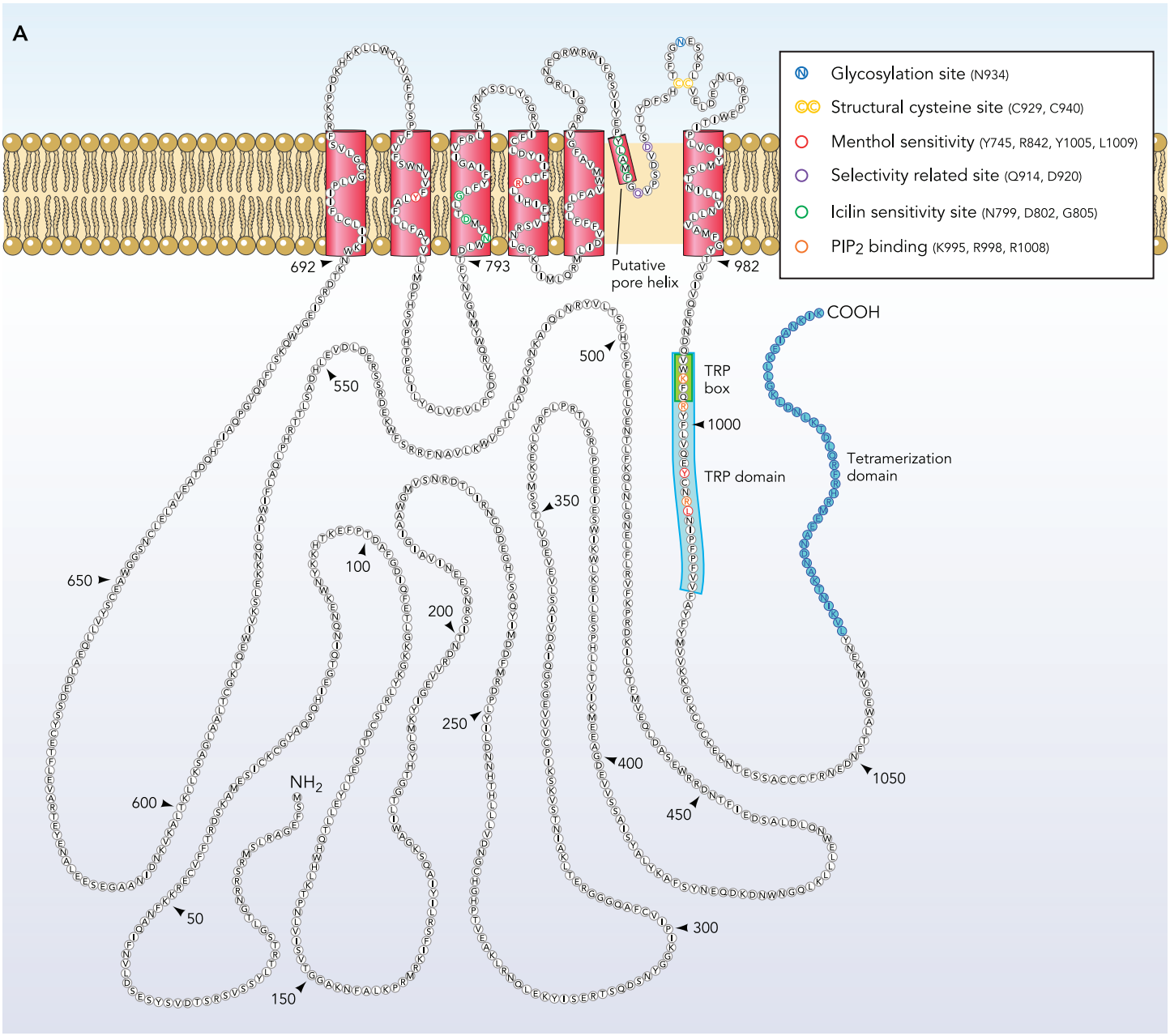
Identified in 2001 as a messenger RNA upregulated in prostate cancer (120), TRPM8 was cloned and characterized in 2002 by two groups independently (75, 90). TRPM8 is a tetramer, with each subunit consisting of six transmembrane domains (S1–S6) and intracellular COOH and $NH_2$ terminals (FIGURE 1). Coiled coil domains located in the distal portion of COOH terminal of the channel appear to be important in the assembly process of TRPM8 (39, 121). Phelps and Gaudet (92) showed that functional channels need the presence of the COOH terminal as well as a region comprised by amino acids 40–86 of the intracellular $NH_2$ terminal. The same authors also showed, however, that deletion of the COOH-terminal region prevents function but not tetramerization.

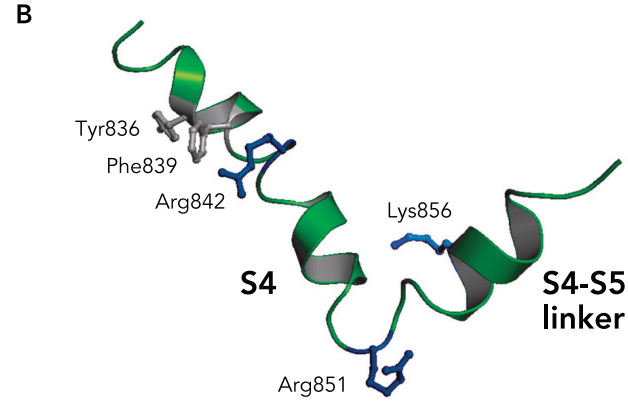
TRPM8 behaves as a polymodal receptor activated by membrane depolarization, cold, and chemical compounds such as menthol, icilin, and several inflammatory agents (16, 66, 75, 90, 125). Also, the activity of TRPM8 appears to require the presence of phosphatidylinositol-4,5-biphosphate [$PI(4,5)P_2$] (31, 102) (see below).

