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Commentary

Frozen images of a cool channel with icy compounds

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The ion channel TRPM8 is the primary sensor of cool temperature. By using cryo-EM, Yin et al. (*Science*, 2019) recently obtained high-resolution images of the channel in complex with ligands, providing detailed molecular insights into how cooling agents such as icilin or menthol can activate the channel to mimic the effect of a cold breeze.

Chemical compounds such as menthol and eucalyptol evoke a fresh feeling when applied to the skin (e.g. in body powder) or the oral mucosa (e.g. in lozenges or mouth wash). The sensation of coolness evoked by these cooling compounds is, however, not associated with an actual drop in temperature. Instead, it represents a phenomenon known as chemesthesis, where chemical compounds elicit a sensation that is normally associated with a physical stimulus detected by the somatosensory system [1].

To evoke a refreshing sensation, the cooling compounds hijack the primary molecular sensor of cool temperatures, the ion channel TRPM8 [2,3]. TRPM8 is one of the so-called thermoTRPs, a subset of the superfamily of transient receptor potential (TRP) cation channels whose gating is highly sensitive to changes in temperature, allowing them to act as molecular thermometers. In particular, thermoTRPs are found at the free endings of sensory neurons in skin and mucosa, where they respond to local thermal stimuli. Opening of thermoTRPs causes cation influx and membrane depolarization in the nerve endings, resulting in the initiation of action potentials that convey thermal information along the axon of the sensory neuron to the central nervous system. Whereas three heat-activated TRP channels play a role in detecting noxious heat [4], the perception of non-noxious cool temperatures (typically between 30 and 15 °C) relies entirely on TRPM8 [1]. Interestingly, TRPM8 also acts as a chemoreceptor for cooling compounds, with menthol being the best studied [2,3]. By directly activating TRPM8, menthol mimics the effect of cooling, and thus fools the somatosensory system to signal a non-existing drop in temperature.

So how does TRPM8 integrate both thermal and chemical cues? TRP channels are tetrameric channels built of four subunits with six transmembrane domains (S1-S6), and belong to the larger superfamily of voltage-gated ion channel proteins. Given that the S4 domain of TRPM8 shows homology to the voltage sensor of classical voltage-gated K^+ channels, it was not too surprising that TRPM8 functions as voltage-gated ion channel, activated by depolarization [5,6]. At a temperature of 37 °C, sizeable currents through TRPM8 can only be recorded upon depolarization to non-physiological voltages > +60 mV. However, upon cooling, the voltage-dependent activation curve shifts to lower voltages, eventually resulting in inward currents carried by Na^+ and Ca^{2+} ions at the typical resting membrane potential of a sensory neurons of about −60 mV (Fig. 1A). Not only does menthol cause a similar shift of the channel's voltage dependence, the effects of cooling and menthol on the voltage-dependent gating are largely additive (Fig. 1A) [5]. This additivity underlies the effect that a low dose of menthol enhances cold sensitivity rather than eliciting a sensation by itself [2,3,5], and explains why a mojito stuffed with mint leaves is extra refreshing on a warm summer day.

Various mutagenesis approaches, dating back already more than a decade, resulted in the identification of amino acid residues in transmembrane domains S1-S4 and in the proximal C terminus of TRPM8 that are key for the action of menthol or the more potent synthetic ligand icilin [6–8]. Most importantly, mutating these specific residues resulted in a channel with impaired ligand responses, while retaining normal cold sensitivity [6–8]. However, while these studies firmly established that activation of TRPM8 by cold and ligands can be dissociated, the picture of the ligand binding sites for cooling agents remained vague. Indeed, based on the mutagenesis experiments one could not unambiguously distinguish between residues that directly interact with the ligands and residues that affect ligand action allosterically. Therefore, the ion channel community was eagerly awaiting structural information that would clarify ligand binding and channel activation of TRPM8.

Such detailed information was recently provided in two papers, in which Yin et al. obtained high-resolution structures of TRPM8 using cryogenic electron microscopy (cryo-EM) [9,10]. In this technique, purified protein complexes in solution are quickly "frozen" in vitrified ice, and subsequently imaged using an electron microscope and direct electron detector device (DDD) camera. By class averaging many thousands of 2D images of particles, structures at near-atomic detail can be obtained.

