Peripheral thermosensation in mammals

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Abstract | Our ability to perceive temperature is crucial: it enables us to swiftly react to noxiously cold or hot objects and helps us to maintain a constant body temperature. Sensory nerve endings, upon depolarization by temperature-gated ion channels, convey electrical signals from the periphery to the CNS, eliciting a sense of temperature. In the past two decades, we have witnessed important advances in our understanding of mammalian thermosensation, with the identification and animal-model assessment of candidate molecular thermosensors — such as types of transient receptor potential (TRP) cation channels — involved in peripheral thermosensation. Ongoing research aims to understand how these miniature thermometers operate at the cellular and molecular level, and how they can be pharmacologically targeted to treat pain without disturbing vital thermoregulatory processes.

Transient receptor potential channels
(TRP channels). A family of cation channels homologous to the product of the *Drosophila melanogaster trp* gene. Several mammalian members of this family function as molecular thermosensors.

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Thermosensation, the ability to estimate temperature, is one of the most ancient sensory processes[1]. All organisms, from bacteria to plants and animals, have processes in place that enable them to react to changes in environmental temperature, and adequate responses to thermal challenges are absolutely crucial for survival. Indeed, temperature can have important and even detrimental effects on the structure and function of key biological macromolecules — including proteins, lipids and nucleic acids — and thus on the physiology and integrity of cells, tissues and organisms[2]. In mammals, changes in perceived temperature often provoke involuntary (physiological) or voluntary (behavioural) thermoregulatory actions. Inadequate thermoregulation, caused by disease, pharmacological treatment or harsh environmental conditions, can rapidly lead to harmful or even lethal hypo- or hyperthermia[3,4].

In recent years, various types of ion channels, including the much-studied transient receptor potential channels (TRP channels), have been identified as highly sensitive molecular thermometers. These discoveries have fuelled detailed studies examining the molecular and cellular mechanisms of thermosensitivity and have led to the development of powerful genetic models and pharmacological tools to analyse the contributions of these channels to thermosensation in health and pathophysiology. In this article, we provide a brief overview of the cellular circuitry that underlies thermosensation and discuss current insights and controversies regarding the molecular and biophysical principles that form the basis of temperature sensing in mammals. In addition, we describe various causes and consequences of pathological thermosensation and the potential of thermosensitive ion channels as targets for pain therapy. Finally, we highlight important lacunas in our knowledge of mammalian thermosensation and provide some perspectives on further developments in this field.

Thermosensing neurons and circuits

In mammals, environmental thermal cues are primarily conveyed by afferent neurons of the somatosensory system. These neurons are individually tuned sensory cells that can convert specific thermal, but also mechanical and chemical, stimuli into electrical signals, which travel from the periphery to the CNS in the form of action potentials[1,5]. Mammalian sensory neurons are pseudounipolar: they contain a single axon that divides into two branches, with one branch extending to peripheral tissues such as skin, mucosa and internal organs, where it gathers information pertaining to environmental stimuli, and the other branch relaying the detected information to second-order neurons in the dorsal horn or the sensory nucleus in the brain (FIG. 1a). The cell bodies of primary sensory neurons that innervate the sensitive parts of the head and face, including the mouth, nose and eyes, are clustered in the trigeminal ganglia adjacent to the brain. By contrast, the cell bodies of sensory neurons that innervate the rest of the body are contained by the dorsal root ganglia (DRGs), which are located in the vertebral column just lateral to the spinal cord[6].

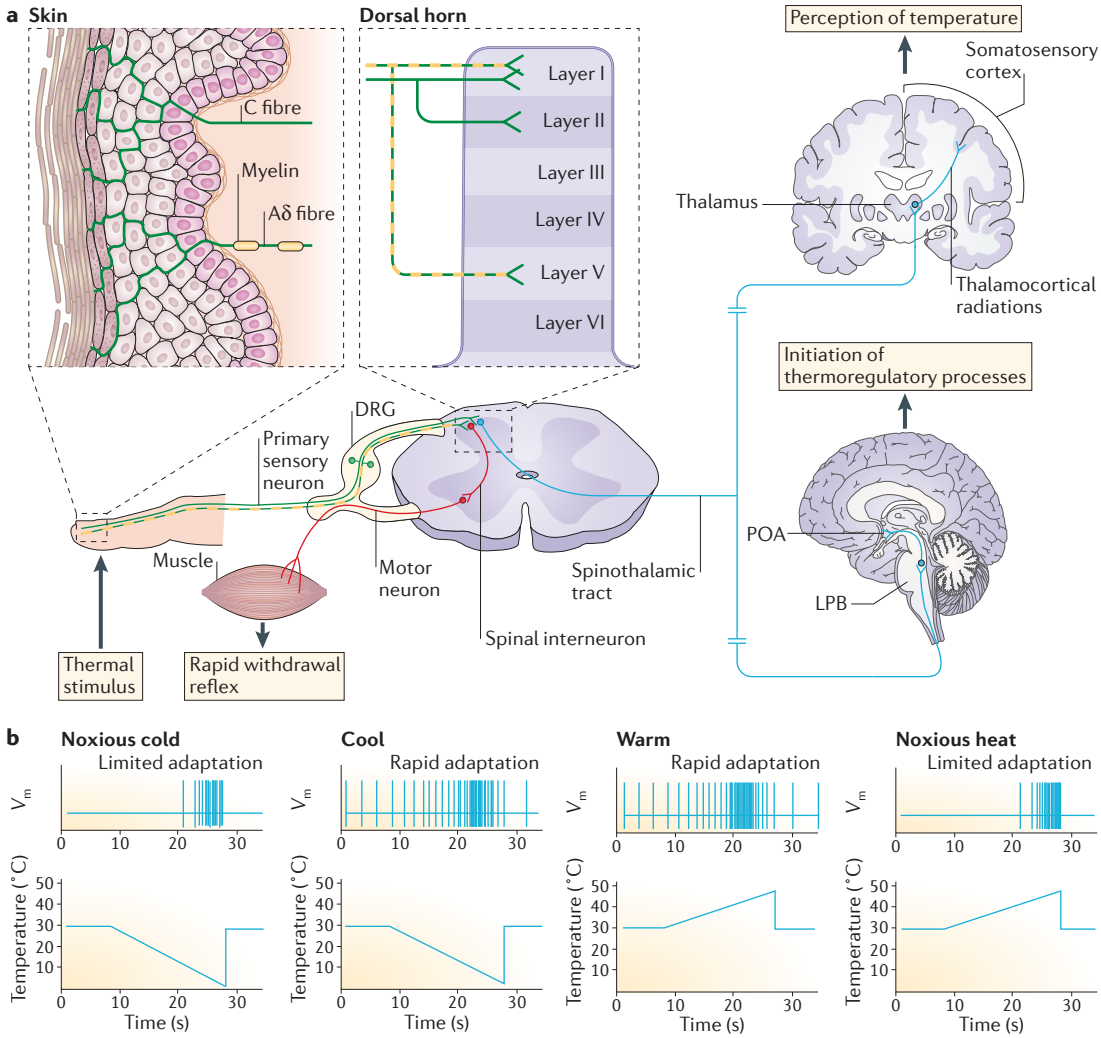


Figure 1 | **Neurons involved in thermosensation.** **a** | Pathways involved in thermal avoidance responses, thermoperception and the initiation of thermoregulatory responses. Cutaneous primary sensory neurons involved in thermosensation (green) include both non-myelinated C fibres and thinly myelinated Aδ fibres. The cell bodies of these neurons are located in the dorsal root ganglia (DRGs) and have axons with two branches. One branch extends towards the periphery, with free endings in the skin, where thermal information is coded in the form of action potentials. These action potentials propagate to the end of the other axonal branch, which forms synapses in layers I and II (for C fibres) or layers I and V (for Aδ fibres) of the dorsal horn. Activity of both Aδ and C primary sensory fibres steers three distinct neuronal pathways and ensuing responses: first, motor neurons can be activated via spinal interneurons, leading to a rapid withdrawal reflex in response to noxious temperatures (red neurons); second, thermosensory information is transmitted via second-order sensory neurons of the ascending spinothalamic tract to the thalamus and further relayed to the somatosensory cortex, where our perception of temperature is formed (blue neurons on the coronal section of the human brain); and third, thermosensory information is transmitted via lateral parabrachial (LPB) neurons, which may also receive input from the spinothalamic neurons, to the pre-optic area (POA) of the hypothalamus (blue neurons on the central sagittal section of the human brain), where thermoregulatory processes are initiated. **b** | Cutaneous thermosensitive neurons as shown in part **a** typically exhibit one of four different thermal response profiles. The first type of thermal response is related to the detection of noxious cold. These fibres are generally silent at the thermoneutral temperature, exhibit thermal thresholds at around 20–10 °C, show a linear increase in firing intensity upon cooling down to 0 °C and exhibit limited adaptation. The second type of response is observed in fibres activated by cool temperatures of 37–20 °C. The majority of these fibres exhibit ongoing activity at thermoneutral skin temperature, which disappears upon moderate warming. Discharge frequency increases upon moderate cooling, reaching a maximum firing rate between 30 and 20 °C. At temperatures of around 20 °C, fibres generally show rapid adaptation, which leads to a reduction in discharge frequency at temperatures below ~17 °C. A similar bell-shaped temperature–frequency relationship and rapid adaptation is observed in warm-sensitive fibres. These exhibit ongoing activity at thermoneutral skin temperature, which disappears upon moderate cooling. Their discharge rate increases upon moderate heating, reaching a maximum at 40–43 °C, but these fibres rapidly fall silent upon further heating. Finally, fibres involved in sensing noxious heat are typically activated at temperatures above 43 °C, with peak discharge occurring at noxious temperatures (45–53 °C), and show little or no adaptation.

