

THERMOTRP CHANNELS AND BEYOND: MECHANISMS OF TEMPERATURE SENSATION

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We possess an acute sense of temperature. Most of us seek shade on a hot summer day, prefer a warm shower to a cold one, and enjoy red wines served at a temperature of 15–18°C. Thermosensation not only affects our comfort, but is also essential for the survival of most organisms. We are now beginning to uncover the molecular identity of proteins that confer thermosensation. The thermoTRPs, a subset of transient receptor potential ion channels are activated by distinct physiological temperatures, and are involved in converting thermal information into chemical and electrical signals within the sensory nervous system.

SENSORY SYSTEMS

PROPRIOCEPTORS

Sensory terminals that are present in muscles, tendons and joint capsules, which receive information about the movements and position of the body.

The peripheral nervous system governs the broadly defined sense of touch, communicating with the central nervous system about the environment, allowing the conscious sensations of balance and coordination, pressure and vibration, pain and temperature. Our sense of touch therefore provides a constant flow of information, ranging from the shape and temperature of objects to the position of our bodies relative to the surfaces on which we walk. Importantly, our sense of touch also acts as an initial alert system that signals when there are potentially dangerous or damaging environmental conditions.

The neurons that allow us to sense these distinct stimuli are located in the dorsal root ganglia (DRG) and within cranial nerve ganglia such as the trigeminal ganglion (FIG. 1). The DRG are clusters of sensory neuron cell bodies that are located in the vertebral column just lateral to the spinal cord. There is functional specialization among the DRG neurons such that they can be partitioned according to their specific sensory modalities¹. That is, on the basis of what environmental stimulus they detect, DRG neurons are functionally classified as PROPRIOCEPTORS, low-threshold mechanoreceptors, and cells that sense pain and/or temperature (FIG. 1a). Nociceptive (pain) neurons detect noxious thermal, mechanical (high-threshold) or chemical stimuli. Thermosensitive neurons, our focus in this article, detect

temperature either in the noxious range (making up a subset of nociceptive neurons) or in the innocuous range.

The DRG neurons are pseudounipolar: one process travels long distances to peripheral tissues such as the skin and muscle, where it detects sensory stimuli, and another branch relays this information to the dorsal horn of the spinal cord (FIG. 1). Whereas the peripheral branches of proprioceptive and low-threshold mechanosensitive neurons terminate in specialized organs in the skeletal muscle and skin, the axons of temperature- and pain-sensing neurons travel to the epidermal and dermal layers of the skin and terminate as free nerve endings (FIG. 1b). Histological studies show the anatomical specialization of these free nerve endings: their branched terminals contain vesicles of unknown function closely apposed to skin cells^{2–6} (see also REF. 7). On the basis of their conduction velocities, both temperature- and pain-sensing neurons are known to be small-diameter, slowly conducting unmyelinated C fibres and larger, more rapidly conducting, thinly myelinated A δ fibres.

Among the temperature- and pain-sensing neurons, there is further biochemical and functional diversity. Some nociceptors are classified as peptidergic, releasing peptides such as calcitonin-gene-related peptide (CGRP) and substance P in response to noxious thermal stimuli

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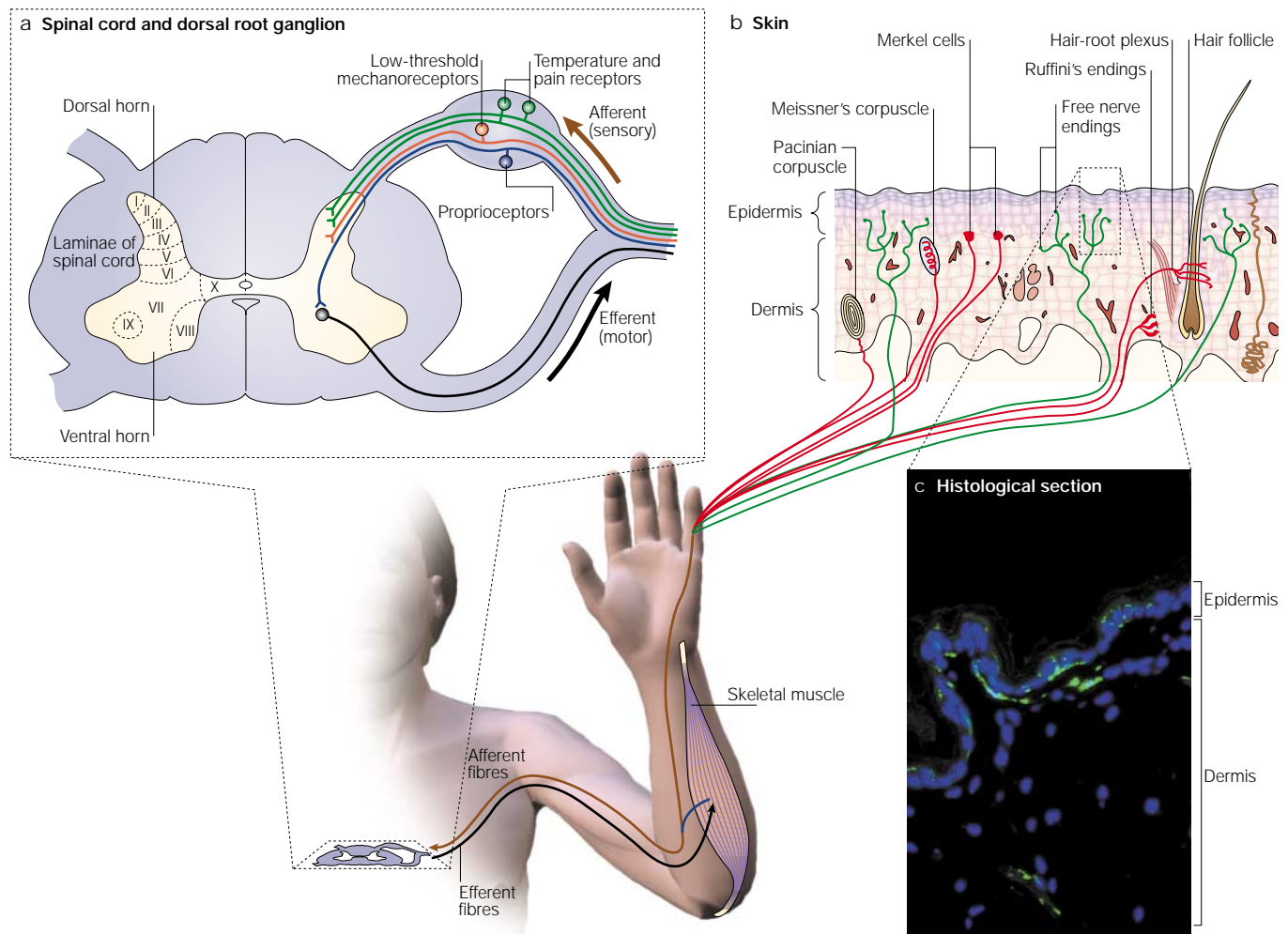