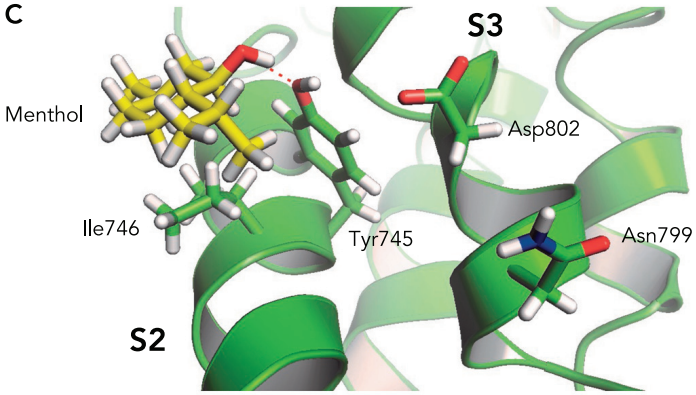
### *Voltage Dependence and Ion Selectivity*

TRPM8 is a channel with a weak voltage dependence, and in the absence of agonists activation requires strongly depolarized membrane voltages (16, 125). Although the voltage sensor domain remains elusive (see Ref. 65), neutralization of positively charged residues in the S4 of TRPM8 causes a decrease in its voltage dependence (FIGURE 1, A AND B)(126). Voets et al. (126) neutralized all the charged residues contained in the S4 segment and in the S4-S5 linker of the TRPM8 channel. They found that the total apparent number of gating charges per channel is 0.85*e* on average and that neutralization of R842 in S4 and K856 located in the S4-S5 linker (FIGURE 1B) decreased this number to 0.7 and 0.5*e*, respectively. These findings suggest that the contribution of these two charges to the total amount of gating charges/channel is not enough to explain the global voltage dependency of the channel (0.85*e*). Therefore, it is possible that at least part of the total gating charge is actually located in another position within the channel structure. It is noteworthy that TRPM2 has a S4 with the same pattern of positively charged residues as the S4 of TRPM8 but is utterly voltage insensitive. However, a chimera containing the putative voltage sensor (S1–S4) of TRPM2 and the

S5-pore-S6 domain of TRPM8 is voltage dependent, albeit with a conductance-voltage curve shifted to hyperpolarizing voltages compared with that of TRPM8 (61). Although this result can be interpreted as a restoration of the coupling between the voltage sensor and the activation gate



induced by the pore exchange, it can be also construed as if part of the TRPM8 voltage sensitivity is due to charges or dipoles located in the pore region.

TRPM8 is a nonselective cation channel. Ion substitution experiments showed little discrimination among monovalent cations but revealed significantly higher permeability for calcium ions ($P_{Ca}/P_{Na}$ = 3.2; $P_K/P_{Na}$ = 1.1; $P_{Cs}/P_K$ = 1.2) (75). Sequence comparison of the S5-S6 loop indicates that this region is well conserved among all members of the TRP family, with highly conserved hydrophobic residues present at the beginning of the region of the pore (Y908 to F912; FIGURE 1A) and an invariant aspartate in position 920 (80, 91, 117). The neutralization of D984 in TRPM4 (D920 in TRPM8) results in a nonfunctional channel with a dominant negative phenotype when co-expressed with wild-type TRPM4. Substitution of Q977 (Q914 in TRPM8) by a glutamate altered the monovalent cation permeability sequence and results in a pore with moderate $Ca^{2+}$ permeability (80).

### *TRPM8 is a Cold Sensor*

TRPM8 is directly activated by slight cooling (temperature threshold of ~22–34°C) and depolarizes sensory neurons (5, 74, 98). A thermodynamic analysis of the TRPM8 induced ionic currents indicate that the amazing temperature-dependence of this channel ($Q_{10}$ of ~25) is mediated by a large enthalpic change [$\Delta H = -150$ kcal/mol (16)] associated with the channel opening reaction. For the opening transition to be reversible, entropy changes must compensate the large enthalpic changes ($T\Delta S = -113$ kcal/mol). The sign of the entropy change indicates that the open state of TRPM8 is more ordered than the closed one. The molecular determinants of the TRPM8 activation by cold are still unknown, but Brauchi et al. (17), by swapping the COOH terminals between TRPM8 and TRPV1 [a heat receptor (24)], showed that this domain confers the temperature-dependence phenotype.

Recently, the Rohács group (134) accomplished the feat of reconstituting TRPM8 into planar lipid bilayer membranes. The TRPM8 channel-forming protein was purified either using bacterial expression or from TRPM8 cDNA-transfected HEK cells, and channel incorporation was obtained by adding a TRPM8 micellar solution to one side of the bilayer only. The importance of this work resides in the fact that it shows irrefutably that TRPM8 channels are directly activated by cold despite the fact of being inserted into a lipid milieu completely different from the cell lipid environment. Although the protein was purified in the presence of detergent and in the absence of lipids, we cannot discard at present the possibility that tightly bound phospholipids to the protein are important in determining the sensitivity to cold of the TRPM8 channel.

### *Agonist Activation*

TRPM8 responds to different agonists such as menthol, icilin, and eucalyptol (75, 90). Unlike menthol, the activation of TRPM8 by icilin requires the presence of intracellular $Ca^{2+}$ and is modulated by intracellular pH (2, 28, 75). The icilin binding site is located within the S2-S3 linker in the analogous zone where capsaicin is stabilized in TRPV1 and N799, D802, and G805 are required for icilin sensitivity of mammalian TRPM8 (28, 89). Bandell et al. (8) showed that residues involved in menthol activation are Y745 in S2 (FIGURE 1C) and Y1005 and L1009 located in the channel COOH terminal; interestingly, the first one is close to S2 and S3, and the last two residues are part of the TRP box domain. Mutations in the S4 and in the S4-S5 linker also affect menthol affinity (126).

All the evidence obtained in ion channels and other proteins indicates that $PI(4,5)P_2$ interactions with the polypeptide chain are electrostatic in origin; the negative charges of the phosphate groups in $PI(4,5)P_2$ interact with positively charged residues in the protein (e.g., Refs. 76, 77, 103). In the case of TRPM8, neutralization of lysines and arginines located in the proximal part of the COOH terminus, the TRP domain, greatly decreases the sensitivity to $PI(4,5)P_2$ (102).

## TRPM8 as the Main Receptor of Cold in Primary Sensory Neurons

TRPM8 is expressed mainly in TG and DRG neurons, although in the peripheral nervous system there is evidence of TRPM8 expression also in nodose ganglia (136) and in the geniculate ganglia (59). TRPM8 has been also identified in prostate

FIGURE 1. **Amino acid sequence of the rat TRPM8 channel subunit**
*A*: the TRPM8 subunit has a very large $NH_2$ terminal (692 amino acid residues) that at difference of most other TRP channels subfamilies lacks ankyrin domains. Each subunit contains six transmembrane domains and, as in voltage-dependent $K^+$ channels, can be divided in a voltage sensor module (S1–S4) and a pore module (S5-pore helix-selectivity filter-S6). The pore module contains sites for glycosilation and two structural cysteines. The COOH terminal contains the TRP domain, a sort of signature sequence for TRP channels, and the TRP box defined by the consensus sequence WKFQR, where W is the only conserved residue among TRP families. The COOH terminal also includes the residues involved in channel modulation by $PI(4,5)P_2$ and a tetramerization domain. Image was modified from Ref. 64 and used with permission. *B*: Arg842 and Lys856 in S4 appear to be the residues in charge of sensing voltage. *C*: residues involved in binding of menthol (Y745, I746) and icilin (N799, D802). The structure of the S4 and S4–S5 linker and of S2–S3 of TRPM8 was obtained using the PDB of the structural model of TRPM8 developed by Pedretti et al. (89) on the basis of the crystal structure of the Kv1.2 and molecular dynamics.