Sensory nerves are classically subdivided into four main categories on the basis of their size and conduction properties[5–7]. Aα and Aβ fibres have large cell bodies, thickly myelinated axons and high conduction velocities; Aδ fibres, by contrast, exhibit medium-sized cell bodies, thinly myelinated axons and intermediate conduction velocities; and C fibres are characterized by small cell bodies, the absence of a myelin sheath and slow conduction velocities. The latter two fibre types — the Aδ and C fibres — include the sensory nerves involved in thermosensation[6,7].

The temperature changes that are encountered by visceral sensory neurons that innervate internal organs are mostly limited to a few degrees around the body's core temperature by strict homeostatic regulation[8]. Cutaneous sensory neurons, however, encounter and convey a much wider range of temperatures. A skin temperature of around 33 °C is perceived as thermoneutral by humans, with lower temperatures perceived to be cool or cold, and higher temperatures perceived to be warm or hot. In general, skin temperatures below ~15 °C or above ~45 °C are associated with pain and considered to be noxious[6,9]. Based on their response profiles, temperature-sensitive neurons can be classified into four main response types[6,10–12] (FIG. 1b). Neurons that are activated by either non-noxious cool or non-noxious warm temperatures show marked basal action potential firing activity. Warm-sensitive neurons respond to moderate heating with a rapid but transient increase in firing frequency and to moderate cooling with a transient reduction of firing activity[11]. Cool-sensitive neurons exhibit a mirror response, with a transient decrease in firing frequency upon moderate heating and a transient increase upon moderate cooling[10,13] (FIG. 1b). The transient nature of the firing response of warm- and cool-sensitive neurons is believed to underlie the typical adaptation to non-noxious temperatures that humans experience, for instance, when stepping into a warm bath or diving into a cool swimming pool. By contrast, sensory neurons that respond to noxious cold or noxious heat are mainly silent at thermoneutral temperatures but show sustained rapid action potential firing in response to prolonged noxious thermal stimuli[6,12] (FIG. 1b). Accordingly, humans experience little or no adaptation to noxious temperatures[14,15]. Prolonged exposure to noxious temperatures also causes tissue damage, leading to the local release of various pro-algesic agents, including ATP, protons, reactive oxygen species and lipid mediators[9,16]. These agents can activate nociceptors and provoke pain even after removal of the painful thermal stimulus.

Temperature-sensitive primary sensory neurons from the DRG enter the spinal column through the intervertebral foramina and form glutamatergic synapses in the dorsal horn[7] (FIG. 1a). Acute avoidance of noxious cold or noxious heat involves a simple spinal reflex, where primary sensory neurons signal via spinal interneurons to motor neurons, resulting in a rapid withdrawal of the challenged body part. Thermal information from cutaneous thermosensory neurons is also transmitted via the spinothalamic tract to the thalamus and then further relayed via thalamocortical radiations to the primary sensory cortex, where the perception of temperature originates and voluntary behavioural actions are initiated[17] (FIG. 1a). Finally, thermosensory information from cutaneous and visceral sensory neurons is transmitted via lateral parabrachial neurons to the pre-optic area (POA; within the anterior portion of the hypothalamus). The POA itself contains intrinsically temperature-sensitive neurons, which not only receive synaptic input relaying the activity of peripheral temperature-sensitive neurons but also respond to small changes in local brain temperature[17,18]. Based on the integrated central and peripheral thermal information, the POA controls thermoregulatory processes, such as cutaneous vasomotion, shivering and brown adipose tissue thermogenesis[9]. Central mechanisms of thermosensation and thermoregulation have been reviewed in detail elsewhere[17–19].

Ion channels as molecular thermometers

At the basis of thermosensation lies the property of specific ion channels to conduct ions in a highly temperature-dependent manner. The steepness of the temperature dependence of an ion channel's conductance can be quantified using the Q_{10} value (BOX 1). Depending on their ionic selectivity, temperature-sensitive ion channels are either excitatory or inhibitory. In the context of a sensory nerve ending, temperature-dependent changes in ionic conductance can determine whether, and at what frequency, action potentials are generated. Over the past two decades, important advances have been made in the molecular identification and characterization of highly temperature-sensitive ion channels. In particular, several temperature-sensitive members of the TRP family of ion channels were identified and were shown to have thermal response profiles collectively covering the entire range of temperatures that mammals can discriminate[20]. These findings led to the proposal that these so-called thermoTRP channels function as the prime or sole molecular thermosensors.

However, for a temperature-sensitive ion channel to be considered a *bona fide* molecular thermosensor, evidence needs to be provided underpinning a role in thermosensation *in vivo*. In recent years, single- and combined-knockout mouse models have been developed to test many putative thermosensors, and these models have enabled detailed phenotyping using various behavioural assays of thermosensitivity. These assays include: acute avoidance tests, in which the latency to withdrawal from a noxious thermal stimulus applied to the tail or paw is measured; pain intensity tests, in which pain behaviours (such as jumping or licking of the paws) in response to sustained noxious stimulation are quantified; and thermotaxis tests, in which an animal's preference to migrate to a specific temperature or temperature zone is analysed[21–24]. Although such studies can reveal important alterations in an animal's thermosensitivity, it should be noted that altered responses in these assays may be due not only to altered thermal sensitivity of primary sensory neurons but also to altered central processing. Therefore, *ex vivo* assays that allow direct measurement of the activity of intact cutaneous

Aδ fibres
Sensory neurons with medium-diameter, thinly myelinated axons and a medium conduction velocity of between 2 and 30 metres per second.

C fibres
Sensory neurons with small-diameter, non-myelinated axons and a low conduction velocity of between 0.5 and 2 metres per second.

Adaptation
The reduction over time of the response of a neuron to a constant sensory stimulus.

Q_{10} value
A dimensionless value to quantify the temperature dependence of a process. It is defined as the ratio between reaction rates or current amplitudes measured at two temperatures 10 degrees apart.

Box 1 | **Quantifying temperature sensitivity of ion channels**

Temperature represents an intensive thermodynamic property of any system — that is, measuring temperature quantifies the average kinetic energy of microscopic particles within the system and determines its total internal energy. As a consequence, any (bio)chemical reaction, including the gating of channels or ion permeation through ion channels, will exhibit some degree of temperature dependence. The steepness of temperature dependence can be quantified using the dimensionless Q_{10} value, which is defined as the relative change in reaction rate upon a 10-degree increase in temperature:

$$Q_{10} = \frac{\alpha_{T+10}}{\alpha_T} \quad (1)$$

where α_T and α_{T+10} are reaction rates at two temperatures 10 degrees apart. This equation can be generalized to reaction rates at any two temperatures ($T1$ and $T2$):

$$Q_{10} = \left(\frac{\alpha_{T2}}{\alpha_{T1}}\right)^{\frac{10}{T2-T1}} \quad (2)$$

Reaction rates vary with temperature according to the Arrhenius equation:

$$\alpha = A\exp\frac{-E_A}{RT} \quad (3)$$

where A is the pre-exponential factor, E_A is the activation energy, R is the universal gas constant and T is the temperature in Kelvin. This yields the following relation between Q_{10} and E_A:

$$Q_{10} = \exp\frac{10 \times E_A}{RT^2} \quad (4)$$

Q_{10} values have also been widely used as benchmarks to quantify the temperature dependence of ion channels, using current amplitudes at two different temperatures (I_{T1} and I_{T2}) in lieu of reaction rates:

$$Q_{10} = \left(\frac{I_{T2}}{I_{T1}}\right)^{\frac{10}{T2-T1}} \quad (5)$$

Whereas most ion channels typically display Q_{10} values between 1 and 3, heat-activated channels typically exhibit much higher Q_{10} values (>7). Conversely, cold-activated channels display positive Q_{10} values much lower than 1 (REFS 108,171).

As current amplitude is not equivalent to simple reaction rate, the interpretation of Q_{10} values determined from current amplitudes requires consideration of the different elements that determine ionic flow through ion channels. The ionic current (I) that is mediated by a specific type of ion channel in a cell is the product of the number of channels in the plasma membrane (N), the ionic current through a single open channel (i) and the mean open probability of these channels (P_{open}):

$$I = N \times P_{open} \times i \quad (6)$$

Consequently, Q_{10} values that are calculated on the basis of temperature-dependent changes in I also depend on the temperature dependence of these three parameters. The first parameter, N, depends on the balance between the incorporation of channels in the membrane (through exocytosis) and removal through endocytosis. Although there are many examples of stimulus-dependent changes in N (for instance, the exocytosis of transient receptor potential A1 (TRPA1)-containing vesicles upon stimulation with mustard oil[172]), there are no reports of abrupt temperature-induced changes in N. Therefore, the global temperature dependence of ionic currents mainly reflects the product of the thermal sensitivity of channel gating (P_{open}) and permeation (i), known as $Q_{10,gating}$ and $Q_{10,permeation}$, respectively:

$$Q_{10} = Q_{10,permeation} \times Q_{10,gating} \quad (7)$$

The rate of ion permeation through an open channel pore exhibits mild thermal sensitivity, with typical $Q_{10,permeation}$ values between 1.2 and 1.5 (REF. 108). This implies that, for steeply temperature-sensitive ion channels, changes in temperature mainly affect P_{open}, reflected in $Q_{10,gating}$ values either greater than 5 or less than 0.2. It should be noted that the highest $Q_{10,gating}$ values for heat-activated channels and the lowest $Q_{10,gating}$ values for cold-activated channels are measured when P_{open} is low (that is, when channel activity is far from saturated).