Figure 1 | Anatomic and functional organization of touch. **a** | Spinal nerves formed by the joining of afferent (sensory) and efferent (motor) roots provide peripheral innervation to skin, skeletal muscle, viscera and glands. Arrows denote the direction of incoming sensory and outgoing motor impulses. The cell bodies of motor neurons are located within the ventral horn (laminae VII–IX) of the spinal cord. Cell bodies of sensory neurons are located in the dorsal root ganglia (DRG). Within the DRG there are subclasses of sensory neurons known as proprioceptive (blue), low-threshold mechanosensitive (red) and temperature- and pain-sensing neurons (green). These neurons project centrally to dorsal horn interneurons (laminae I–VI of the spinal cord) and peripherally to target tissues. Proprioceptive neurons (blue fibre) project to specialized structures within target tissues such as muscle, and sense muscle stretch. **b** | Low-threshold mechanosensitive neurons (red fibres) project to end organs that transmit mechanical stimuli. Five types of mechanosensitive assemblies have been described and are illustrated in the figure. Temperature and pain sensing neurons (green) do not project to specialized end organs; instead they terminate as free nerve endings in all layers of the skin, and near blood vessels and hair follicles. **c** | Section of skin showing free nerve endings (green fibres) stained with the pan-neuronal marker PGP9.5. The nuclei of skin cells are stained (blue) with 4,6-diamidino-2-phenylindole (DAPI). Free nerve endings are found in both the epidermal and dermal layers.

and inflammation. These neurons express TrkA, the nerve growth factor (NGF) receptor⁸. Other nociceptors bind isolectin B4 and express c-RET, the glial-derived neurotrophic factor (GDNF) receptor⁹. Among these nociceptors, some are specialized to detect a unique noxious modality, whereas others are polymodal nociceptors that respond to painful levels of heat, cold and mechanical stimuli^{10,11}. Clear biochemical markers for neurons that sense innocuous temperature have not been identified, but functional distinctions have been described. Warm- and cool-sensitive spots studied in the skin show the presence of innocuous thermosensitive neurons that do not respond to any other stimuli such as mechanical deformation^{6,12,13} (FIG. 2).

The specific molecules that are involved in detecting and conveying thermal stimuli are currently under intense investigation. Studies of other sensory modalities have shown that sensory ion channels can be either directly gated by the sensory stimulus (mechanical, in the case of hearing), or activated indirectly through a signalling pathway that involves G-protein-coupled receptor activation (in the case of taste, olfaction and vision)¹⁴. For thermosensation, the process is thought to begin through specific receptor proteins that are located within the free nerve endings in the skin. A key advance in our understanding of temperature sensation has come from the recent cloning and characterization of temperature-activated transient receptor potential (TRP)

ANKYRIN
A protein domain that attaches integral membrane proteins to cytoskeletal elements.

Q₁₀
The change in the rate of activity resulting from a 10°C increase in temperature. The higher the Q₁₀, the more sensitive to temperature the reaction is.

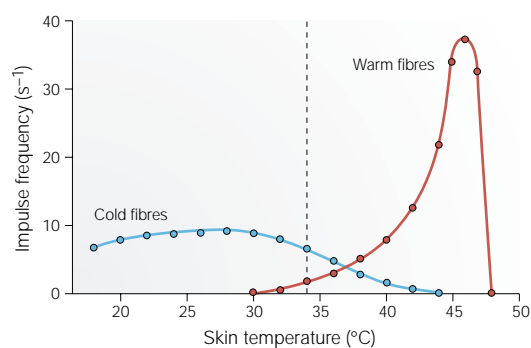


Figure 2 | Average discharge frequency of individual cold- and warm-sensitive fibres in response to changes in skin temperature. The dotted line indicates the normal skin temperature (33°C). Cold-sensitive fibres respond only to cooling, whereas warm-sensitive fibres respond to warming. Neither type of fibre responds to mechanical stimulation. Adapted, with permission, from REF. 13 © (1969) The Physiological Society.

ion channels, which we have dubbed thermoTRPs. Whereas the activity of many ion channels is thermodynamically modulated by temperature, thermoTRPs have the distinctive feature that temperature alone can activate them. In addition, individual thermoTRPs are specialized to detect distinct temperature ranges; some thermoTRPs