and genitourinary tract (112, 115), lung (105), liver (40, 48), vascular smooth muscle (54, 132), bladder (112), sperm (32, 73), and odontoblasts (38), and has been also associated with an important variety of tumors (14, 27, 120, 131, 133), where its role is not entirely understood. Anatomical evidence of the expression pattern of TRPM8 was recently provided by using transgenic mice expressing GFP protein under control of the channel promoter (34, 88, 113, 114). Central afferent projections of TRPM8-positive DRG neurons are restricted mainly to the lamina I and IIo into the spinal cord (34, 113, 128). Somatosensory neurons that express this channel show immunoreactivity to some classical somatosensory and nociceptive markers. In subpopulations of primary sensory neurons, TRPM8 co-localizes with peripherin, a marker of C-fibers, with intermediate filaments NF200, a marker of Aδ fibers, and does not appear to co-express with the neuronal marker for nonpeptidergic neurons isolectin-IB4 (113). Both functional and immunohistological evidence suggest that, in a subset of somatosensory neurons, TRPM8 also co-expresses with classical nociceptor markers, such as TRPV1 channels, calcitonin gene-related peptide (CGRP), and substance P (4, 6, 34, 85, 113, 124, 130).

In parallel to the molecular cloning of TRPM8, a subpopulation of TG and DRG neurons that respond specifically to low temperatures was identified in culture by using $Ca^{2+}$-imaging and patch-clamp recordings (100, 116, 124). These neurons are sensitive to menthol (FIGURE 2, A AND B), and they respond to cooling with the development of a depolarizing inward current (FIGURE 2, C–E) with biophysical and pharmacological properties consistent with TRPM8 currents observed in heterologous expression systems (68, 69, 99). 4-(3-Chloro-pyridin-2-yl)-piperazine-1-carboxylic acid (4-tert-butyl-phenyl)-amide (BCTC) is a strong blocker of TRPM8 ion channels. In trigeminal neurons in culture, BCTC produces a dose-dependent and reversible inhibition of the cold and menthol responses of TRPM8-positive cold-sensitive neurons (69) (FIGURE 2). Three groups developed genetically modified mice that lacked functional expression of TRPM8 ion channels (10, 29, 35). Behavioral and electrophysiological experiments in these knockout mice show that their cold sensitivity is strongly compromised, advocating a central role for TRPM8 in the detection of innocuous cold in vivo.

There is a remarkable difference in the activation threshold by cooling between recombinant and native TRPM8 channels, which are more sensitive to temperature than the recombinant form. This discrepancy is not due to differences in the expression levels of the protein (33). In 2007, Mälkiä et al. (72) demonstrated that voltage-dependence of native channels is shifted toward hyperpolarizing values compared with the recombinant TRPM8, which results in a shift of the thermal threshold of cold-sensitive neurons to warmer temperatures. The molecular bases underlying this difference are still poorly understood.

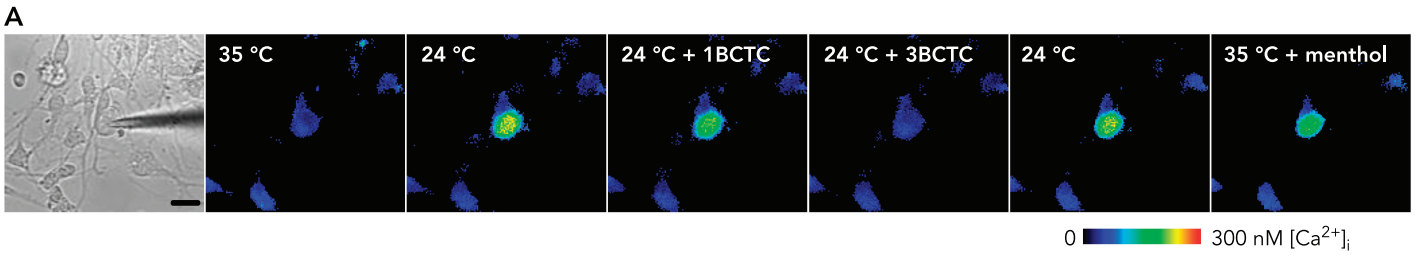
### *Modulation of TRPM8 in Sensory Neurons*

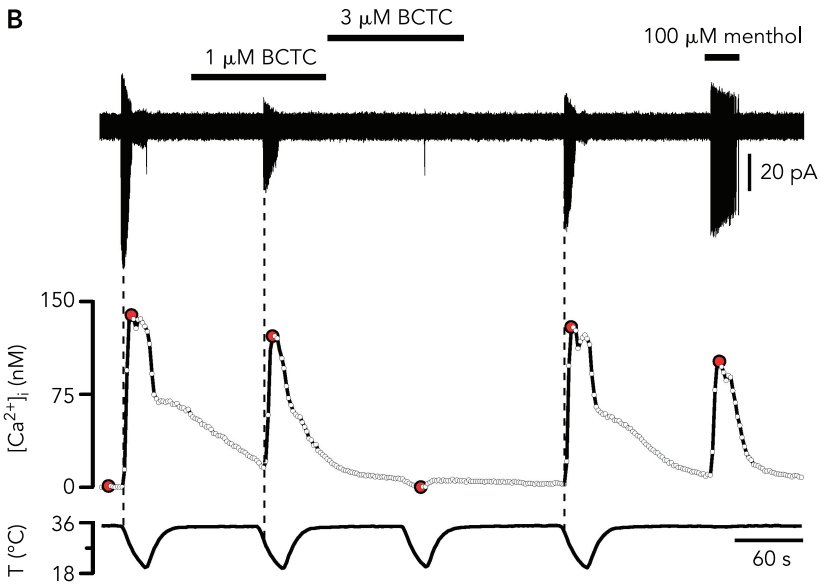
Several cellular signaling cascades may be involved in the regulation of TRPM8 activity in sensory neurons (FIGURE 3A). Activation of TRPM8 by physical (cold) or chemical (menthol) stimulation is followed by channel desensitization, which depends on extracellular $Ca^{2+}$ (75, 99), and there is evidence for an involvement of $PI(4,5)P_2$ in this process. $PI(4,5)P_2$ acts as a positive modulator of cold or menthol sensitivity of TRPM8, most likely by shifting the voltage-sensitivity of activation toward physiological voltages, and prevents current run-down in cell-free patches (55, 102). It has been proposed that $Ca^{2+}$ influx through TRPM8 leads to activation of $Ca^{2+}$-dependent phospholipase C, inducing a reduction of $PI(4,5)P_2$ levels and channel desensitization (102). A complementary pathway for TRPM8 desensitization may occur via activation of $Ca^{2+}$-dependent protein kinase C (PKC) (1, 95), which indirectly causes dephosphorylation of TRPM8 via protein phosphatase 1, leading to down-regulation of the channel (95). The sensitivity of