Multiple studies have also used the term 'thermal threshold' ($T_{threshold}$) as an indication of the temperature at which a temperature-sensitive channel is first activated, but the use of this parameter should be discouraged. Thermal activation of ion channels is a gradual process; it is not governed by a specific threshold[101], and $T_{threshold}$ is an ill-defined value that merely represents the temperature at which current is detected above background[108,171].

sensory nerve endings are an important complementary approach[12]. Overall, knockout-mouse studies have confirmed the role of some but not all thermoTRP channels and indicate that additional TRP-independent mechanisms may play a prominent part in the transduction of thermal stimuli in the somatosensory system.

TRP channels. In mammals, the TRP superfamily consists of 28 members, which can be subdivided into 6 subfamilies (TRPC, TRPV, TRPM, TRPA, TRPML and TRPP)[25]. Humans only express 27 TRP channel members as, during the evolution of higher primates, the gene encoding TRPC2 became a pseudogene[25]. Using responsiveness to capsaicin (the hot constituent of chilli peppers) as a screening readout, vanilloid receptor 1 (VR1; now known as TRPV1) was cloned from a cDNA library that was created from rat sensory neurons[26]. TRPV1 is expressed in a subset of nociceptive, small-diameter Aδ and C neurons that originate from DRGs and trigeminal ganglia. Like all members of the TRP channel superfamily, TRPV1 subunits have six transmembrane domains, and four of these subunits are required to form a functional cation channel[25,27]. The heterologous expression of TRPV1 not only resulted in capsaicin-gated currents but also revealed that TRPV1 is strongly activated upon heating[26], with Q_{10} values >10 (FIG. 2).

TRPV1 is responsive to various noxious stimuli, including: noxious heat; acidic and basic solutions; aversive chemicals produced by certain plants and animals; and endogenous lipid signalling molecules, such as anandamide and eicosanoids[28–31]. The activation of temperature-sensitive ion channels by a ligand provides a molecular explanation for chemesthesis, the phenomenon whereby certain chemical compounds mimic a physical stimulus (BOX 2). In support of TRPV1's role as a sensor of thermal and chemical stimuli, currents evoked by heat (>43 °C) and capsaicin were virtually absent in cultured neurons from TRPV1-deficient mice,

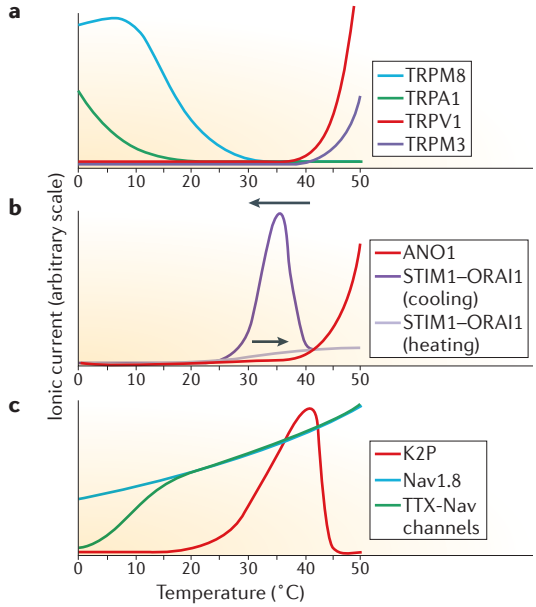


Figure 2 | **Temperature dependence of ion channels involved in thermosensation.** **a** | Members of the transient receptor potential (TRP) channel family function as excitatory cation channels and respond to cooling or heating, depending on their subtype. **b** | The Cl^- channel anoctamin 1 (ANO1) acts as an excitatory heat-activated channel. Heating leads to only minimal activation of ORAI1 by heat-activated stromal interaction molecule 1 (STIM1) (light purple), but a large excitatory 'off' response occurs upon cooling following a heat stimulus (dark purple). **c** | Two-pore-domain K^+ (K2P) channels and voltage-gated Na^+ (Nav) channels determine the extent of sensory neurons' overall excitability by influencing the membrane's depolarization threshold above which action potentials may be fired. At cold temperatures, tetrodotoxin (TTX)-sensitive Nav channels (TTX-Nav channels) inactivate and thermosensation becomes fully dependent on Nav1.8.

although higher-threshold (>55 °C) heat responses were intact[21,22]. Moreover, in behavioural experiments, TRPV1-deficient mice showed a longer latency in their responses to noxious heat but normal latencies to painful mechanical stimuli[21]. The key role of TRPV1 as a noxious heat sensor becomes even more pronounced in the setting of inflamed tissue: TRPV1-deficient mice do not exhibit the prominent inflammation-associated heat hyperalgesia that is observed in wild-type animals[21,22]. Through the use of TRPV1 antagonists, the noxious-heat-sensing activity of TRPV1 has been further confirmed in other mammals, including humans[32,33].

The residual heat-sensitivity observed in TRPV1-deficient mice suggested the existence of an additional heat sensor (or sensors), and three closely related members of the TRPV subfamily, TRPV2, TRPV3 and TRPV4, were obvious candidates. TRPV2, the closest homologue of TRPV1, has been a long-standing candidate for a high-threshold molecular sensor of noxious heat, because it was found to be expressed in a subset of medium- to large-diameter Aδ (but also Aβ) neurons, and heterologous expression of rodent TRPV2 produced a cation current that was activated at temperatures exceeding ~52 °C[34]. However, a recent report demonstrated that genetic ablation of TRPV2 in mice had no discernible effect on acute pain responses to noxious heat or on inflammatory heat hyperalgesia, even when TRPV1 activity was simultaneously suppressed[35], indicating that TRPV2 can be excluded as a key thermosensor in the somatosensory system.

TRPV3 and TRPV4 have been proposed to be molecular sensors that are involved in the detection of non-noxious warmth. In heterologous expression systems, these channels show a steep increase in activation in response to increases in temperature between 25 and 35 °C[36–40]. Interestingly, these two channels are abundantly and functionally expressed in epidermal and hair-follicle keratinocytes, whereas in sensory neurons, expression levels of mRNA encoding TRPV3 and TRPV4 are low or below the detection limit of northern blot or quantitative PCR analyses[37,38,41,42]. This expression pattern has led to the hypothesis that TRPV3 and TRPV4 could mediate the sensation and discrimination of warm temperatures by keratinocytes, which then communicate with sensory neurons by releasing diffusible messengers such as ATP or nitric oxide[43–45]. In line with this view, initial papers describing the behaviour of TRPV3- or TRPV4-deficient mice reported significant abnormalities in thermal preference in the warm temperature range[24,46]. In particular, TRPV4-deficient mice preferred warmer floor temperatures on a thermal gradient, whereas TRPV3-deficient mice showed a relative indifference to temperatures ranging from 20 to 35 °C[24,46] However, more recent studies indicate that the alteration in temperature preference in TRPV3-deficient mice is highly dependent on the genetic background and sex of the mice[44,47]. On a 129S1 background, deficits in innocuous warmth discrimination were observed in TRPV3-deficient female but not male mice[44]. On a C57BL6 background, mice that lacked both TRPV3 and TRPV4 exhibited thermal preference behaviour that was virtually indistinguishable from that of wild-type C57BL6 mice, even when TRPV1 function was pharmacologically blocked[47]. Although the possibility that TRPV3 and TRPV4 have more specialized functions cannot be excluded, these results argue against a general role for TRPV3 and TRPV4 in thermosensation and stress the importance of considering the sex and genetic background of mutant mouse strains when interpreting thermosensory behaviour[48].

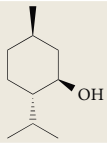
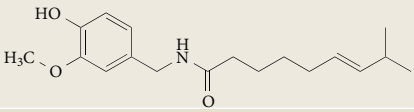
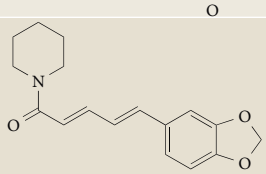
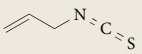
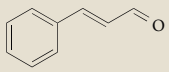
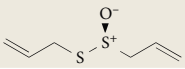
Recently, TRPM3 was identified as an alternative noxious-heat sensor in a large subset of sensory neurons, including nociceptors, from DRGs and trigeminal ganglia. This member of the TRPM subfamily can be activated by chemical ligands, such as the neurosteroid pregnenolone sulphate, as well as by heat[49,50]. Heterologously expressed TRPM3 is activated by heating, with a current–temperature relationship curve that is shifted slightly towards higher temperatures compared with that of TRPV1 (REF. 50) (FIG. 2). TRPM3-deficient mice exhibit deficits in avoidance responses to noxious heat and in the development of heat hyperalgesia in inflamed tissue[50]. Similarly, pharmacological inhibition of TRPM3 using citrus fruit flavanones reduced the

Hyperalgesia
Increased sensitivity to a painful stimulus.

Box 2 | **Thermal chemesthesis**

Several temperature-sensitive ion channels, in particular transient receptor potential (TRP) channels, are not only sensitive to changes in temperature but also function as ligand-gated channels. In particular, various plant-derived natural compounds can directly bind to and activate temperature-sensitive TRP channels (see the table for some examples). When such ligands reach their targets in sensory nerve endings — for example, in the oral mucosa or following topical application and penetration through the skin — they excite thermosensitive sensory neurons and thus provoke a feeling akin to a thermal stimulus, although there is no actual temperature change. This phenomenon, where a chemical compound mimics a physical stimulus, is called chemesthesis.

TRP channel ligands are typically hydrophobic molecules that readily enter and permeate the plasma membrane. Structure–function studies indicate that several of these hydrophobic ligands directly and reversibly interact with their target TRP channels at a binding site within the first four transmembrane α-helices of each subunit[173–175], a notion that is confirmed by the recent high-resolution electron microscopy structure of TRPV1 in complex with capsaicin[109]. In the case of the TRPM8–menthol interaction, it has been demonstrated that each channel can bind to up to four ligand molecules, with one ligand per subunit. Each bound menthol molecule reduces the difference in enthalpy between the closed and open states (ΔH_{gating}) by approximately 3.1 kJ mol^{-1}, which has the equivalent effect on the channel's open probability as would cooling by ~5 °C[176]. An alternative mode of action has been described for the activation of TRPA1 by electrophiles such as allyl isothiocyanate, cinnamaldehyde and allicin, which activate the channel by covalently binding to intracellular cysteine or lysine residues[177,178]. The activation of TRPV1 by allicin (but not by allyl isothiocyanate) has also been attributed to covalent binding of the ligand to a cytosolic cysteine residue[179].