In a first paper, Yin et al. reported the structure of TRPM8 from the collared flycatcher (*Fidecula albicollis*; Fig. 1B) in the unliganded conformation and at an overall resolution of 4.1 Å [10]. The structure revealed fourfold symmetric channel with a three-layered architecture (Fig. 1C). The upper layer contains the transmembrane domains: domains S5 and S6 and the interconnecting loops from the four subunits assemble to form the central pore, surrounded by four peripheral

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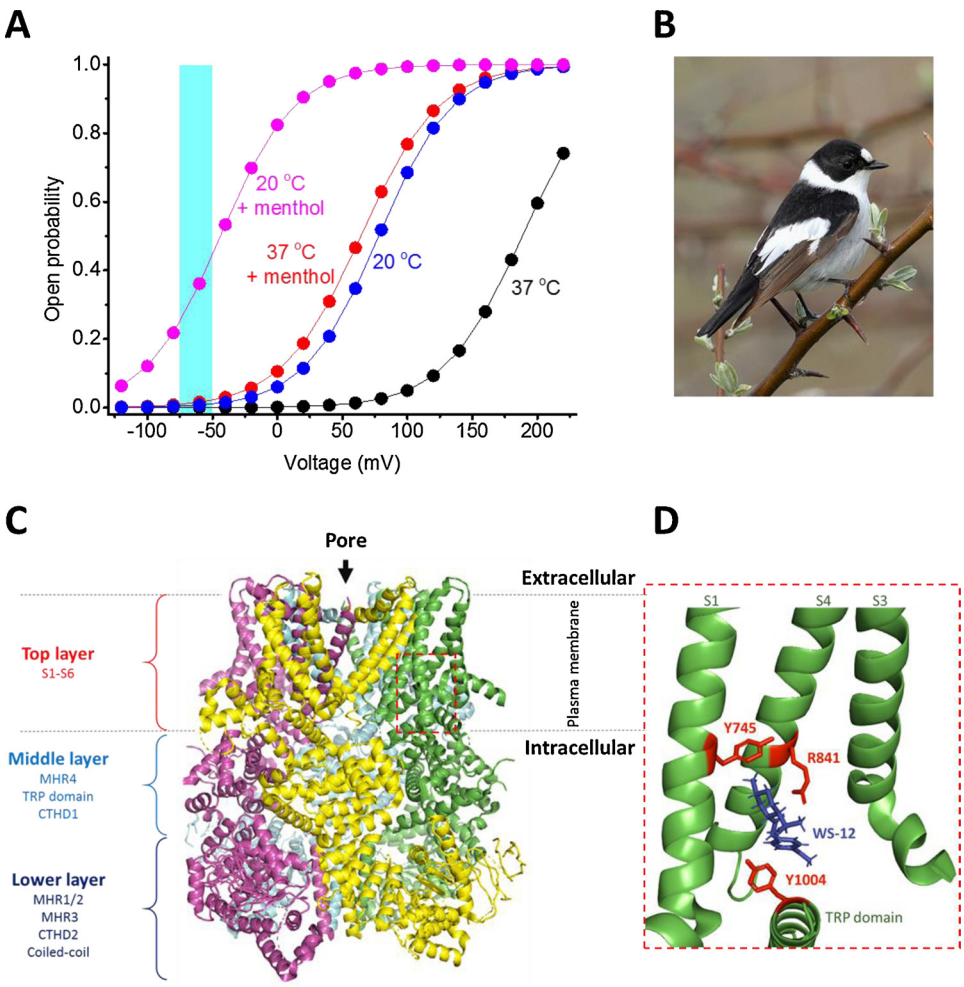