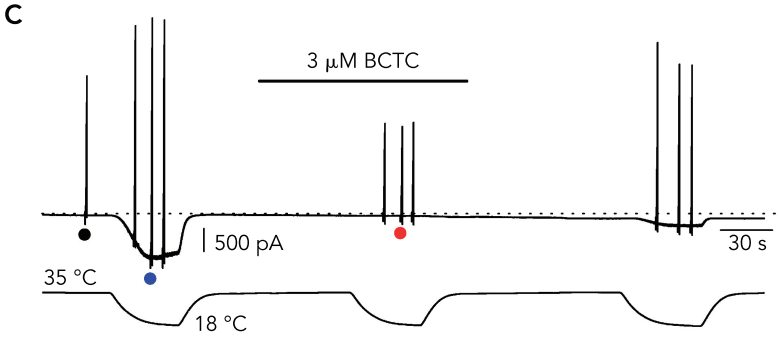
FIGURE 2. **Excitatory response of cold-sensitive trigeminal neurons to mild temperature reductions depends on TRPM8**
*A*: Transmitted (left) and pseudocolor ratiometric $[Ca^{2+}]_i$ images showing the effects of BCTC on cold-evoked $[Ca^{2+}]_i$ signals in a cold-sensitive trigeminal neuron. Note also the response to menthol. A patch pipette was positioned in close apposition to the cold-sensitive cell before initiating the sequence of cell-attach recordings. The fluorescence images correspond with the time points marked in red in (*B*). *B:* Simultaneous recording of action currents in cell-attached (top trace), $[Ca^{2+}]_i$ signals (middle trace) and bath temperature (bottom trace) during 4 consecutive cooling ramps. The two insets at the bottom show the action currents and the temperature change on an expanded time scale, in control (left) and 1 μM BCTC. The temperature threshold is marked by red arrowheads. The black arrow marks, on the control trace, the temperature threshold in 1 μM BCTC. Note the strong shift in temperature threshold in this condition. *C*: Simultaneous recording of membrane current (top trace) and bath temperature (bottom trace) during application of 3 consecutive cooling ramps to a cold-sensitive neuron; $V_{hold}$= −60 mV. The spike-like currents are the responses to voltage-ramps (−100 to +100 mV). Application of saturating concentration of BCTC (3 μM) fully blocked $I_{cold}$. The dotted line represents the zero current level. *D:* Current-voltage relationship of the cold-sensitive (blue trace) and BCTC-sensitive (red trace) current obtained during the voltage ramps in (*C*). To obtain the cold-sensitive current, the ramp current at 35°C (black dot) was subtracted from the current at 20°C (blue dot). To derive the BCTC-sensitive current, the ramp current at 20°C in BCTC (red dot) was subtracted from the current at 20°C in control solution (blue dot). Note that BCTC is less effective at positive membrane potentials. *E*: Current-temperature relationships for $I_{cold}$ in a different neuron in control (blue trace) and in the presence of 1 μM (black trace) and 3 μM BCTC (red trace). Note the marked shift in temperature threshold and the strong effect of the TRPM8 blocker on the cold-induced current. Modified from Madrid *et al.* (69).

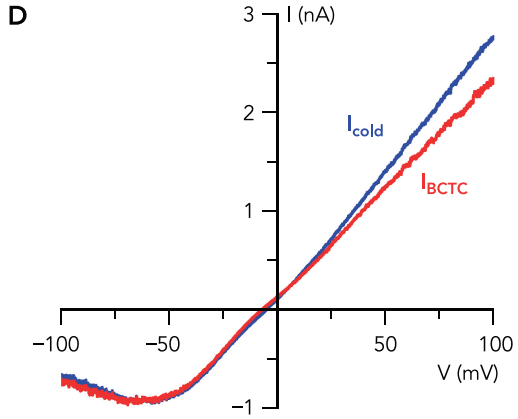
TRPM8 to PKC and $PI(4,5)P_2$ suggests that in vivo TRPM8 could be highly sensitive to stimulation of phospholipase C (PLC)-coupled receptors. Activation of PLC would have a dual inhibitory effect on the channel via a reduction of cellular $PI(4,5)P_2$ levels and via a diacyl glycerol-induced activation of PKC. Recently, Daniels et al. (31) showed evidence supporting the notion that PLC activity mediates adaptation of TRPM8 to thermal stimuli.

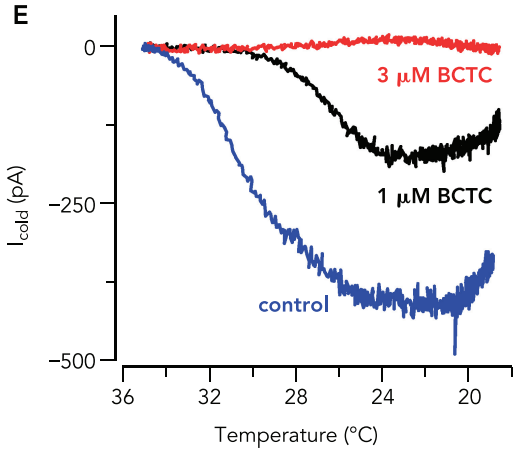
Bradykinin and prostaglandin E2, two pro-inflammatory mediators, applied acutely reduce the responses to cold and menthol in putative TRPM8-positive neurons, possibly by PKC and protein kinase A (PKA)-dependent mechanisms, respectively (66). Phospholipase A2 (PLA2) could also affect the function of TRPM8, by generating polyunsaturated fatty acids (PUFAs) and lysophospholipids from glycerophospholipids that modulate the channel in opposite directions (3, 42). On the other hand, $G_i$-coupled α2A-adrenoreceptor (α2A-AR), which is also expressed in sensory neurons, could modulate the function of TRPM8; in DRG neurons, stimulation of



α2A-AR reduces the activity of the channel by a PKA-dependent phosphorylation mechanism (11). TRPM8 can also co-express with trkA, the high-affinity tyrosine kinase receptor for NGF (90). Application of NGF results in an upregulation of the channel function in cultured DRG neurons (6). The physiological relevance of these regulatory mechanisms on TRPM8 channel function has not yet been entirely clarified.