Compound	Compound structure	Natural source	Targets	Mode of action	Refs
Menthol		Mint plant (*Mentha* spp.)	TRPM8	Reversible interaction	54,55
			TRPA1	Reversible interaction	180
Eucalyptol		Eucalyptus tree (*Eucalyptus polybractea*)	TRPM8	Reversible interaction	54
Capsaicin		Chilli peppers (*Capsicum* spp.)	TRPV1	Reversible interaction	26
Piperine		Pepper corns (*Piper nigrum* and *Piper longum*)	TRPV1	Reversible interaction	181
Allyl isothiocyanate		Mustard seeds (*Brassica nigra*)	TRPA1	Covalent binding	62,66
			TRPV1	Reversible interaction	182
Cinnamaldehyde		Cinnamon bark (*Cinnamomum* spp.)	TRPA1	Covalent binding	62
Allicin		Garlic (*Allium sativum*)	TRPA1	Covalent binding	183
			TRPV1	Covalent binding	179,183

sensitivity of mice to noxious heat[51]. These results suggest that TRPM3 is a *bona fide* thermosensor that is involved in the detection of noxious heat. Nevertheless, pharmacological inhibition of TRPV1 in TRPM3-deficient mice did not fully abrogate avoidance responses to noxious heat[50], implying the existence of additional mechanisms for sensing noxious heat. Interestingly, selective ablation of TRPV1-expressing neurons in adult mice, using a diphtheria toxin-mediated strategy, leads to a complete lack of heat avoidance at temperatures up to 50 °C: a much more pronounced noxious-heat-sensing phenotype than was seen following combined elimination of TRPM3 and TRPV1 (REFS 52,53). These findings suggest that TRPV1-expressing cells are essential for heat responses but express additional heat sensors besides TRPV1 and TRPM3. This interpretation must be taken with some caution, as ablating a large proportion of sensory neurons in an adult animal might have unpredictable effects on neighbouring neurons in the sensory ganglia or dorsal horn.

TRPM8, the first cold-activated TRP channel to have been identified, plays a crucial part in cold detection by the somatosensory system. In heterologous expression systems, TRPM8 activity steeply increases upon cooling (FIG. 2) and in the presence of substances that are known to produce a cooling sensation, including menthol, eucalyptol and the 'super-cooling agent' icilin[54,55] (BOX 2). TRPM8 is expressed in a small subpopulation of

C and Aδ fibres that originate from DRGs and trigeminal ganglia. Although functional studies indicate that a small but significant proportion (approximately 20%) of TRPM8-expressing neurons respond to the TRPV1 agonist capsaicin[23,54], TRPM8 shows no co-expression with typical markers of nociceptor neurons, such as calcitonin gene-related peptide (CGRP), substance P or isolectin B4 (REF. 55). Intact sensory nerve fibres and cultured sensory neurons from TRPM8-deficient mice exhibited profoundly diminished responses to non-noxious cool temperatures[56–58]. In particular, these mice exhibited a strong reduction in the number of cold-activated C and Aδ fibres, and the remaining cold-sensitive C fibres exhibited strongly reduced basal activity at thermoneutral skin temperature[56]. Behaviourally, TRPM8-deficient mice exhibited a striking deficit in avoiding cool temperatures: whereas wild-type mice strongly prefer a temperature of around 30 °C to cooler temperatures, TRPM8-deficient mice showed relative indifference to temperatures between 18 and 30 °C[56–58]. Moreover, whereas mild cooling can evoke analgesia in wild-type mice, cooling-induced analgesia was absent in TRPM8-deficient mice[58]. Importantly, however, a subpopulation of DRG and trigeminal ganglion neurons from TRPM8-deficient mice responded to noxious cold, and these mice still showed robust avoidance behaviour in response to temperatures below 15 °C[56–58]. These data indicate that TRPM8 functions as a thermosensor in non-nociceptive Aδ and C fibres that are involved in the perception of cool temperatures. Interestingly, cold sensing by TRPM8-expressing neurons that innervate the cornea also helps to regulate basal tearing[59]. These neurons respond to reductions in corneal temperature as small as 1 °C, which occur upon evaporation of the tear film, and thus indirectly function as sensitive wetness sensors[59]. Whereas genetic deletion of TRPM8 elicits certain deficits in cold avoidance, selective ablation of TRPM8-expressing neurons in adult mice yields a stronger cold-avoidance deficit and a partial reduction in the avoidance of noxious cold[52,60]. These results suggest that TRPM8-expressing neurons also participate in detecting painful cold but imply the existence of additional cold sensors as well, especially for the detection of noxious cold.

TRPA1, which is the only mammalian member of the TRPA subfamily, is a prominent but controversial candidate to mediate (part of) the sensing of noxious cold. Several studies report that, in heterologous expression systems, TRPA1 is activated by cooling — with a current–temperature relationship that is shifted further towards colder temperatures compared with that of TRPM8 (REFS 23,61–65) (FIG. 2) — and by a plethora of chemically diverse noxious or irritant compounds[62,66,67] (BOX 2). TRPA1 is specifically expressed in a subset of small-diameter mammalian sensory ganglion neurons that co-express typical nociceptor markers such as CGRP, substance P and isolectin B4, as well as TRPV1 (REF. 61). These properties are consistent with the suggestion that TRPA1 acts as a noxious-cold sensor to elicit cold-induced pain. Indeed, several *in vivo* studies have shown that TRPA1-null mice have marked deficiencies in the nocifensive responses to noxious cold[23,68,69].

However, whereas the role of TRPA1 as a sensor for noxious chemicals is generally accepted, crucial aspects of its proposed function in (noxious) cold sensing remain controversial and highly debated. First, although several studies have demonstrated cold-induced activation of heterologously expressed human, mouse and rat TRPA1, in both whole cells and cell-free membrane patches[23,61–64], there are conflicting reports showing that cold fails to activate TRPA1 from these and other mammals[66,70,71]. The reasons for these discrepancies are not clear. Studies showing cold-induced activation of TRPA1 report that cooling is a 10- to 30-fold less potent stimulus than chemical agonists such as mustard oil[23], suggesting that differences in assay sensitivity may explain why some studies fail to detect cold-induced responses. In addition, as TRPA1 is highly sensitive to intracellular Ca^{2+} levels[70], changes in which can potentiate or desensitize channel activity, differences in the Ca^{2+} content or Ca^{2+}-buffering capacity of experimental solutions may underlie some conflicting results. Second, whereas some studies report marked deficits in acute noxious-cold sensing in TRPA1-deficient mice[23,68,69], others report that TRPA1-null mice exhibit normal responses to noxious cold[72,73]. Furthermore, others found that *Trpa1*-knockout mice only show reduced cold-sensitivity under conditions in which the sensitivity of TRPA1 is enhanced by endogenous or exogenous chemicals[74]. Based on the available data, it is not clear whether these conflicting results reflect differences in genetic background, assays, experimental conditions, researchers or a combination thereof.

Irrespective of whether TRPA1 is involved in sensing cold, there is little doubt that additional TRPM8- and TRPA1-independent molecular mechanisms of cold sensation must exist, as mice that lack both TRPM8 and TRPA1 still avoid noxious cold[73]. One potential, but not yet fully explored, candidate for cold sensing within the TRP superfamily is TRPC5. In a heterologous expression system, TRPC5 channels are sensitive to cold in the temperature range of 37–25 °C. Although TRPC5 is expressed in sensory neurons, TRPC5-null mice do not exhibit obvious deficits in temperature-sensitive behaviour[75]. Overall, these studies illustrate that our understanding of the molecular sensors involved in cold sensation remains incomplete and suggest the existence of additional cold-activated depolarizing mechanisms in sensory neurons.

Ca^{2+}-activated Cl^- channels. Anoctamin 1 (ANO1; also known as TMEM16A), a Ca^{2+}-activated Cl^- channel[76], has recently been proposed to be a potential noxious-heat sensor[77]. ANO1 is expressed in small-diameter sensory neurons that also express the nociceptor markers isolectin B4 and CGRP, and is steeply activated by heat in the noxious range (FIG. 2), even at low intracellular Ca^{2+} concentrations. At typical intracellular Cl^- concentrations of ~45 mM, the activation of ANO1 induces a depolarizing current in isolated DRG neurons that is sufficient to induce action potentials[77]. However, as the intracellular Cl^- concentration can be dynamically modulated (for example, by inhibition of

Na^+–K^+–Cl^- cotransporter 1), situations can be envisaged wherein ANO1 activation actually counteracts the depolarization of sensory neurons. Genetic ablation or pharmacological inhibition of ANO1 in mice reduced heat-induced nociceptive behaviour[77,78], suggesting that ANO1 is an additional heat sensor involved in the perception of painfully hot temperatures. ANO2, the closest homologue of ANO1, is also sensitive to heat, but its physiological relevance remains to be determined[77].