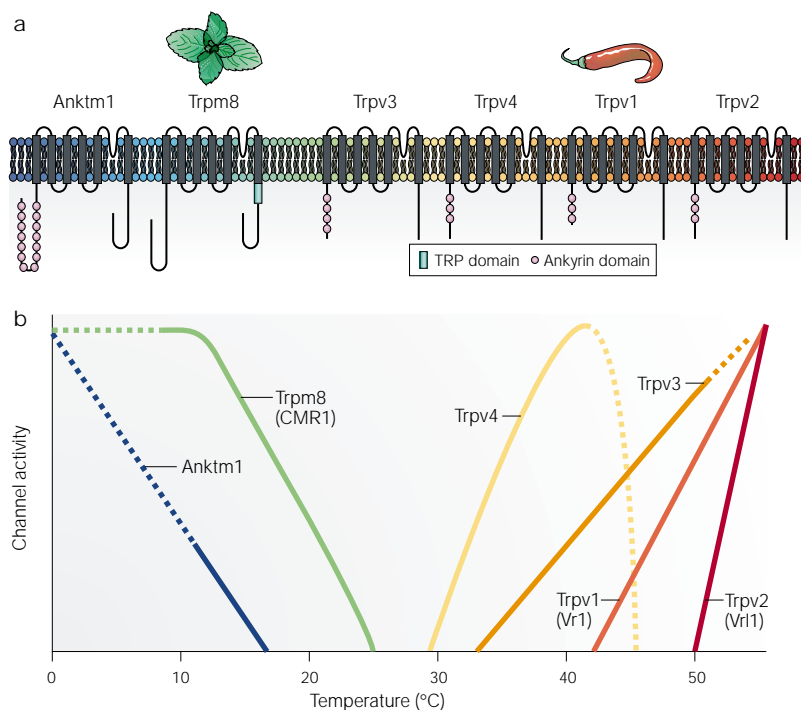


Figure 3 | Domain organization and temperature thresholds of temperature-activated transient receptor potential ion channels (thermoTRPs). **a** | TRP channels are composed of six putative membrane-spanning units and cytoplasmic amino and carboxyl termini. Some TRPs also have variable numbers of ankyrin repeats at the amino terminus, or a conserved TRP domain of 25 amino acids after the transmembrane regions. **b** | Temperatures ranging from noxious heat to noxious cold activate several members of the TRP family. The cooling compound menthol and capsaicin (the hot ingredient of chilli pepper) act as non-thermal activators of Trpm8 and Trpv1, respectively. The thresholds of activation and maximal activation are based on activity of these channels in heterologous systems; some of these thresholds are averaged values from different studies. Dashed lines indicate an uncertainty in the exact slope of the lines.

are activated by heat, others by cold. Collectively, these channels detect almost the entire range of temperatures that are sensed by most mammals. The identification of these ‘temperature receptors’ will no doubt be key to our understanding of thermosensation.

Hot and spicy

Physiological studies have shown two types of noxious heat responses in sensory fibres that are classified on the basis of their response thresholds. Some fibres respond to a moderate threshold (~43°C), whereas a smaller percentage respond to high-threshold (~52°C)¹⁵. A subset of both C and A δ fibres are believed to respond to moderately noxious stimuli (type II), whereas only a subset of A δ fibres respond to temperatures above 52°C (type I)¹⁶. The 43°C point is in the range at which we perceive a shift from innocuous warmth to noxious heat. Heat-activated signal transduction occurs in the nerve terminal at skin level. However, the signalling molecules that are involved in this process are also present in the DRG cell soma, as heat-activated currents are also evoked in dispersed cultures of DRG neurons¹⁷. Responsiveness to capsaicin (the hot ingredient of chilli peppers), a vanilloid compound, was shown to be a primary pharmacological trait of a main subpopulation of the heat-sensitive neurons, particularly those with small- to medium-sized fibres that are activated at about 45°C¹⁸.

Using capsaicin responsiveness as a screening readout, the first vanilloid receptor was cloned from a cDNA library from rat sensory neurons¹⁹. The cloned receptor — **Trpv1** (Vr1) — belongs to the TRP family of cation channels²⁰. The prototypical TRP channel was originally identified in *Drosophila* and is involved in phototransduction²¹. This *Drosophila* TRP is a calcium-permeable cation channel that is activated downstream of rhodopsin receptor signalling through phospholipase C. Over the past few years, several invertebrate and vertebrate homologues of the *Drosophila* TRP channels have been cloned. These proteins are thought to have intracellular amino and carboxyl termini with six putative transmembrane segments, and a predicted pore region between segments five and six^{22,23} (FIG. 3a). TRP channels have been classified into different subfamilies on the basis of overall homology and the presence of structural domains such as ANKYRIN repeats²³.

Trpv1 is highly expressed in a subset of peptidergic and isolectin B4-binding DRG and trigeminal ganglion neurons. Heterologous expression of Trpv1 resulted in capsaicin-gated currents similar to the responses that are evoked in sensory neurons by this agent. Importantly, Trpv1 is also activated by noxious temperatures that are equal to or higher than 42°C (FIG. 3b) with a Q_{10} value of 20.6 (REFS 19,24). Trpv1 shows a much higher sensitivity to heat than most ion channels, which show small linear increases in current flow with Q_{10} values lower than 2. Trpv1 is also activated and potentiated by low pH, indicating a role for Trpv1 as a molecular integrator of nociceptive stimuli²⁵. Trpv1 was therefore proposed to be the receptor that transduces the type II currents that mediate nociception at the pain threshold ($\geq 42^\circ\text{C}$)²⁶.

Table 1 | Properties of ion channels involved in thermal transduction

Nomenclature	Other names	Temperature sensitivity	Non-thermal agonists	Blockers	Tissue distribution	References
Transient receptor potential (TRP) channels involved in thermosensation						
Trpv1	Vr1	≥ 42°C	capsaicin, lipoxygenase, acidic pH, resiniferatoxin, NADA, anandamide and ethanol	Ruthenium red, capsazepine	PNS, brain, spinal cord, skin, tongue, bladder	19,25,32,35,41,45
Trpv2	Vr11	≥ 52°C	Growth factors (mouse)	Ruthenium red	PNS, brain, spinal cord, widely expressed	46,48
Trpv3	Vr13	> 33°C		Ruthenium red	Skin, PNS (human)	51–53
Trpv4	OTRPC4, VR-OAC, Trp12, Vr12	~ 27–42°C	Hypotonic, 4- α phorbol	Ruthenium red, gadolinium	Kidney, PNS, skin, inner ear, brain, liver, trachea, heart, skin, hypothalamus, fat endothelium	54–59,106
Trpm8	CMR1	≤ 25°C	Menthol, icilin, eucalyptol		PNS, prostate (human)	71,72,76
Anktm1		≤ 17°C	Icilin	Ruthenium red	PNS	77
Non-TRP proteins that might be involved in thermosensation						
TREK-1	Kcnk2	Cold	Membrane stretch, polyunsaturated fatty acids, intracellular pH		PNS, brain	88,89
P2X3		Warmth	ATP		PNS	94
Na/K ATPase	?	Cold?		Ouabain	PNS?	90,91
BNC1, ASIC, DRASIC		Cold	Acidic pH (potentiated)	Amiloride	PNS	92

PNS, peripheral nervous system.

Two groups have carried out functional studies in mice lacking Trpv1. In support of its role as a sensor of thermal and chemical stimuli, heat ($\geq 42^\circ\text{C}$) and capsaicin-gated currents were completely absent in cultured neurons from mutant mice, whereas high-threshold ($> 55^\circ\text{C}$) heat responsiveness was not^{27,28}. Somewhat unexpectedly, physiological tests on mutant animals only uncovered small differences in response latencies to mild noxious stimuli ($\geq 42^\circ\text{C}$), but reduced pain sensitivity at temperatures higher than 50°C was observed. Perhaps the threshold temperature for activation of a thermoreceptor *in vitro* is lower than the *in vivo* threshold for initiating action potentials in a deeply insulated nerve fibre²⁹. Alternatively, another receptor might partially compensate for the lack of Trpv1 in the mutant mice. It is clear, however, that noxious heat sensation is disrupted, but not abolished in these mice.