### *TRPM8 in Acute and Chronic Cold-Induced Pain and Dysesthesias*

There is important evidence showing that TRPM8-expressing neurons could participate in sensing of not only innocuous but also unpleasant or painful cold (10, 12, 21, 43, 68, 113, 127, 130). The fact that deletion of this channel dramatically reduces the number of fibers sensitive to cold temperatures, even in the noxious cold range, suggests that

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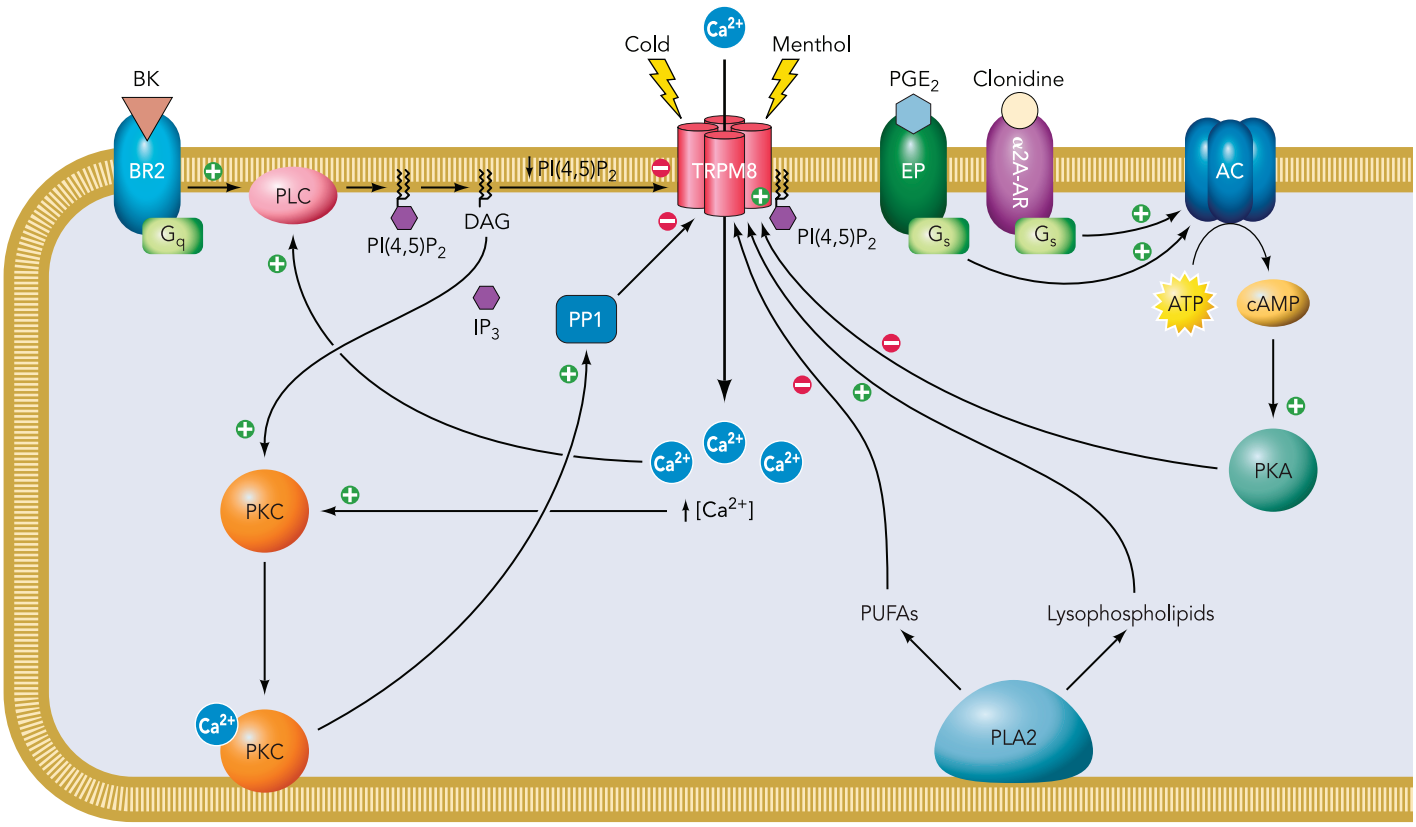


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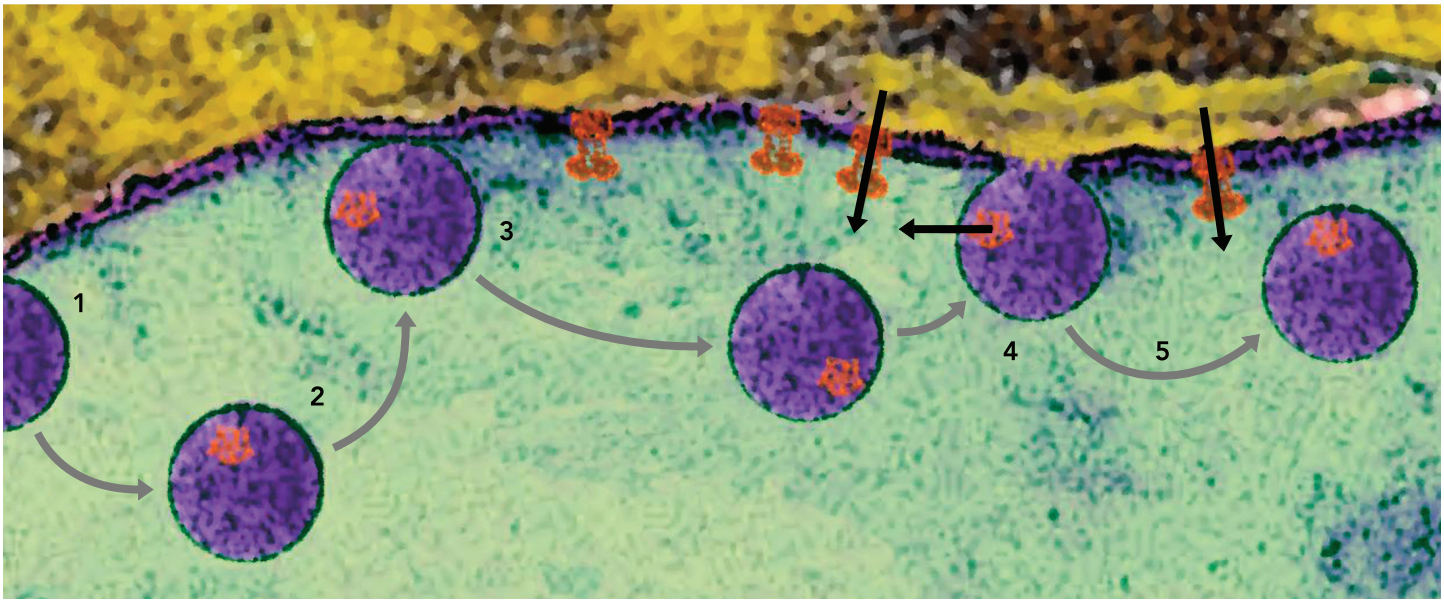