STIM1–ORAI1. Another potential instrument in the repertoire of heat-sensitive ion channels is stromal interaction molecule 1 (STIM1), which has a well-established function as the endoplasmic reticulum (ER) Ca^{2+} sensor of store-operated Ca^{2+} entry. Upon ER Ca^{2+}-store depletion, STIM1 clusters at ER–plasma membrane junctions, where it interacts with and opens Ca^{2+}-permeable ORAI1 ion channels[79]. Recently, it was reported that mild heating of cells to temperatures above 35 °C induces clustering of STIM1 independently of Ca^{2+}-store depletion[80]. As temperatures above 35 °C also prevent the physical interaction between STIM1 and ORAI1, this STIM1 clustering does not lead to Ca^{2+} entry during heat stimulation. However, subsequent cooling provokes a transient Ca^{2+} influx through ORAI1 channels (FIG. 2), because the ability of the STIM1 clusters to activate ORAI1 channels is temporarily restored before the clusters are disassembled[80]. As both STIM1 and ORAI1 are functionally expressed in sensory neurons, a potential role for them in the regulation or modulation of thermosensation can be envisaged but remains to be established.

Na^+ and K^+ channels. Temperature-dependent excitation of a sensory neuron depends not only on the activation of a depolarizing current, which causes depolarization of the sensory nerve ending (the receptor potential), but also on the combination of K^+ channels and voltage-gated Na^+ (Nav) channels that together set the threshold voltage for the generation of action potentials. Modulation of these channels can enhance or dampen the effect of activation of a depolarizing current, thereby drastically influencing the temperature sensitivity of a neuron[81].

Members of the two-pore-domain K^+ (K2P) channel family give rise to the background (leak) K^+ conductance and thus function as brakes on excitability in many cell types[82]. Interestingly, three members of the TREK/TRAAK subfamily of K2P channels — namely, TREK1 (encoded by *KCNK2*), TRAAK (encoded by *KCNK4*) and TREK2 (encoded by *KCNK10*) — show notable heat sensitivity, with Q_{10} values of 5–10 in the 20–40 °C temperature range, followed by a steep drop in activity at higher temperatures[83,84]. These three channels are expressed in sensory neurons, in which they modulate stimulus sensitivity in a highly temperature-dependent manner[82]. Indeed, both TREK1- and TRAAK-deficient mice displayed an enhanced sensitivity to warmth at the transition between non-noxious and noxious heat, as well as hypersensitivity to mechanical stimuli[85,86]. Mice without both TREK1 and TRAAK also display a hypersensitivity to cold that is not observed in the single-knockout mice[86].

The firing of action potentials in neurons requires the activity of Nav channels, and of the nine mammalian Nav channels, at least five (namely, Nav1.1 and Nav1.6–Nav1.9) are abundantly expressed in sensory neurons[87]. Nociceptors express both tetrodotoxin (TTX)-sensitive Nav channels (for example, Nav1.7) and the TTX-resistant Nav channels Nav1.8 and Nav1.9 (REF. 87). At noxiously cold temperatures, most Nav channels in sensory neurons undergo slow voltage-dependent inactivation, thereby strongly reducing excitability[88]. Nav1.8, however, is much less sensitive to cold-induced inactivation and is thereby able to sustain action-potential initiation at noxiously cold temperatures[88]. As a consequence, action-potential generation at low temperatures fully relies on Nav1.8, and Nav1.8-deficient mice, as well as mice in which all sensory neurons expressing Nav1.8 are ablated, show negligible responses to noxious cold and mechanical stimulation at low temperatures[88,89].

Temperature sensitivity of ion channels

A fascinating but largely unresolved question in the field of thermosensation is what determines the steep thermosensitivity of ion conductances in sensory neurons. Temperature mainly affects the opening and closing (gating) of the underlying channels (BOX 1), and several distinct — but not mutually exclusive — mechanisms may contribute to this temperature dependence (FIG. 3).

The effects of temperature on channel gating. First, changes in temperature may provoke local or global phase transitions in the plasma membrane (FIG. 3a). For instance, the cell membrane's transition between the liquid-crystalline and gel-like phases has profound and abrupt effects on its tension and/or thickness[90], which in turn can affect ion channel gating. In such a model, the ion channel itself would have to be responsive to mechanical stress or to changes in membrane thickness. In this respect, it is interesting to note that some channels, in particular the K2P channels TREK1, TREK2 and TRAAK, are both mechano- and thermosensitive, which suggests that the effects of temperature on membrane tension, thickness or curvature may modulate channel gating[82,84]. However, given that temperature-dependent channel activation is mostly a gradual phenomenon, whereas phase transitions in membranes are much more abrupt, it is unlikely that mechanosensitivity is the prime mechanism of thermosensitive gating for most thermosensitive channel types.

Second, changes in temperature may alter the concentration of channel ligands by, for example, increasing or decreasing the activity of a temperature-dependent ligand-producing and/or ligand-degrading enzyme (FIG. 3b). Notably, in such a model, the temperature dependence of ligand metabolism does not need to be extremely steep, as long as the ligand activates the channel in a highly cooperative manner. For instance, if the ligand concentration has a Q_{10} of 2 (that is, a doubling of the concentration upon a 10-degree increase in temperature), and this ligand activates the channel in a cooperative manner, with a Hill coefficient of 4, then the overall channel activation has a maximal Q_{10}

Tetrodotoxin
(TTX). A toxin from puffer fish that potently blocks several types of voltage-gated Na^+ channels.

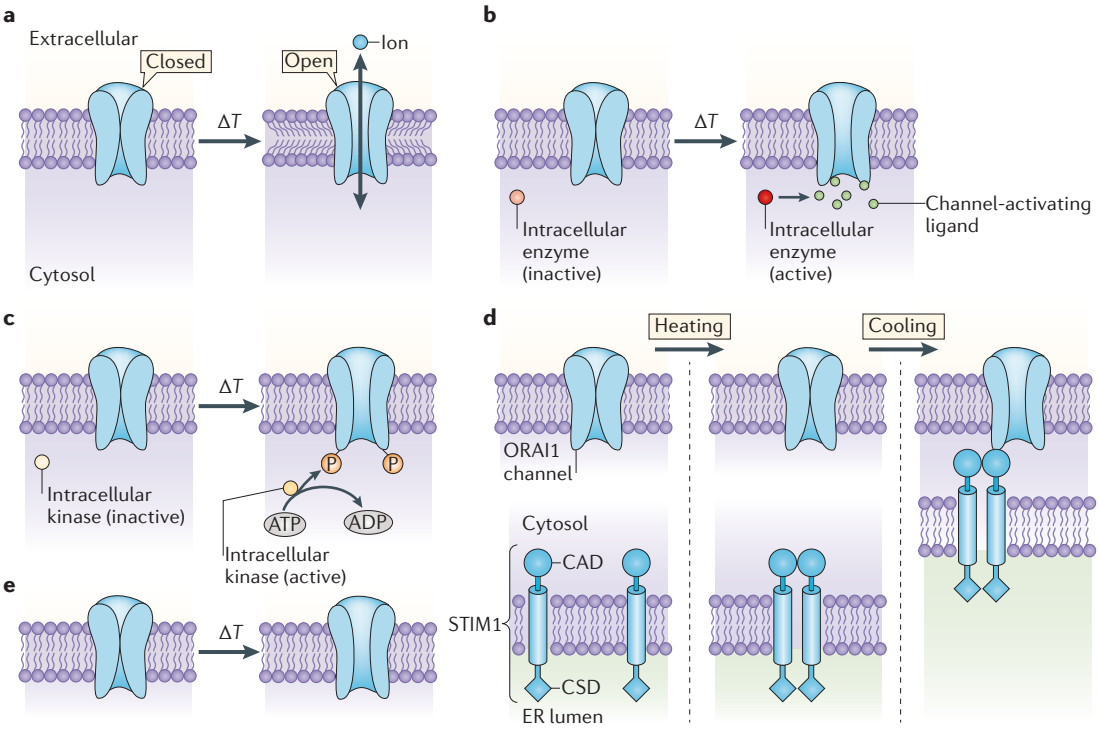


Figure 3 | **Potential mechanisms underlying temperature-sensitive gating of ion channels.** Open channels conduct ions into or out of the cell, depending on channel type and ionic conditions. Δ*T* represents an activating change in temperature, which can be either negative (for cold-activated channels) or positive (for heat-activated channels). **a** | Temperature-dependent changes in membrane properties. Here, the channel is mechanosensitive and responds to temperature-dependent alterations in membrane tension, thickness or curvature. **b** | Temperature-dependent production of channel ligand. In this mechanism, an intracellular enzyme is activated by the change in temperature, leading to the production of channel-activating ligands. **c** | Temperature-dependent channel phosphorylation. An intracellular kinase is activated by the change in temperature, leading to phosphorylation and activation of the channel. **d** | Temperature-dependent activation of Ca^{2+}-dependent Cl^- channel ORAI1. The endoplasmic reticulum (ER)-resident protein stromal interaction molecule 1 (STIM1) contains a luminal Ca^{2+}-sensing domain (CSD) and a cytosolic Ca^{2+}-activating domain (CAD). When the temperature is below 37 °C and the ER Ca^{2+} content is high, Ca^{2+} that is bound to the CSD prevents clustering and activation of ORAI1 at the plasma membrane. Heating leads to clustering of STIM1 but prevents the interaction between the CAD and ORAI1. Subsequent cooling, however, allows the CAD domains to bind to and activate ORAI1, resulting in a transient 'heat-off' response that lasts until the STIM1 clusters are disrupted. **e** | Intrinsic temperature sensitivity. Here, changes in temperature directly induce conformational changes in the channel complex, leading to channel opening.

of $2^4 = 16$. We are not aware of temperature-sensitive channels for which such an indirect, ligand-dependent pathway represents the prime activation mechanism. Nevertheless, temperature-induced changes in the cellular concentration of phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P_2) and Ca^{2+} have been proposed to modulate the activity of the TRP channels TRPM8 and TRPA1 (REFS 70,91).