The most striking phenotype of *Trpv1*^{-/-} mice is a severe deficit of inflammation-induced thermal HYPERALGESIA, indicating that Trpv1 activity might be modulated by elements that are present in the inflammatory milieu. Indeed, Trpv1 activity is facilitated by substances that are known to participate in inflammation such as mild acidification, NGF, bradykinin, lipids (including several lipoxygenase products), prostaglandins, protein kinases A and C, and ATP^{25,30–39} (TABLE 1). For example, bradykinin and NGF potentiate Trpv1 activity by modulation of intracellular phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P₂)³⁴. Recently, PtdIns(4,5)P₂ has been shown to inhibit Trpv1 gating through interaction with the carboxy-terminal portion of the channel⁴⁰. Furthermore, NGF leads to an increase in Trpv1 levels in culture and in the inflamed hindpaw through the

activation of the p38 mitogen-activated protein kinase (MAPK)^{37,38}. In response to NGF, Trpv1 protein levels in the skin were about 25 times higher than in controls. By contrast, Trpv1 was unchanged in the central terminals of sensory neurons, indicating that complex post-transcriptional and trafficking pathways regulate Trpv1 expression. In addition, the endocannabinoid anandamide can activate Trpv1, and ethanol has also recently been shown to activate and sensitize Trpv1 (REFS 35,41,42). Collectively, these studies indicate that Trpv1 is not simply a thermoreceptor, but its activity is modulated by various molecules that act through distinct pathways. This is not surprising given the range of inflammatory mediators to which nociceptive neurons respond.

Roles of Trpv1 outside of the sensory nervous system are also emerging. Trpv1 is expressed in nerve fibres that innervate the bladder and also in urothelial cells. *Trpv1*^{-/-} mice have impaired bladder function and disrupted stretch-evoked purine release from urothelial cells⁴³. Further studies are needed to determine whether the role of Trpv1 in the bladder is a new mechanosensitive property or whether it depends on the release of factors that activate Trpv1.

Trpv1 expression is also observed in the brain, consistent with reports that capsaicin evokes a response in various brain nuclei⁴⁴. The expression of Trpv1 in the brain argues for the presence of an endogenous agonist of this channel. Recently, capsaicin-like substances that are present in the brain, such as *N*-arachidonoyldopamine (NADA) and *N*-oleoyldopamine (OLDA), were shown to activate Trpv1 (REF 45). Further evaluation of the Trpv1-knockout mice might assist in clarifying the role of this receptor in the brain.

HYPERALGESIA
Repeated application of a noxious stimulus leads to a progressive increase in the response of nociceptors. This process, known as hyperalgesia, manifests as a prolonged pain sensation even after the stimulus is removed.

Based on its 50% sequence identity to Trpv1, the vanilloid receptor-like channel **Trpv2** (Vr11) was identified⁴⁶ (TABLE 1). Functional analysis in mammalian cells and *Xenopus* oocytes showed that Trpv2 is insensitive to capsaicin and low pH, and its threshold for activation by heat is $\geq 52^\circ\text{C}$ (FIG. 3b). In the DRG and the trigeminal ganglion, Trpv2 is predominantly expressed in neurons of medium to large diameter that are believed to project their myelinated axons to the superficial laminae of the dorsal horn. It has therefore been proposed that Trpv2 is the molecular transducer of high-threshold, type I currents that are observed in sensory neurons distinct from neurons that express Trpv1. The size distribution of Trpv2-immunoreactive neurons is also similar to that of the high-threshold, heat-responding cells in cultured primary neurons⁴⁷. As is the case with Trpv1, Trpv2 transcripts are expressed outside the sensory nervous system, implying that there are additional, unidentified functions of this channel. To date, none of the modulators of Trpv1 have been shown to affect Trpv2 activity. Interestingly, insulin-like growth factor 1 has been reported to cause the translocation of mouse Trpv2 to the membrane, where it forms a constitutively active channel at room temperature⁴⁸. Studies of Trpv2-knockout animals should provide more direct evidence for the role of this channel in high threshold heat transduction and possible additional functions.

Warm and fuzzy

Whereas most of the attention in the search for heat transducers has focused on the mechanisms for the detection of noxious temperatures ($> 42^\circ\text{C}$), innocuous warmth ($34\text{--}42^\circ\text{C}$) is also important for the survival of an organism. Warmth is thought to be sensed by a subset of specialized thermosensitive neurons (FIG. 2). Afferent impulses that are elicited by warming have been recorded from nerve fibres that innervate the skin, tongue and nose of several species, including primates^{12,13,49}. In these studies, recordings were made from nerve fibres while the skin was perfused with warm buffer. These experiments showed that warm-sensitive fibres were not excited by mechanical stimulation and, on the basis of their conduction velocity, they seemed to be non-myelinated. Unlike mechanoreceptors that are silent in the absence of tactile stimuli, warm receptors fire action potentials continuously at low rates at skin temperatures of 34°C (REF. 6). As temperature increases, warm-sensitive fibres show an equivalent increase in firing frequency, with the maximum response scattered over a temperature range of $41\text{--}47^\circ\text{C}$. At spinal levels, distinct warm-sensitive neurons have also been reported in lamina I of the dorsal horn⁵⁰.