TRPM8 expression is not only limited to innocuous cold-sensing neurons but that the channel is also expressed in neurons connected with nociceptive routes. On the other hand, an increase in TRPM8 expression has been linked to the development and maintenance of cold allodynia and hyperalgesia after peripheral nerve injury in somatosensory neurons (41, 129). However, strong evidence suggests that these pathological states are not necessarily correlated with significant variations in TRPM8 channel expression (23, 58, 79). Interestingly, it has been suggested that TRPM8 may also have an important role in analgesia under chronic pain states (96). Recently, Parra et al. (88) provided evidence of a new physiological role of TRPM8-expressing sensory neurons. TRPM8-dependent cold thermoreceptors of the cornea maintain a tonic ongoing impulse activity exquisitely sensitive to cold. Suppression of ongoing and cold-evoked activity by abrogation of TRPM8 reduces the basal tear secretion. This work unveiled an unknown role for peripheral TRPM8-positive cold thermoreceptors as regulators of surface wetness of the eye and a possible role for TRPM8 in pathologies related to dryness of body mucosae. Thus the picture of the role of TRPM8 in pain and dysesthesias is still emerging.

## Near Membrane Trafficking

TRPM channels have been described not exclusively at the plasma membrane (PM) but also at intracellular membranes (36, 118). Since several members of the TRPM family have been implicated in human diseases (81), knowledge regarding channel intracellular localization, trafficking, and recruitment to the PM may help researchers and clinicians in the control of disease onset and progression.

### *TRPM8 Channels at Intracellular Membranes*

It is not rare nowadays to read reports about the presence of TRPM channels at intracellular membranes or associated to exocytic vesicles. This is the case of TRPM1(83), TRPM2(63), TRPM4 (30), and TRPM7 (15, 60).

Following the same trend, TRPM8 channels have been reported to be localized at the ER membrane. In the absence of external calcium, menthol or cold induces an increase of $[Ca^{2+}]_i$ in human prostate cancer cells (LNCaP), opening the possibility for temperature-dependent signaling cascades operating within micro-domains near the ER/golgi compartment. This increase was shown to be sensitive to thapsigargin, suggesting that TRPM8 stimulation causes release of $Ca^{2+}$ from ER stores (135). Later results suggest that this reticular TRPM8 would support $Ca^{2+}$ release from the ER, further activating an androgen-dependent store-operated calcium conductance (115). On the contrary, TRPM8 expressed in HEK-293 cells exclusively targets the PM (115). However, a separate study challenged those results by showing the inefficiency of the antibody used in previous studies and that the intracellular menthol-dependent $Ca^{2+}$ response can be independent of TRPM8 expression (70). Further experiments are needed to clarify the potential role of TRPM8 channels at the ER membrane.

### *TRP Channel Translocation as Part of a Regulatory Machinery*

Many membrane receptors and channels undergo regulated exocytosis to and endocytosis from the plasma membrane (PM). Some examples include regulated translocation of AMPA, NMDA, and GABA receptors (9, 119). Regulated exocytosis has also been reported to control TRP channel-mediated currents (25, 118). Insertion of vesicles containing TRPs into PM can alter current amplitude by regulating the number of functional channels at the cell surface as demonstrated for TRPV2 (57), TRPC5 (13), TRPC6 (26), TRPV1 (111, 137), TRPM7 (84), TRPV5 (62), TRPA1 (109), TRPM4 (30), and TRPM8 (122). Although the mentioned TRP channels have been convincingly demonstrated to increase at the PM in response to specific stimuli, the mechanisms associated with this dynamic control of TRP

FIGURE 3. **Modulation of TRPM8 by intracellular signaling and vesicle trafficking**
*A*: TRPM8 can be modulated by diverse signaling cascades in sensory neurons. TRPM8 ion channel is represented with four subunits in the scheme. Activation of phospholipase C (PLC) through the activation of bradikynin B2 receptor (B2R) induces a reduction in $PI(4,5)P_2$ levels in the plasma membrane by cleavage of $PI(4,5)P_2$ in dyacilglycerol (DAG) and inositol triphosphate ($IP_3$), leading to desensitization of the channel. Activation of prostaglandin receptors (EP) or $\alpha$2A adrenoreceptors ($\alpha$2A-AR) induces an increase in cyclic adenosine monophosphate (cAMP) by activation of adenylate cyclases (AC). cAMP activates protein kinase A (PKA), which induces a downregulation of the channel by an unknown mechanism. Activation of TRPM8 ion channels by cold or menthol can induce an increase in the intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$). An intracellular $Ca^{2+}$ increase activates PKC, which can modulate protein phosphatase 1 (PP1) leading to the inhibition of the channel by dephosphorylation. On the other hand, increases in phospholipase A2 (PLA2) activity can generate polyunsaturated fatty acids (PUFAs) and lysophospholipids from glycerophospholipids; PUFAs exert inhibitory effects on TRPM8, and lysophospholipids upregulate the channel function. *B*: TRPM8 hop-diffusion process. The picture portrays the process of a vesicle (purple) containing TRPM8 channels (orange). Yellow shades correspond to the extracellular space. Gray arrows indicate vesicle transit, and black arrows indicate TRPM8 conductance. Numbers indicate the different steps of the process. *1* and *3* represent the docked vesicle waiting for a specific signal to fuse; *2* represents the absence of an adequate signal allowing the vesicle to continue with its hopping process; *4* corresponds to the fused vesicle contributing to the whole cell current; *5* corresponds to the detachment of the intact vesicle. Note that we hypothesize that vesicle fusion occurs near an active TRPM8 patch, amplifying the calcium signal. For details, see Ref. 122.

channel density at the PM are still largely unknown.

### *TRPM8's Hop-Diffusion and a Kiss that is Sure to Linger*

At least three modes of vesicle fusion into the PM have been proposed: full collapse, kiss-and-run, and kiss-and-linger (104). PM recruitment of TRPC5-, TRPV5-, and TRPM8-containing vesicles are characterized by longer membrane dwelling times (in the order of seconds) than what would be expected for a kiss-and-run fusion. Considering the fact that these TRP-transporting vesicles retain their integrity after fusion, these observations are in good agreement with a kiss-and-linger mechanism for channel insertion (13, 62, 122).