Third, temperature may influence channel gating by influencing post-translational modification of the channel (FIG. 3c). For instance, the activity of many channels is strongly modulated by phosphorylation by kinases such as protein kinase C and protein kinase A[92], and in some cases this modulation is remarkably enhanced at increased temperatures, thus contributing to temperature dependence[93]. The steepness of the temperature dependence may be further increased if the effects of post-translational modification on channel gating show cooperativity, as described above for the effects of non-covalent ligands.

Fourth, temperature may alter the conformation and/or localization of a channel-regulating auxiliary subunit. A striking example is the temperature-dependent multimerization of the ER-resident protein STIM1 and the ensuing ORAI1-mediated 'off' response to a heat stimulus described above[80] (FIG. 3d). This process may be a more general mechanism of thermosensitive modulation of plasma membrane channels given that STIM1 has also been proposed to regulate other membrane channels, including voltage-gated Ca^{2+} channels[94,95] and TRPC channels[96], although the lattermost interaction has been disputed[94,97].

Last, the pore-forming part of the channel itself may exhibit considerable intrinsic thermal sensitivity (FIG. 3e), which manifests itself through steeply temperature-dependent changes in the channel's mean open probability P_{open}. Compelling evidence for such an intrinsic activation mechanism has recently been provided for cold-activated TRPM8 and heat-activated TRPV1, both of which were shown to retain high

Enthalpy
A thermodynamic measure of the internal energy of a system. In a protein, formation of stabilizing intramolecular bonds causes a reduction in enthalpy.

temperature sensitivity following purification and subsequent reconstitution into artificial lipid bilayers[98,99]. The temperature dependence of several other cold- or heat-activated TRP channels, including TRPM3, TRPM4, TRPM5 and TRPA1 (REFS 23,50,100), can be described using a unifying two-state gating model that was initially developed for TRPV1 and TRPM8 (REF. 101). Therefore, it is reasonable to assume that these TRP channels are all intrinsically thermosensitive. In recent years, a growing number of studies have provided insight into the mechanistic basis of this steep, intrinsic thermosensitivity, which is described further below.

Box 3 | Thermodynamic aspects of channel gating

The equilibrium between two global states of a protein or protein complex is determined by:

$$K_{eq} = \exp\left(\frac{-\Delta G}{RT}\right) \quad (8)$$

where K_{eq} represents the ratio between the two states, ΔG is the difference in Gibbs free energy between the two states, R is the universal gas constant and T is the temperature in Kelvin[102]. Applying this to a hypothetical ion channel that has only one open and one closed state yields the following expression for the channel's open probability (P_{open}):

$$P_{open} = \frac{1}{1 + \exp\left(\frac{\Delta G_{gating}}{RT}\right)} \quad (9)$$

in which ΔG_{gating} is the change in Gibbs free energy when transiting from the closed to the open state. Although ion channels generally exhibit multiple closed and open states, such a two-state reduction generally also yields a reasonable description of the gating equilibrium between the principal closed and open state (or states)[102,108].

ΔG_{gating} depends on the change in enthalpy (ΔH_{gating}) and entropy (ΔS_{gating}) upon channel opening, according to:

$$\Delta G_{gating} = \Delta H_{gating} - T\Delta S_{gating} - E \quad (10)$$

where E represents other possible forms of energy that affect the opening of the channel. For instance, several temperature-sensitive transient receptor potential (TRP) cation channels (including TRPV1, TRPA1, TRPM3 and TRPM8) are also voltage gated, which implies that E includes the change in electrostatic energy $E = zFV$, where z is the gating charge, F is the Faraday constant and V is the transmembrane voltage[101,175].

At low P_{open}, $Q_{10,gating}$ is maximal and directly related to ΔH_{gating}, in accordance with:

$$Q_{10,gating} = \exp\left(10 \times \frac{\Delta H_{gating} - E}{RT^2}\right) \quad (11)$$

Half-maximal activation occurs at a temperature T_{50}:

$$T_{50} = \frac{\Delta H_{gating} - E}{\Delta S_{gating}} \quad (12)$$

From this analysis, it can be appreciated that the opening of a heat-activated channel (that is, one with a $Q_{10,gating}$ value much greater than 1) is a process with a marked and positive value for ΔH_{gating}. Thus, the channel's opening represents an endothermic reaction: a reaction that absorbs heat from the surroundings. By contrast, the opening of a cold-activated channel (where the $Q_{10,gating}$ value is less than 1) represents an exothermic reaction with negative ΔH_{gating} and ΔS_{gating} values. By fitting experimental data to a two-state gating model, estimates for ΔH_{gating}, ΔS_{gating} and z have been obtained for several TRP channels implicated in thermosensation (see the table)[23,50,100,101].

Channel	ΔH_{gating} (kJ mol^{-1})	ΔS_{gating} (kJ mol^{-1} K^{-1})	z	$Q_{10,gating}$ (at –70 mV)	T_{50} (°C at –70 mV)
TRPM3	130	400	0.55	5.30	61
TRPV1	185	590	0.71	10.80	49
TRPM8	–160	–550	0.89	0.14	8
TRPA1	–125	–440	0.41	0.22	5

Basis of intrinsic thermosensitivity. Thermodynamic considerations, as explained in BOX 3, dictate that the opening of a heat-activated channel (where $Q_{10,gating} >> 1$) is associated with a marked increase in both enthalpy ($\Delta H_{gating} >> 0$) and entropy ($\Delta S_{gating} >> 0$), whereas cold-activated channels ($Q_{10,gating} << 1$) exhibit negative values for both ΔH_{gating} and ΔS_{gating}. Understanding the origin of intrinsic thermosensitive gating of ion channels requires knowledge of the structural rearrangements that provoke these significant changes in enthalpy and entropy during channel gating.

Intrinsically temperature-sensitive TRP channels, such as TRPV1 and TRPM8, function as tetrameric complexes of ~4,000 amino acids and interact with a large number of membrane lipids and water molecules. The enthalpy of a specific state of a channel complex is determined by all of the interatomic interactions in the system, which include not only covalent bonds but also weaker interactions, such as van der Waals interactions, hydrogen bonds, salt bridges (formed between acidic and basic amino-acid side chains) and cation–π interactions (formed between aromatic and basic amino-acid side chains). The disruption or formation of such weaker interactions leads to increases or decreases in enthalpy, respectively. Smaller changes in enthalpy are associated with van der Waals interactions (0.1–2 kJ mol^{-1}) and hydrogen bonds (~5 kJ mol^{-1}), whereas larger amounts of energy are associated with cation–π interactions (10 kJ mol^{-1}) and salt bridges (20 kJ mol^{-1})[102]. Concomitantly, entropy, which is a thermodynamic measure of the disorder of a system, also generally increases when weaker interactions are disrupted. *In silico* analysis of the recent high-resolution cryo-microscopy structure of the unliganded (and therefore most probably closed) TRPV1 channel[27] indicates that one tetrameric channel complex comprises at least 70 cation–π interactions, 100 salt bridges and 1,500 hydrogen bonds. Thus, disruption of only a small percentage of these interactions upon channel opening would suffice to explain the reported values for the gating enthalpy of TRPV1 (REFS 101,103), which are in the range of 180 to 400 kJ mol^{-1}.

In the past decade, several studies have searched for specific structural elements that underlie steep temperature dependence, in particular in TRP channels, by evaluating the consequences of channel mutagenesis on temperature-induced channel activity. On the basis of these studies, several authors have proposed the existence of confined 'thermosensor modules' in temperature-sensitive TRP channels, akin to the voltage-sensor modules found in voltage-gated cation channels[104–107]. In FIG. 4, the regions that have been implicated in the temperature sensitivity of TRPV channels are indicated. The observation