Hensel and Iggo¹² observed that cutaneous warm fibres of the hairy skin of primates could be divided into two groups. Both populations showed activity in the range of $30\text{--}47^\circ\text{C}$. However, one group of neurons had an average maximum response at 41°C , whereas the second group continued to increase its firing rate above this temperature. Remarkably, these electrophysiological characteristics are roughly comparable to the properties of two recently identified TRP channels that respond to warm temperatures (FIG. 3 and TABLE 1). The first of them

— **Trpv3** — was cloned by sequence homology to other heat-activated TRP channels, and shares 40% identity with Trpv1 (REFS 51–53). In heterologous systems, Trpv3 showed an activation temperature of $\sim 34\text{--}38^\circ\text{C}$, and the current continued to increase in the noxious temperature range. The second channel that is activated by warm temperatures is **Trpv4**. Trpv4, which is $\sim 50\%$ identical to Trpv1, was originally identified as an osmosensor^{54–57}. The channel was activated by cell swelling that was induced by hypotonic solutions, and inhibited by increases in osmolarity. More recently, acute changes in ambient temperature have been shown to activate Trpv4 independently of osmolarity^{58,59}. Heat-evoked responses in *Xenopus* oocytes and mammalian cells expressing Trpv4 were detected once temperatures reached $\sim 27\text{--}34^\circ\text{C}$. Sustained heating beyond 42°C typically resulted in a decline in the amplitude of the Trpv4 current even as the temperature continued to rise. Collectively, the characterization of Trpv3 and Trpv4 heat responses is reminiscent of the earlier electrophysiological data on native sensory fibres, but there is no physiological evidence yet that these ion channels are involved in sensing warmth *in vivo*.

An emerging characteristic of TRPV channels is their varied responses to repeated heat challenges. For example, repeated heating ($> 42^\circ\text{C}$) of Trpv1-expressing cells elicits only slight sensitization with a lower heat-activation temperature^{46,60}. By contrast, Trpv3 shows marked sensitization with repetitive heat challenges^{51,52}. The first response to a step increase in heat is often small, but the current response grows significantly with repeated heating. The mechanism that underlies this behaviour is not known, but given that Trpv3 is active at both warm and noxious temperatures, this phenomenon could warn an organism of a potentially damaging stimulus. The use of pharmacological inhibitors and other loss-of-function studies should clarify if Trpv3 has a role in thermosensation and thermal nociception.

By contrast to Trpv3, the responses of Trpv4 to heat show desensitization on repeated heat applications. Prolonged exposure to a suprathreshold temperature also causes Trpv4 responses to desensitize. However, once Trpv4-expressing cells are acclimated to 37°C , the channel can still respond to increases in temperature up to 42°C (REF. 58). Although the activation threshold of Trpv4 indicates that the channel might be constitutively active at body temperature, the fact that it can detect changes after adapting to 37°C would allow Trpv4 to be activated by small fluctuations in body temperature.

Neurons that are sensitive to highly ($> 50^\circ\text{C}$) and moderately ($> 43^\circ\text{C}$) noxious temperatures have been identified in dissociated DRG cultures^{17,47}. However, warm-sensitive neurons with an activation threshold lower than 40°C have not been identified. This might be attributable to issues that are associated with neuronal culture conditions. Typically, the sensory terminals are completely removed during the isolation of DRG neurons, and although many properties are retained in culture, some are probably lost during this process. Interestingly, the Trpv4 mRNA, but not the protein, was found in DRG neuronal bodies, implying that the Trpv4

protein is also rare in dispersed cultures⁵⁸. There could be a mechanism for the regulation of the Trpv4 protein transport to sensory terminals. Such a mechanism would not make cultured DRG cells an ideal setting in which to study warm-activated thermosensitive neurons. Similarly, high levels of Trpv3 mRNA have not been observed in mouse sensory ganglia by NORTHERN BLOT or by *in situ* hybridization, although transcripts were detected by RT-PCR (PCR after reverse transcription of RNA)⁵¹. By contrast, readily detectable levels of expression were observed in the mouse skin. However, monkey and human TRPV3 expression in DRG neurons and other tissues has been observed^{52,53}. Smith *et al.*⁵³ showed co-expression of TRPV1 and TRPV3 in human DRG sections using immunohistochemistry, and established that these two proteins could be co-precipitated in heterologous expression systems. The apparent disparity between primate and rodent TRPV3 expression in sensory ganglia could reflect inter-species variability. However, our analysis of human TRPV3 expression using Northern blot showed high levels of expression in human skin, but not in some of the other tissues that are reported to express TRPV3 (unpublished observations). Further studies will be needed to determine whether TRPV3 is expressed at physiologically relevant levels within the DRG.

Irrespective of expression levels in sensory neurons, it is clear that both Trpv3 and Trpv4 are expressed in skin cells^{51,52,58}. Although keratinocytes contribute to hyperalgesia by releasing molecules in response to injury and inflammation, their direct role in thermosensation has not been established. Given their proximity to both the external environment and free nerve endings, it is intriguing to postulate that skin cells also contribute to temperature sensation. Future studies aimed at evaluating heat responses in native skin cells, and tissue-specific gene knockouts of Trpv3 and Trpv4 should help address this issue.

In addition to sensing cutaneous temperature, Trpv4 might also participate in regulating thermogenesis. The preoptic and anterior hypothalamus is the control centre of thermogenesis in the brain, and has specialized warm- and cool-sensitive neurons that are also activated by hypo-osmolarity^{61–64}. Trpv4 protein expression and currents with an activation threshold similar to that of Trpv4 have been recorded in neurons from this region^{57,58,61}. Similar Trpv4-like responses have been observed in endothelial cells, and Northern blot analysis has shown expression of this channel in the endothelium^{56,59}. As heating can result in local vasodilation, activation of Trpv4 in the endothelium could contribute to this thermoregulatory process. Although an analysis of Trpv4-knockout mice was recently reported, sensitivity to warm temperature or thermogenesis was not assessed⁶⁵.

Cool, minty and cold

Similar to warm-specific fibres, a class of cold-sensitive afferent fibres was identified to respond specifically to moderately cool temperatures^{5,6} (FIG. 2). These fibres were described as innocuous cold-specific, and did not respond to warming or to non-thermal stimuli such as

strong mechanical deformation¹². Conversely, a distinct class of cold-sensitive fibres has been described as polymodal nociceptors, responding to noxious cold, heat and pinch^{10,11,66}.

The cooling sensation of mint-derived menthol is well established. Several studies of skin cold-receptive fields indicated a strong correlation between menthol and cold sensitivity of individual free nerve endings (for example see REF. 67). Menthol also modulates the activity of cool-induced currents. In recordings from lingual and nasal cold-sensitive afferents, menthol application enhanced the activity of these fibres at warmer temperatures^{67,68}. Similarly, electrophysiological and calcium-imaging experiments of cultured sensory neurons identified a population of cells that responded to both cool stimuli and menthol^{69,70}. Finally, Reid and Flonta suggested that menthol and cooling stimuli are transduced through a non-selective cation channel that is located within the cutaneous peripheral projections of DRG neurons⁶⁹.