By means of TIRF microscopy and single-particle tracking, TRPM8 was observed in vesicles that constitutively undergo distinct patterns of movement, including rapid lateral movement in or very near the PM, and axial (*z*) movements into and out of the near field (i.e., exo- and endocytosis) (122). TRPM8-transporting vesicles approach the PM and fuse retaining integrity, resulting in dwelling times up to a few seconds confined within defined PM corrals. Moreover, TRPM8-transporting vesicles undergo hop-diffusion, jumping from one corral to the next, lingering for few seconds at every time. Such stabilization of the fused vesicle for longer times may allow channels access to the extracellular solution via the lumen of the fused vesicle, thereby permitting vesicle-associated channels to contribute to membrane currents that are measured with whole-cell voltage clamp (122) (FIGURE 3B). It is worth noticing that the mechanism of hop-diffusion/kiss-and-linger described by Veliz et al. (122) for TRPM8 channels differs from the rapid vesicular insertion (RiVIT) mechanism proposed by Bezzerides et al. (13) for TRPC5 channels, where the rate of vesicular fusion was clearly shown to increase. Such differences may point toward different regulatory elements specific for different TRP channel members. Future studies in native systems (e.g., DRG neurons), will be required to determine whether the mechanisms controlling TRPM8 channel residency on the PM are similar to those observed in heterologous expression systems.

### *Regulated Exocytosis of TRPM8 Channels as Part of a Cellular Signal-Amplification Mechanism*

From biophysical and physiological studies, we know that TRPM8 channels are capable of integrating multiple concomitant channel-activating signals (i.e., cold, menthol, and voltage) and transduce these stimuli via calcium influx and membrane depolarization. On the other hand, from trafficking studies, we can speculate that vesicle stabilization at the plasma membrane arises from a positive feedback loop, where immobile TRPM8 channels at the PM could be thought of as signal feed and the dynamic hop-diffusing pool that transits between corrals as the potential amplification power (FIGURE 3B). If this mobile population represents a reserve of TRPM8 channels that are "on-hold" awaiting an appropriate docking spot and/or specific stimuli for PM insertion, adding these channels could increase the number of available channels and thereby provide a significant amount of signal amplification. Additional regulatory complexity would be provided if TRPM8 channel activity also controls the trafficking of TRPM8-containing vesicles (e.g., by TRPM8-mediated $Ca^{2+}$ flux).

Some important questions remain to be addressed. *1*) Is TRPM8 channel activity relevant to a specific cell signaling process? *2*) Do TRPM8 channels normally mediate ionic conductance in intracellular membranes? If so, what controls channel opening? *3*) Does TRPM8 channel conductance affect the trafficking of vesicles on which they are being transported? *4*) Is regulated exocytosis a common theme for the control of TRP channel-dependent cell response?

## TRPM8 in the Big Picture of Cold Transduction

The view of TRPM8 as the molecular sensor for cold sensation has dominated since its cloning (75, 90), although it was not truly confirmed until the characterization of $TRPM8^{(-/-)}$ mice (10, 29, 35). However, these "molecular" years were preceded by more than 50 years of physiological characterization of cold thermoreceptors (7, 37, 45, 47, 52). Other proteins have also been proposed to play the role of transducing cold into electrical potentials, and growing molecular evidence shows that TRPM8 is a piece to be fit into a more complex picture.

### *The Complex Life of Cold Receptors*

Mammalian thermoreceptor nerve endings that respond in the innocuous cold temperature range behave somewhat differently than other somatosensory receptors. They show a regular ongoing spiking activity at normal skin temperature that can be of a tonic or bursting nature and which is accelerated upon cooling the receptive field and suppressed by warming (18, 20). The change of firing rate upon a temperature change suffers a strong adaptation in less than a minute, and the relationship between temperature and stationary (adapted) firing rate has a bell shape with a

maximum between 20 and 30°C that cannot encode the stimulus unambiguously. This is apparently solved thanks to a dramatic change of firing pattern (FIGURE 4A): at low steady temperatures, the interval between spikes is increased, but at the same time bursting is promoted so that single spiking events are rarely seen. On the contrary, at temperatures above 30–32°C, single spikes prevail and "skipping" events are evidenced as intervals that are the double (or higher multiples) of the mean (18). The variety of firing patterns is reproduced by mathematical models (19, 51, 67) based on a general model of slow wave bursting (94). In this type of model, regular spiking or bursting is driven by a slow membrane potential oscillation, and all that it takes to change its firing pattern from tonic to bursting as temperature decreases is to consider the usual effects of temperature on ion channels