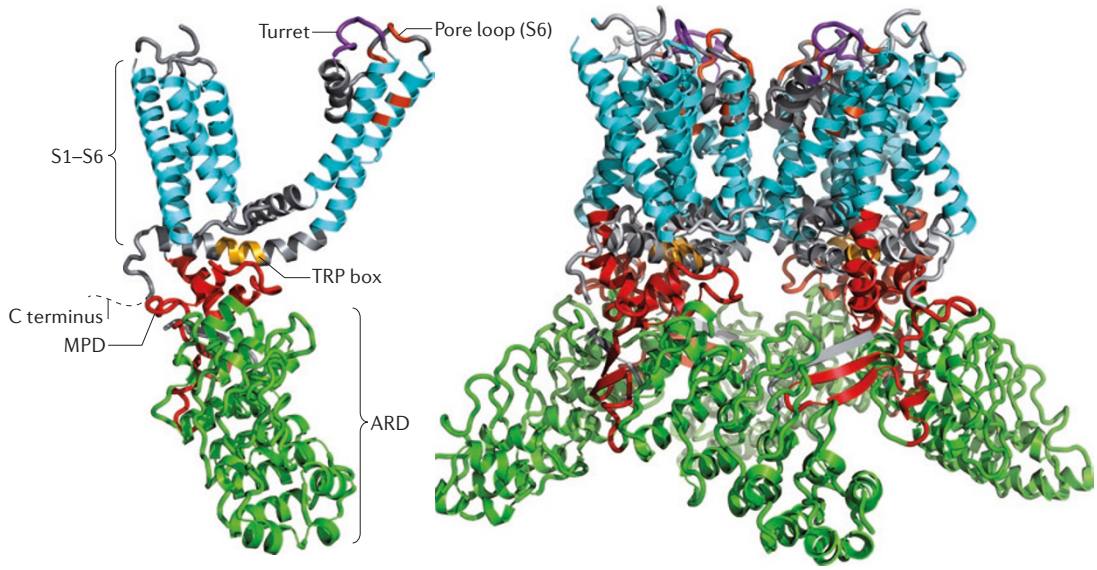


Figure 4 | **Putative regions implicated in the heat sensitivity of TRPV channels.** The structure of a single transient receptor potential V1 (TRPV1) subunit (left) and entire tetrameric channel (right) viewed from the side[27], showing the ankyrin-repeat domain (ARD), the transmembrane domains S1–S6 and various regions implicated in the heat sensitivity of TRPV channels. These include: first, the membrane-proximal domain (MPD; between the ankyrin repeats and the first transmembrane domain), which has been put forward as a crucial modular thermal sensor in TRPV channels[184]; second, the pore turret, a 25-amino-acid loop that connects S5 with the pore helix, which was reported to be required for normal heat-induced but not capsaicin-induced activation of TRPV1 (REF. 185); third, several residues in the outer pore region and in the initial part of S6, alterations to which impair heat-induced activation of TRPV1 and TRPV3 (REFS 106,186); and last, the carboxy-terminal TRP box, alterations to which were found to reduce the Q_{10} for heat-induced activation of TRPV1 (REF. 187). Note that a further proximal C-terminal part, which was reported to confer heat-induced activation to the cold-sensitive TRPM8 (REFS 104,188), a more distal C-terminal region, which has been put forward as another major structural determinant of thermal sensitivity in TRPV1 (REFS 30,189), and a large part of the pore turret[185] are not visible in the structure. These segments were removed to improve the biochemical stability of the expressed TRPV1 channel, which nevertheless retained sensitivity to heat in functional assays[27]. Based on similar structure–function experiments on TRPA1 from different animals, including *Drosophila melanogaster*, snakes and mammals, it was proposed that elements in the ARDs, S5 and the pore region dictate thermal sensitivity[71,107,190]. The RCSB Protein Data Bank identifier for TRPV1 is 3J5P.

that these regions are spread over large parts of the channel does not seem to provide strong support for the view that thermosensitivity specifically arises in conserved and/or demarcated regions but rather suggests that alterations in different parts of temperature-sensitive TRP channels can affect their response to temperature[108]. Conceivably, the marked difference in enthalpy between closed and open states may arise from multiple submolecular rearrangements occurring simultaneously during gating in dispersed areas of the channels. As the structural effects of point mutations and domain swaps on channel structure and gating rearrangements remain unpredictable, a detailed understanding of the structural basis of thermosensing remains some way off. However, the recent advances in high-resolution cryo-electron microscopic structural analysis, which yielded detailed pictures of TRPV1 in closed and ligand-bound states, have paved the way towards visualization of the structural rearrangements that occur during temperature-induced channel gating of ion channels[27,109]. This may ultimately enable calculation of the relative contributions of domains, residues and atoms to the unique thermodynamic properties that underlie thermosensitivity.

Entropy
A thermodynamic measure of the disorder of a system. It is determined by the number of different configurations (microstates) that correspond to a specific macrostate.

Pathophysiology of thermosensation

The thermal sensitivity of sensory neurons, including nociceptors, is determined by the balance of the activities of a blend of depolarizing and repolarizing ion channels. Unbalanced activity of one or more of these channels causes a distorted perception of thermal stimuli, which may result in thermal dysaesthesias, pain or improper thermal homeostasis.

Genetic factors influencing thermosensation. Reduced acuity of thermosensation and/or thermoregulation is among the symptoms of various pleiotropic disorders — including certain hereditary forms of neuropathies, ataxia and congenital insensitivity to pain — all of which are associated with more generalized sensory disturbances[110]. Even in healthy humans, there is substantial interindividual variation in thermosensitivity. Multiple factors, including age, sex, body-mass index, prior temperature experience and psychological state, are known to influence an individual's sensitivity and response to cold or heat as well as the subjective experience of pain evoked by a noxious thermal stimulus[111,112]. In addition, there is evidence for a genetic influence on temperature sensitivity, such as the strong variation in pain response to noxious

temperatures between ethnic groups[97]. Studies investigating whether genetic variants in the genes encoding temperature-sensitive ion channels can contribute to this interindividual variability have reported that variations in the gene encoding TRPA1 account for a small part of the variation in cold-withdrawal latency in healthy subjects[113] and that variants in the genes encoding TRPA1 and TRPV1 strongly correlate with thermosensory abnormalities in individuals with neuropathic pain[114]. The full consequences of these mutations on channel expression and function remain to be explored.

A more striking example of genetic variation in a temperature-sensitive TRP channel is provided by the rare autosomal dominant disease familial episodic pain syndrome (FEPS), which has been characterized in a single Colombian family[65]. The FEPS-associated mutant TRPA1 channel exhibits a gain of function, including an increased sensitivity to cold and chemical agonists. Individuals with FEPS show normal thresholds for cold- or heat-evoked pain but experience episodes of debilitating upper-body pain that can be triggered by fasting or cold stimuli, such as swimming in cold water, and terminated by eating and warming[65].

Acquired thermal hypersensitivity. Several acquired pathologies are characterized by large shifts in temperature sensitivity. For instance, inflamed or wounded tissue typically exhibits hypersensitivity to both mechanical and thermal stimuli[7,9]. Thermal hypersensitivity is characterized by lower thresholds for both cold- and heat-evoked pain (thermal allodynia) as well as by the exacerbation of pain responses to noxious thermal stimuli (thermal hyperalgesia)[9]. Whereas hyperalgesia and allodynia have a useful purpose — namely, to warn and protect already-injured tissue from further damage — hypersensitivity can become chronic and debilitating. Both peripheral sensitization, which occurs when local mediators alter the function and sensitivity of thermal sensors at the sensory nerve ending, and phenotypic switching, which involves changes in gene expression owing to pathological status, have been shown to contribute to hypersensitivity[101]. Central-sensitization mechanisms, whereby hyperalgesia and allodynia result from enhanced processing of thermal input from the somatosensory system in the CNS, have been discussed elsewhere[9,115].

A large body of evidence links inflammatory heat hyperalgesia to TRPV1 activity. Initial studies on TRPV1-deficient mice showed that these animals fail to develop heat hyperalgesia in response to experimental local inflammation induced by injection of complete Freund's adjuvant (CFA) or carrageenan[21,22]. Inflamed tissue, including skin injured by ultraviolet B irradiation, releases various extracellular factors, including bradykinin, lipid mediators, ATP, nerve growth factor (NGF) and protons (collectively called the inflammatory soup), which can all sensitize or potentiate TRPV1-mediated heat responses[116,117]. Whereas protons can directly bind to the extracellular part of TRPV1, resulting in sensitization to heat[118], other factors act through their cognate membrane receptors[119], which subsequently activate diverse intracellular signalling pathways.

First, the heat-sensitizing effects of NGF and bradykinin have been proposed to reflect relief of tonic inhibition of TRPV1 by PtdIns(4,5)P_2, as the binding of NGF or bradykinin to their respective receptors leads to the activation of the PtdIns(4,5)P_2-hydrolysing enzyme phospholipase C (PLC)[119,120]. Although the inhibitory effect of PtdIns(4,5)P_2 on TRPV1 activity was recently confirmed in reconstitution experiments in artificial lipid bilayers[99], several other studies have indicated that PtdIns(4,5)P_2 can also potentiate TRPV1 activity and that its breakdown correlates with channel desensitization[121,122]. Second, diacylglycerol — which is formed upon PLC-mediated hydrolysis of PtdIns(4,5)P_2 — activates protein kinase C, which in turn phosphorylates TRPV1 on multiple sites, leading to the sensitization of the channel to heat[123,124]. Similar sensitization is observed following TRPV1 phosphorylation by other serine/threonine kinases, including protein kinase A and Ca^{2+}/calmodulin-dependent protein kinase II[125,126]. Third, there is evidence that, within minutes, receptor activation can increase the number of functional TRPV1 channels in the plasma membrane. In the F-11 cell line, which mimics rat DRG neurons, activation of the NGF receptor TRKA (also known as NTRK1) promotes the exocytotic insertion of TRPV1 through a PLC-independent mechanism that involves phosphatidylinositol 3-kinase[121]. In addition, several chronic painful pathologies — including chronic inflammation, nerve injury, diabetic neuropathy and bone cancer — can lead to increased expression of TRPV1 (REFS 127–129). Indeed, there is evidence for both transcriptional and post-transcriptional regulation of TRPV1 expression under these conditions, which can result in prolonged heat hypersensitivity[127,128,130].

In addition to TRPV1, several other temperature-sensitive channels have been linked to heat hyperalgesia. Most strikingly, similarly to TRPV1-deficient mice, TRPM3-null mice fail to develop heat hyperalgesia in inflamed tissue after injection of CFA[50]. In addition, mice that lack expression of ANO1 in DRG neurons show reduced heat hyperalgesia under conditions of inflammation or nerve injury[77,78], whereas TREK1-deficient mice exhibit exacerbated heat hyperalgesia[85]. Although it remains to be established how the activity and/or expression of these channels is modulated during inflammation or other pathological conditions, it is becoming clear that TRPV1 should no longer be regarded as the sole mediator of heat hyperalgesia.

Cold allodynia and cold hyperalgesia are severe dysaesthesias that are common following chemotherapy or are associated with conditions such as diabetes mellitus, herpes virus infections, inflammation and nerve compression[9,131]. Recent research has highlighted the role of temperature-sensitive ion channels in the development of cold hypersensitivity. TRPM8 expression and activity are augmented in rat DRG neurons affected by nerve injury, leading to increases in both the proportion of cold-sensitive neurons and their responsiveness to cold or menthol[131]. Moreover, genetic disruption or pharmacological inhibition of TRPM8 was found to strongly reduce cold hypersensitivity caused by inflammation or nerve constriction[57,132]. The mechanisms that modulate

Allodynia
Pain in response to a stimulus that does not normally evoke pain.

Complete Freund's adjuvant
(CFA). An emulsion of inactivated and dried mycobacteria. When injected subcutaneously, it induces local inflammation and hypersensitivity to thermal and mechanical stimuli.