Two groups independently cloned and characterized a cool and menthol receptor — Trpm8 (CMR1)^{71,72}. Trpm8 was identified by expression cloning, taking advantage of the fact that menthol is a known stimulant of sensory neurons, and of its low, but significant sequence similarity to other sensory TRP channels. In heterologous systems, Trpm8 is activated by menthol and cooling, with an activation temperature of ~25–28°C (FIG. 3 and TABLE 1). As is the case with native cold-sensitive neurons, subthreshold concentrations of menthol that are present during cooling shift the activation temperature of Trpm8 to warmer temperatures (from ~25°C to ~30°C). This is similar to the change in activation temperature of Trpv1 in the presence of mildly acidic conditions²⁵. Trpm8 is also robustly activated by icilin (a synthetic cooling compound) and by eucalyptol (the naturally occurring *Eucalyptus* derivative)^{72,73}. The temperatures that activate Trpm8 are similar to those that activate native innocuous-cold-sensitive fibres in the skin. However, as Trpm8 is only activated at about 25–28°C in heterologous systems, the baseline activity of some cold-sensitive fibres at 33°C (skin temperature) is not readily accounted for by the activity of Trpm8 alone (FIGS 2 and 3)^{12,74}.

The expression pattern of Trpm8 also argues for a physiological role for this ion channel in innocuous thermosensation. It is specifically expressed in the DRG and trigeminal ganglion neurons with the fibres of the smallest diameter (probably C fibres). At the cellular level, Trpm8 does not co-localize with any known markers of nociceptive fibres such as CGRP, substance P, Trpv1 or isolectin B4 (REF. 71). However, its expression is lost in newborn *trkA*-null mutant mice, indicating that Trpm8 is expressed in a subset of pain- or temperature-sensing neurons. As none of the markers that are specific for pain-sensitive fibres are co-expressed with Trpm8, we hypothesize that this ion channel specifically marks cool- and menthol-sensing neurons. Recent single-cell analysis showed a strong correlation between cold- and menthol-responsiveness and Trpm8 expression, reinforcing the theory that Trpm8 is the endogenous menthol receptor⁷⁵. Trpm8 is also expressed in the prostate, but its function in this organ is unknown⁷⁶.

NORTHERN BLOT

A molecular technique in which RNA molecules are separated by electrophoresis, transferred to nitrocellulose, and subsequently identified with a suitable probe.

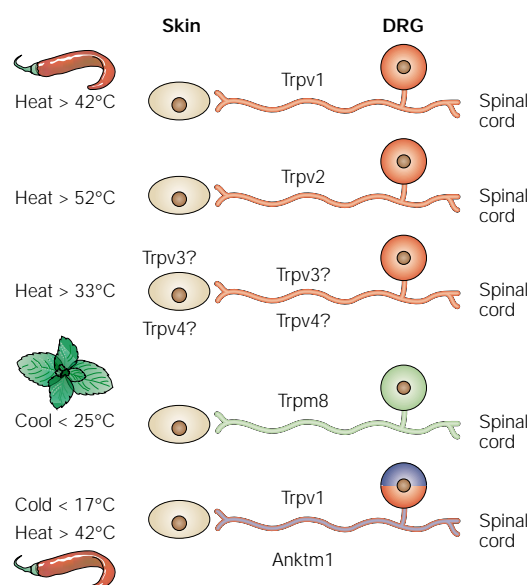


Figure 4 | Expression and temperature sensitivity of temperature-activated transient receptor potential ion channels (thermoTRPs) label distinct populations of sensory neurons. This figure shows the cell bodies and peripheral projections of sensory neurons. ThermoTRPs with unique temperature thresholds that are expressed in distinct subsets of neurons have been identified. The model presented here is mainly based on pairwise comparisons of expression patterns, and a more complicated scenario might be more realistic. The relevant expression of the warm-activated channels Trpv3 and Trpv4 is not clear. The last neuron in this figure illustrates a putative ‘polymodal nociceptor’. The expression of Anktm1 in a subset of the noxious-heat-sensing Trpv1-expressing cells indicates that these neurons are tuned to respond to multiple types of painful stimuli. DRG, dorsal root ganglia.

Anktm1, a distantly related TRP channel, is also activated by cold with a lower activation threshold as compared to Trpm8 (REF. 77). In heterologous expression systems, Anktm1 is activated at $\sim 17^\circ\text{C}$, a temperature that is reported as painfully cold by humans (FIG. 3 and TABLE 1). Anktm1 is insensitive to menthol but is activated by icilin, although more modestly than Trpm8. Unlike Trpm8, Anktm1 is specifically expressed in a subset of sensory neurons that express the nociceptive markers CGRP and substance P. Strikingly, Anktm1 is expressed within a subset of neurons that express the noxious-heat receptor, Trpv1 (FIG. 4). This raises the interesting possibility that Anktm1 and Trpv1 mark and mediate the function of a class of polymodal nociceptors — neurons that respond to both noxious heat and cold. Unlike the cold-activated currents of Trpm8, electrophysiological studies of Anktm1 in both CHO cells and *Xenopus* oocytes show strong desensitization during a single cooling ramp and during repeated cooling steps. The physiological relevance of such strong inactivation is not understood. Interestingly, a similarly robust desensitization was not observed in calcium-imaging experiments, which points to important differences that should be considered when comparing data obtained with the two techniques.

Studies of cultured DRG neurons support a physiological role of two groups of cold-sensitive neurons with Trpm8- and Anktm1-like properties⁷⁷ (see also REF. 75). Overall, such culture studies agree quite well with the *in vivo* co-expression analysis of the various thermoTRPs in DRG neurons. One exception is the co-localization of Trpv1 and Trpm8. Whereas *in situ* hybridization studies do not show any co-expression, experiments on cultured DRG have shown clear menthol and capsaicin co-responsiveness from individual neurons^{72,78}. This discrepancy has been proposed to depend on the high levels of NGF that are present in these cultures, which sensitizes and upregulates Trpv1 levels⁷⁷.