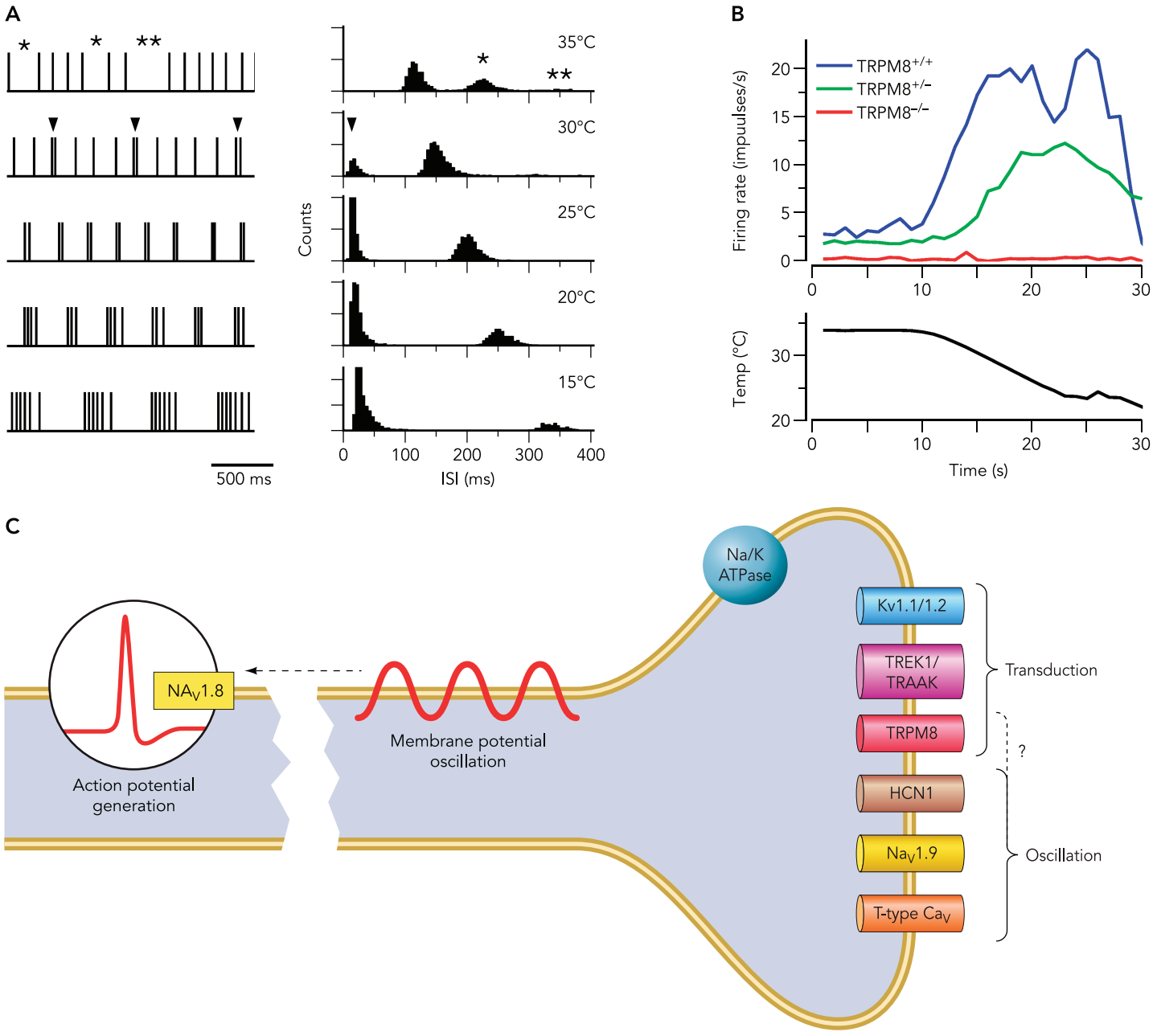


FIGURE 4. **The complex life of cold receptors**
*A*: typical firing patterns (*left*) and inter-spike intervals (ISI) histograms (*right*) that can be observed in extracellular recordings of cold-sensitive nerve endings or nerve fibers at the static temperatures indicated. Asterisks denote skipping events, corresponding to intervals that are multiples of the main peak of the histogram. Arrowheads indicate bursting events. The figure was generated using a conductance-based model based on Ref. 19 and tuned to resemble experimental recordings found in the literature (7, 18, 107). The model includes fast voltage-dependent sodium and potassium currents, a slow voltage-dependent sodium/calcium current, intracellular calcium dynamics, and a calcium-activated potassium current. Gating kinetics have a $Q_{10}$ of 3, and ionic currents have a $Q_{10}$ of 1.3. *B*: mean firing rate (*top*) of corneal cold-sensitive nerve endings from $TRPM8^{(+/+)}$, $TRMP8^{(+/-)}$, and $TRPM8^{(-/-)}$ mice during a cooling ramp (*bottom*). Adapted from Figure 2A of Ref. 88 and used with permission. *C*: the variety of molecular mechanisms presumed to be involved in cold transduction and their proposed roles. The site of actual cold transduction (*right*) is depicted apart from the site of action potential generation (*left*) (22).

($Q_{10}$ values of 3 for kinetics and 1.3 for conductance). Regarding the actual molecular mechanisms involved, experimental evidence points to the involvement of slow TTX-resistant sodium channels (20), probably $Na_v1.9$ (50), and the entry of extracellular calcium through T-type voltage-activated calcium channels (108).

So where does TRPM8 fit in this aspect of cold transduction? It is remarkable that the whole variety of stationary firing patterns is reproduced in a model that lacks TRPM8 or any conductance specifically modulated by temperature (19). Then, it is tempting to assume that this channel does not play a role in the generation of regular firing patterns, especially at normal skin temperatures at which it is supposed to be closed. However, nerve endings and fibers from $TRPM8^{(-/-)}$ mice not only lack any response to cold but also lack any regular ongoing activity (10, 88). Remarkably, cold-sensitive nerve endings from $TRPM8^{(+/-)}$ mice show an ongoing activity and response to cold with approximately half the frequency of wild-type nerve endings (88) (FIGURE 4B). Whether TRMP8 plays a rather passive role helping to set the membrane potential or plays a dynamical role in the slow oscillation remains to be elucidated.

The molecular determinants of the acute response to temperature changes (the dynamic response of cold receptors), on the other hand, remain to be determined, and there is no mathematical model that accounts for it. As mentioned before, TRPM8 undergoes an adaptation or desensitization that is dependent on extracellular calcium and involves phospholipase-C activation and $PI(4,5)P_2$ depletion (31, 75, 99, 102). The temporal scales of this process and of the adaptation in cold receptors are similar, suggesting that TRPM8 adaptation underlies the dynamic response in cold nerve endings. This, however, remains to be tested experimentally.

### *Other Channels Involved in Cold Transduction*

The first proposals of cold transduction mechanisms were related to the function of the $Na^+/K^+$ pump, since the treatment of cold receptors with ouabain increases their firing rate (93, 106, 110). Afterward, the use of cell culture and patch clamp tools revealed that its contribution is minor (100). However, these techniques, together with molecular cloning tools, have shown that several ion channels are involved in transducing cold.

When cultured cold-sensitive neurons from the trigeminal or dorsal root ganglions are exposed to a cold stimulus, a notorious inward current is developed, which is now attributed to the opening of TRPM8 channels (33, 69, 99). However, there is also a decrease of membrane conductance (100, 124) attributed to the cold-induced closing of background $K^+$ channels from the two-pore domain family, TREK1 and/or TRAAK (56, 71). Moreover, mice lacking these channels have an altered cold and warm perception (82). Another channel reported to play a role in cold transduction is HCN1, which provides a hyperpolarization-activated and cyclic nucleotide-modulated current that reverses around −30 mV (87, 101). This current is present in cultured cold-sensitive neurons (86, 124), and although it does not seem to be involved in the response to acute cooling, mice lacking the HCN1 gene show an altered cold perception (86). Also, cold sensitivity in these cells is dampened by the expression of $I_{KD}$, an inhibitory outward slow-inactivating $K^+$ current (68, 116, 124). The low activation threshold of $I_{KD}$ and its slow inactivation implies that this current acts as an excitability brake that counteracts the depolarizing effect of cold in primary sensory neurons (68, 124). Last but not least, cold affects inactivation of sodium channels in a way that may prevent the generation of action potentials. The reliability of cold and noxious cold receptors is maintained due to the presence of $Na_V1.8$, a TTX-resistant sodium channel whose inactivation properties are not affected by cold (138). How the role of all these channels, together with TRPM8, is orchestrated to originate the phenomenon of cold transduction is not completely clear. The big picture of cold transduction is slowly emerging but still requires further studies.

## Conclusions

From the wealth of information provided by molecular biology and electrophysiological studies, it is clear that TRPM8 is an allosterically gated polymodal receptor. Its importance as the main ionic mechanism involved in innocuous cold transduction is now widely accepted and has been strongly supported by the studies using TRPM8-defficient mice, which show severe impairments in cold detection in behavioral tests. We are at present having the first glimpses about the regulation and handling of TRPM8 by the cell and how this amazing molecular machine contributes to give shape to the firing properties of mammalian cold thermoreceptor neurons. ■

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