Carrageenan
A sulphated polysaccharide extracted from red edible seaweeds. When injected subcutaneously, it induces local inflammation and hypersensitivity to thermal and mechanical stimuli.

TRPM8 expression and function under pathophysiological conditions are not fully understood. Several studies provide *in vitro* evidence that TRPM8 activity is reduced after activation of PLC-coupled receptors, an effect that has been attributed to either depletion of PtdIns(4,5)P_2 or activation of protein kinase C[133–136]. Surprisingly, however, one study provided evidence that inhibition of TRPM8 function following the activation of bradykinin and histamine receptors was independent of PLC, PtdIns(4,5)P_2 depletion and protein kinase C and was instead mediated though direct binding of $G\alpha_q$ to the channel[137].

There is evidence that TRPA1 is involved in the development of cold hypersensitivity following nerve injury or inflammation, but whether this is due to increased expression or sensitivity of TRPA1 remains controversial[74,138,139]. TRPA1 also has a crucial role in the cold allodynia that occurs after intoxication with ciguatoxins, a class of fat-soluble compounds that are produced by microalgae and that accumulate in large tropical fish[140]. These toxins shift the voltage dependence of Nav channels in TRPA1-expressing cold-activated nociceptors such that these neurons fire action potentials at non-noxious cool temperatures, resulting in severe pain[141]. Finally, TRPA1 is involved in the development of cold hypersensitivity associated with the use of anticancer drugs such as paclitaxel and oxaliplatin[142–144]. In addition to sensitizing TRPA1, oxaliplatin has been shown to reduce the expression of the temperature-sensitive K2P channels TREK1 and TRAAK, and to increase the expression of hyperpolarization-activated cyclic nucleotide-gated (HCN) channels, thus greatly increasing the excitability of cold-sensitive sensory neurons[145].

TRP channels as therapeutic targets

An estimated one in every five adults experiences moderate to severe chronic pain, and about one half of these individuals report inadequate pain control[146]. The elucidation of the essential role of temperature-sensitive TRP channels in various forms of acute and chronic pain has created the opportunity to investigate their potential as molecular targets for novel, more specific and/or effective analgesic drugs[147].

Inspired by the finding that TRPV1-deficient mice are generally healthy but fail to develop thermal hyperalgesia, intensive efforts have been made to develop TRPV1 antagonists as novel analgesic drugs[32]. This has resulted in multiple classes of highly potent and selective compounds that effectively inhibit TRPV1 activity both *in vitro* and *in vivo*[32]. Several of these antagonists have shown encouraging analgesic effects in animal models of inflammatory, neuropathic, bone-cancer and post-operative pain[148–150], as well as in some limited studies of post-operative pain in humans[33,151]. However, the first generation of TRPV1 antagonists was associated with mild to severe hyperthermia (up to 40 °C) in humans and other animals[33,148,151,152]. This hyperthermia is a general, on-target effect, as it is observed in rodents, dogs, primates and humans but not in TRPV1-deficient mice[153–155]. Mechanistically, inhibition of TRPV1 in the peripheral sensory neurons that innervate the skin and viscera causes the thermoregulatory centres in the brain to underestimate the actual core and environmental temperatures, leading to the unnecessary activation of thermoregulatory processes such as skin vasoconstriction and thermogenesis that are aimed at increasing body temperature[154]. In line herewith, humans dosed with TRPV1 antagonists regularly describe feeling cold and exhibit visible shivering before the onset of hyperthermia[155]. This unwanted hyperthermia has resulted in the termination of several drug development programmes.

More recent evidence suggests that modality-specific antagonists, which inhibit TRPV1 activity induced by heat or capsaicin but not by protons, do not markedly affect body temperature[156]. However, whether such compounds are equally effective analgesics remains to be established. As an alternative, compounds that inhibit sensitization of TRPV1 — for instance, by disrupting its interaction with the scaffolding protein AKAP79 (A-kinase anchor protein 79) — have shown analgesic effects in inflammatory pain in mice without causing hyperthermia[157]. Interestingly, the hyperthermic effects of TRPV1 antagonists are mirrored by the hypothermia that is induced by TRPV1 agonists such as capsaicin, which fool the body into believing that it is hot, resulting in thermoregulatory responses such as cutaneous vasodilation and sweating that are aimed at increasing body-heat loss. It has been proposed that mild, TRPV1 agonist-induced hypothermia could be of therapeutic use in, for example, reducing neurological injury in survivors of cardiac arrest[158,159].

TRPM8-activating stimuli, including cold temperatures or natural products such as menthol or eucalyptol, have long been used for topical pain relief, and recent experiments using TRPM8-deficient mice confirm that the analgesic effects of such treatments are fully dependent on TRPM8 activation[58,160]. These findings suggest that TRPM8-expressing, non-nociceptive sensory neurons can exert inhibitory control over nociceptive signalling[160]. More recently, TRPM8 antagonists have been developed as potential drugs to treat cold hypersensitivity and cold-related pain. The first results in rodent models confirm that TRPM8 is a promising target to treat cold hyperalgesia associated with inflammation and peripheral nerve injury but also show that, in contrast to the hyperthermic effects of TRPV1 antagonists, systemic inhibition of TRPM8 causes mild and transient hypothermia[132,161]. TRPM8 antagonists cause a reduction in the activity of cool-sensitive sensory neurons innervating the skin, which leads to an overestimation of the environmental temperature, resulting in the inhibition of cold-avoidance behaviour, cold-induced cutaneous vasoconstriction and non-shivering thermogenesis[161]. The inverse is seen with TRPM8 agonists such as icilin or menthol, which provoke warm-seeking behaviour and shivering, leading to hyperthermia[132,162].

Thus, whereas TRPV1 and TRPM8 antagonists have demonstrated their effectiveness in reducing thermal hypersensitivity, they have also revealed that their target channels have a central role in thermal homeostasis, acting as molecular thermometers that provide essential information about the environmental and core body temperature to the body's thermostat. This may put constraints on these antagonists' future therapeutic use,

especially under conditions in which thermal homeostasis is already compromised, such as fever or severe climatological circumstances. Pharmacological targeting of TRPM3 and TRPA1, which detect more extreme temperatures (FIG. 2), may provide a safer alternative. Initial preclinical tests indeed suggest that antagonists of these channels can inhibit certain forms of thermal pain without affecting core body temperature[51,163].

Conclusion and perspectives

Over the past two decades, we have witnessed the identification of various ion channels as molecular thermometers that translate environmental and internal thermal cues into electrical activity in the somatosensory system. Advanced approaches towards the dissection of the molecular and cellular logic of mammalian thermosensation have provided genetic and pharmacological tools to modulate thermosensitivity *in vivo* and have opened the way to the development of novel drugs to treat various forms of acute or chronic pain. Nevertheless, it should be pointed out that our understanding of the fundamentals of thermosensation is still fragmentary for the following reasons.

First, whereas for some time it was believed that temperature-sensitive TRP channels could fully explain the ability of the mammalian somatosensory system to detect and discriminate temperatures covering the 0–60 °C range, more recent research calls the universal role of TRP channels into question. In particular, responses to noxious cold and noxious heat are partially blunted, but by no means abrogated, following combined elimination of the established cold- and heat-sensitive TRP channels[50,73]. This indicates the existence of multiple redundant molecular mechanisms to detect these potentially life-threatening stimuli, including thermosensitive elements that remain to be identified. In recent years, several novel classes of ion channels (for example, the mechanosensitive PIEZOs[164], the channels related to the Ca^{2+}-activated Cl^- channel ANO1 (REFS 165–167) and the store-operated ORAI channels[168,169]) have been identified using powerful techniques such as expression profiling, genome-wide or rational RNAi screens, expression cloning and bioinformatics. Similar approaches may lead to the discovery of additional novel classes of ion channels representing the currently missing links in thermosensation.

Second, despite numerous structure–function studies, we are still far from understanding the mechanisms that underlie the striking temperature sensitivity of the several ion channels that are involved in thermosensation. Well-studied activating mechanisms for ion channels, such as transmembrane voltage changes or ligand binding, act on clearly delineated channel domains. By contrast, temperature seems to be particularly intractable, because it affects all atoms in the channel and its surroundings, and alterations in various parts of temperature-sensitive channels affect temperature dependence. Therefore, a full understanding of the basis of the steep thermosensitivity in ion channels will most probably require atomic-detail structural information of the conformational changes that occur during gating. Although crystallization of entire TRP channels has proven to be extremely difficult, recent advances in structure determination using single-particle cryo-electron microscopy may hold the potential to obtain detailed structures of different channel conformations during temperature-dependent gating[27,109].

Last, initial enthusiasm for the use of antagonists of the prototype heat sensor TRPV1 as potent new analgesics has been tempered by the finding that such compounds can dangerously disturb normal temperature homeostasis[32]. This unforeseen consequence of tampering with temperature-sensitive channels illustrates our limited knowledge of the processes that steer thermoregulation in mammals but has also fuelled investigations that have revealed important aspects of the neuronal pathways that translate thermosensory information into thermoregulatory responses[17,18]. Furthermore, powerful tools that will undoubtedly expand our knowledge of the physiology of thermoregulation further have been developed, including genetic techniques that enable controlled elimination of specific thermosensitive neurons *in vivo*[52] and genetically encoded sensors that enable visualization of thermogenesis with subcellular resolution[170]. A more comprehensive understanding of the physiology of thermoregulation, in combination with deeper insights into the molecular and submolecular aspects of thermosensation in normal and pathophysiological conditions, may provide the basis for the development of more efficacious and safer therapies that target temperature-sensitive channels to treat thermal hypersensitivity and pain.

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Competing interests statement

The authors declare no competing interests.

DATABASES

RCSB Protein Data Bank TRPV1 (3J5P): http://www.rcsb.org/pdb/explore/explore.do?pdbId=3j5p

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