Coding of temperature information

DRG neurons are highly specialized to sense distinct ranges of temperatures at the skin. Emerging evidence also points to a great deal of specialization within the central targets of thermosensitive neurons, the dorsal horn of the spinal cord. In lamina I of the dorsal horn, functionally distinct interneurons have been identified that respond to specific sensory stimuli. ‘Cool’ neurons respond to innocuous cool temperatures; ‘HPC’ neurons respond to noxious heat, pinch and cold; ‘NS’ (nociceptive specific) neurons respond to noxious heat and pinch; and rare ‘warm’ neurons only respond to innocuous warm temperatures^{50,79}. In addition, combined histological and electrophysiological studies show morphological distinctions among the three most abundant classes of lamina I interneurons⁸⁰. Furthermore, the spinal cord interneurons that respond to innocuous temperature changes versus noxious thermal stimuli have different thalamic projections⁸¹.

Collectively, these data indicate a labelled-line hypothesis, where distinct sets of sensory neurons are tuned to convey specific sensory information through dedicated pathways to the central nervous system. However, the exact connectivity between the primary thermosensitive neurons and their spinal interneuron targets is not clear. The characterization of thermoTRP expression in DRG cell bodies and the projections of these cells will shed light on the connectivity of thermosensitive neurons. So far, preliminary analysis indicates that Trpv1, Trpv2 and Trpm8 might be expressed in unique populations. One clear exception is the expression of Anktm1 in a subset of Trpv1 neurons (FIG. 4). Will Trpv1/Anktm1-positive neurons specifically project to polymodal HPC interneurons? Will Trpm8-expressing neurons project to ‘cool’ interneurons? The answers to these and similar questions might help us decode sensory processing, which is undoubtedly complex. Several sensory enigmas (PARADOXICAL HEAT AND COLD, the THERMAL GRILL ILLUSION and cold ALLODYNIA) illustrate this anticipated complexity^{82–84}.

How are thermoTRPs gated?

So far, the mechanisms that underlie thermal activation of TRP channels are not known. Given that ankyrin domains are thought to couple membrane proteins to the cytoskeleton, one possibility is that channel gating by heat requires cytoskeletal components. However,

PARADOXICAL HEAT AND COLD
Conditions in which a cold stimulus produces the sensation of being hot and *vice versa*.

THERMAL GRILL ILLUSION
A sensation of painful heat that is elicited by touching interlaced warm and cool bars. It was first shown by T. Thunberg in 1896.

ALLODYNIA
A heightened sensitivity to a normally innocuous stimulus such that it is perceived as painful. An example is an increased sensitivity of sunburned skin to light touch.

heat-activated Trpv1 single-channel recordings have been obtained from excised membrane patches, showing a lack of overt dependence on an intact cytoplasmic architecture²⁵. By contrast, Trpv4 single-channel recordings could only be evoked by heat in cell-attached patches⁵⁹. These findings indicate that Trpv4 activity might require cytoplasmic anchoring and/or an additional cellular messenger. Recent experiments have identified the third transmembrane domain of Trpv1, and its amino and carboxyl termini, as essential for capsaicin sensitivity^{60,85}. However, the temperature sensitivity of Trpv1 (or any other thermoTRP) has not been mapped to specific residues or domains.

Beyond thermoTRPs

The discovery of thermoTRPs indicates that thermosensation is mediated in part by a common molecular mechanism that uses these ion channels as the primary transducers of thermal stimuli. However, given that humans can detect changes in temperature as small as 1°C, it is likely that temperature sensation involves a complex interaction of specialized receptors that work in concert with a repertoire of ion channels and additional proteins to allow the fine detection of thermal energy. Both Anktm1 and Trpm8 are activated by cold stimuli, but as cold temperature decreases the activity of many enzymes, there might be inactivating mechanisms that help depolarize and excite neurons. For example, recent electrophysiological studies have raised the possibility that cold transduction involves the inhibition of a background potassium channel, which causes depolarization and firing of action potentials in cold-sensitive neurons^{86,87}. The molecular nature of this channel is not known. One candidate is TREK-1, a member of a family of mammalian two-pore domain K⁺ channels⁸⁸ (TABLE 1). TREK-1 is expressed throughout the brain with high levels of expression in the preoptic and anterior hypothalamus⁸⁹. TREK-1 is also expressed in small- to medium-diameter mouse DRG neurons. Recently, TREK-1 channels were shown to open gradually and reversibly in response to heat⁸⁹. A 10°C increase in temperature increased the TREK-1 current amplitude by ~7-fold. At physiological temperatures, TREK-1 would be open and help keep the neuron near its resting potential, whereas at cooler temperatures the channel would close and therefore contribute to the depolarization and activation of the neuron in response to cold. Whether TREK-1 is sufficient for cold transduction remains to be determined. It will be of interest to evaluate whether TREK-1 is co-expressed in Trpm8- or Anktm1-positive neurons. However, a recent study did not detect TREK-1 mRNA in single-cell RT-PCR experiments in DRG neurons⁷⁵.

In addition to the inhibition of K⁺ channels, early work in the mollusk *Aplysia* and in mammals indicated that cold transduction was dependent on the inhibition of a Na⁺/K⁺ ATPase^{90,91} (TABLE 1). Ouabain, a relatively specific inhibitor of this pump, was found to induce excitatory responses in cold receptors. The operation of a temperature-sensitive Na⁺/K⁺ pump was proposed to maintain the resting potential of the thermosensitive

neuronal membrane so that a drop in temperature would decrease pump activity and lead to depolarization. Recent experiments tested the role of this ATPase in cultured DRG neurons⁸⁶. Although repeated cooling induced depolarization and action potentials in a subpopulation of DRG neurons, treatment with ouabain in these cold-sensitive neurons only elicited currents that were 10–50% of that induced by cooling. Furthermore, ouabain-evoked responses never elicited action potentials in these cells. These findings indicate that this might not be a primary mechanism for cold transduction.

In addition to channels and proteins that are directly activated by temperature, other molecules are modulated or indirectly activated by temperature. For example, members of the degenerin/epithelial sodium channel (DEG/ENaC) family of sodium channels are potentiated by cold⁹² (TABLE 1). The acid-gated currents generated by some of these family members (ASIC and DRASIC) show a marked temperature dependence. Lowering the temperature enhanced the current by slowing desensitization in the presence of an agonist, but cold temperatures alone did not seem to induce channel gating. In another example, P2X3, an ATP-gated cation selective ion channel that is expressed in sensory neurons, seems to have a role in the sensation of warmth⁹³ (TABLE 1). P2X3-knockout mice respond normally to noxious temperatures (> 45°C), but showed little neuronal activity to warm temperatures (32–45°C) when recorded from spinal cord interneurons⁹⁴. The channel has not been directly activated by warm temperature in heterologous systems, nor have P2X3 currents been potentiated by temperature. Mechanistically, how P2X3 receptors are involved in the coding of innocuous warmth remains to be determined.