Fig. 1. Biophysical and structural features of the cold-activated channel TRPM8. (A) Voltage-dependent activation curves of TRPM8 at different temperatures, in the absence and presence of menthol. At body core temperature, detectable channel activation only occurs at non-physiological voltages. Lowering temperature or adding menthol shift the activation curves towards negative voltages, resulting in significant inward currents at the typical resting membrane potential of sensory neurons (indicated in cyan). The effects of menthol and cooling on voltage-dependent activation are additive, resulting in a strong enhancement of inward currents. Activation curves were simulated based on an established TRPM8 gating model [5,6]. (B) The collared flycatcher (*Ficedula albicollis;* picture by Andrej Chudý, https://commons.wikimedia.org/w/index.php?curid=36448767), a model species used in both ecology and genetics, whose TRPM8 ortholog turned out to be well amenable for cryo-EM structural analysis. (C) Architecture of TRPM8. The channel is built as a tetramer of four identical subunits. See text for details. (D) Close-up of the binding site of the menthol analog WS-12, interacting with specific residues in S1, S4 and the TRP domain. Structures in panels (C) and (D) were based on PDB 6NR2 [9], and rendered using PyMOL 2.3 (https://pymol.org).

voltage sensor-like domains (VSLDs) formed by S1-S4. Overall, the structure of the transmembrane domains of TRPM8 resembles that of other members of the voltage-gated ion channel. The middle and lower layer consist of the long cytosolic N- and C-terminal domains of TRPM8. The N terminus contains four melastatin homology regions (MHRs) and a pre-S1 α-helical domain that dips into the cytosolic leaflet of the plasma membrane. The proximal C terminus encompasses the so-called TRP domain (a conserved structure in TRPM, TRPV and TRPC channels), which has been implicated in channel regulation by the membrane phospholipids, followed by two C terminal domain helices (CTDH1 and CTDH2) and finally a C terminal coiled coil domain implicated interconnecting the four subunits in the center of the lower layer [10].

In their most recent paper, Yin et al. provide two additional highly informative structures of TRPM8: one in complex with the menthol analog WS-12, and a second in complex with icilin and Ca^{2+} [9]. Importantly, both structures also encompass phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$). This phospholipid is an important positive regulator of the activity of TRPM8, and its breakdown by phospholipase C contributes to desensitization upon prolonged channel stimulation. Gratifyingly, the structures confirm the predictions from earlier studies that the cooling compounds interact with the VSLD of TRPM8. WS-12 is sandwiched between Tyr^{745} (previously assumed to be in S2, but now assigned to S1), Arg^{841} (in S4) and Tyr^{1004} (in the TRP domain), three residues that were identified by mutagenesis as critical for menthol activation of the channel (Fig. 1D) [6,7]. Since Arg^{841} also carries part of the gating charge of the voltage sensor of TRPM8 [6], these results provide a structural correlate for the intricate interplay between voltage sensing and ligand action (Fig. 1A).

Icilin is a very potent TRPM8 agonist, but only so when there is a concomitant increase in cytosolic calcium [8]. Moreover, it was known from earlier studies that the chicken TRPM8 ortholog is fully insensitive to icilin [8], and the same appears to be the case for TRPM8 from *Ficedula albicollis* [9]. Yin et al. therefore introduced a single alanine-to-glycine mutation in S3 to confer icilin sensitivity to the bird TRPM8 protein, and imaged particles obtained in the combined presence of icilin and Ca^{2+} [9]. The binding site of icilin largely overlaps with that of WS-12, revealing interactions of the ligand with residues in S1, S4 and the TRP domain. In a previous study, residues Asn^{799} and Asp^{802} had been identified as crucial for icilin action [8]. Interestingly, the structure shows that these residues do not directly interact with icilin; instead, these residues, located at the cytosolic side of S2 and S3, participate in coordinating Ca^{2+}. Possibly, binding of Ca^{2+} may cause a conformational change in the VSLD that facilitates subsequent icilin binding.

These studies not only provide detailed fundamental insights into the action of cooling compounds on their primary biological target, but may also be important in steering the rational development of novel drugs targeting TRPM8 for a variety of human conditions, including cold hypersensitivity, urinary urgency and migraine. A major challenge for future studies is to identify the structural rearrangements that occur during cooling-induced activation of the channel. This may require approaches that allow “freezing” the channel at different well-specified temperatures, ideally with simultaneous control of the transmembrane voltage.

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