Thermosensation in invertebrates. Although thermal adaptation mechanisms in invertebrates might be essentially different from those of homeothermic mammals, invertebrates also need to detect environmental temperature. Invertebrates have sensory systems to monitor temperature fluctuations and show preference for distinct temperature ranges. For example, when confronted by a thermal gradient, the nematode *Caenorhabditis elegans* migrates towards the initial temperature at which it was cultivated⁹⁵. These worms also migrate away from the temperature at which they were previously starved. Laser ablation studies have shown that the neural circuit of thermotaxis in the 15–25°C range is composed of a pair of sensory neurons (termed AFD) in addition to two pairs of interneurons (AIY and AIZ). In addition to thermotaxis, *C. elegans* also shows a reflexive withdrawal reaction to an acute heat stimulus, possibly through a neuronal and molecular pathway that is different from the one that mediates thermotaxis, although this is not well understood⁹⁶. Similarly, *Drosophila* adult flies show a strong preference for ambient temperatures around 24°C, and ablation studies have revealed that thermoreceptors are housed in the third antennal segment⁹⁷. In *Drosophila* larvae, terminal sensory organ neurons and body-wall neurons are thermosensitive⁹⁸.

Table 2 | TRP channels in sensory transduction.

Gene name	Sensory process	Mechanism*	References
Trpv1–4	Mammalian temperature	Direct	19,46,51–53,58,59,107
Trpm8	Mammalian temperature	Direct	71,72
Anktm1	Mammalian temperature	Direct	77
Trpc2	Mammalian pheromone	Indirect	108
Trpm5	Mammalian taste	Indirect	109,110
Pkd1–2	Mammalian shear stress	Direct?	111
Trp	<i>Drosophila</i> vision	Indirect	112
Nompc	<i>Drosophila</i> mechanical	Direct	113
Painless	<i>Drosophila</i> noxious temperature and mechanical (polymodal)	?	100
dAnktm1	<i>Drosophila</i> temperature	Direct	101
TRPVs (i.e. OSM-9)	<i>Caenorhabditis elegans</i> (polymodal)	Indirect?	102
Yvc1p	Yeast mechanical	Direct	105,114

TRP, transient receptor potential. *The use of the word 'direct' as a mechanism of activation implies that the channel functions as the sensory receptor. An 'indirect' mechanism indicates that the channel functions downstream of the sensory stimulus, typically downstream of a G-protein-coupled receptor.

Although the involvement of invertebrate TRP ion channels in various sensory modalities such as vision, olfaction, osmosensation and mechanosensation has been clear, a direct role in thermosensation was lacking⁹⁹. However, recent experiments indicate that TRP channels have a crucial role in invertebrate thermosensation as well. A new Anktm1 homologue in *Drosophila* named *painless* is expressed in thermosensory multidendritic neurons, and is shown to be required for the response of larvae to both noxious thermal and mechanical stimuli¹⁰⁰. It is not yet clear if *painless* is a direct sensor of temperature or if it acts downstream of a signalling pathway, reminiscent of the role of *Drosophila* TRP in the visual system. A direct role of a *Drosophila* TRP channel in sensing heat is indicated by another recent study; dAnktm1, the *Drosophila* sequence orthologue of Anktm1, is activated by a warming stimulus when expressed in heterologous systems¹⁰¹. This is the first invertebrate temperature-activated ion channel to be characterized. However, whether dAnktm1 has a physiological role in sensing temperature is not known.

The *C. elegans* genome contains predicted dAnktm1- and *painless*-related TRP channels. However, these proteins have not been characterized yet. On the other hand, the TRPV-family members of *C. elegans* OSM-9 and OCR-1–4 (OSM-9/capsaicin receptor related genes) are well characterized and are required for responses to

mechanical stimuli, light osmolarity and avoidance of noxious chemicals¹⁰². Heterologous expression of OSM-9 and OCR-2 has not shown any currents when stimulated with capsaicin, heat or osmotic pressures. *C. elegans* TRPVs seem to function downstream of G-protein-coupled receptor signalling in the sensory transduction pathway (TABLE 2). Interestingly, the introduction of mammalian Trpv1 in OSM-9-expressing nociceptor ASH neurons in *C. elegans* induces an avoidance to capsaicin. This indicates that ASH neuron activity is labelled as an avoidance signal, and depolarizing this neuron by a foreign TRP channel can activate this specific behaviour. Other protein families have also been implicated in *C. elegans* thermosensation. Two cyclic nucleotide-gated channels — *tax-2* and *tax-4* — form heteromers that are required for thermotaxis, olfactory transduction and sensory-neuron outgrowth⁹⁵. Last, the LIM homeobox gene *ceh-14* confers thermosensory function when expressed ectopically in chemosensory neurons in *C. elegans*¹⁰³, and mutations in *ttx-1*, another homeo-domain-containing protein, affect AFD neuronal function¹⁰⁴.

Concluding remarks

Although great strides have been taken towards understanding the fundamental process of sensing temperature, many questions remain unanswered. With the cloning and characterization of thermoTRPs, the tools are now available to build a molecular understanding of temperature sensation and coding. With respect to thermoTRPs, loss-of-function studies will be crucial for dissecting their physiological roles in thermosensation and pain. The characterization of thermoTRPs has coincided with findings that highlight an expanded role of TRP channels in sensory signalling. Both invertebrate and vertebrate TRP channels seem to be involved in the signalling pathways of diverse sensory modalities including mechanosensation, vision, taste and pheromone sensing (TABLE 2). Notably, the single TRP channel that has been found in the yeast genome has recently been shown to have mechanosensitive properties¹⁰⁵. Sensory TRPs fall in two classes: those that seem to be the sensory receptor and are therefore activated by sensory stimuli (such as thermoTRPs), and those that are activated indirectly by sensory stimuli, downstream of G-protein signalling. Why TRP ion channels are used repeatedly in these diverse sensory mechanisms is not completely clear. It is evident, however, that TRP channels are 'truly remarkable proteins'.

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Acknowledgements
We thank N. Hong, T. Jegla, U. Mueller and L. Stowers for critically reading the manuscript.

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