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Molecular mechanisms of temperature adaptation

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Abstract

Thermal perception is a fundamental physiological process pertaining to the vast majority of organisms. In vertebrates, environmental temperature is detected by the primary afferents of the somatosensory neurons in the skin, which express a ‘choir’ of ion channels tuned to detect particular temperatures. Nearly two decades of research have revealed a number of receptor ion channels that mediate the perception of several temperature ranges, but most still remain molecularly orphaned. Yet even within this well-researched realm, most of our knowledge largely pertains to two closely related species of rodents, mice and rats. While these are standard biomedical research models, mice and rats provide a limited perspective to elucidate the general principles that drive somatosensory evolution. In recent years, significant advances have been made in understanding the molecular mechanism of temperature adaptation in evolutionarily distant vertebrates and in organisms with acute thermal sensitivity. These studies have revealed the remarkable versatility of the somatosensory system and highlighted adaptations at the molecular level, which often include changes in biophysical properties of ion channels from the transient receptor potential family. Exploiting non-standard animal models has the potential to provide unexpected insights into general principles of thermosensation and thermoregulation, unachievable using the rodent model alone.

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Abbreviations

AITC, allyl isothiocyanate; DRG, dorsal root ganglia; K_{2P} channel, two-pore-domain potassium channel; TG, trigeminal ganglia; TRP, transient receptor potential.

Temperature sensitivity: basic principles and experimental approaches

In vertebrates, environmental temperature is perceived in the skin by primary afferents of the somatosensory neurons. Somas of the neurons are housed in the trigeminal ganglia (TG), which innervate the head, or dorsal root ganglia (DRG), which innervate the body. TG and DRG neurons form specialized, but not exclusive, functional groups, tuned to detect a particular range

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of temperatures. The molecular basis of temperature detection relies on the ability of specialized ion channels in the plasma membrane of primary afferents to initiate and propagate action potentials. Several major classes of ion channels contribute to this process: (i) receptor channels, which detect temperature changes and depolarize neurons via a non-selective cation influx; (ii) voltage-gated sodium and potassium channels, which open in response to receptor-mediated depolarization and propagate action potentials; and (iii) leak channels, which dynamically regulate the membrane potential at rest through voltage-independent potassium efflux (Fig. 1). A combination of the three classes of ion channels is thought to constitute the core of the mechanism that fine-tunes neuronal sensitivity to a particular temperature range.

Somatosensory neurons are intrinsically thermosensitive, i.e. they do not require other tissue components to convert temperature changes into excitatory ionic current. Temperature-evoked electrical activity in these cells is usually studied either by an ex vivo skin–nerve preparation, whereby afferent signals are recorded from exposed afferent fibres, or in dissociated neuronal cultures using patch-clamp electrophysiology or ratiometric calcium imaging. A disadvantage of dissociated culture is that functional responses are studied in neuronal soma, whereas in vivo the signal is detected in the distal part of primary afferents. Nevertheless, dissociated neurons appear to provide an accurate description of neuronal specialization in vivo, and the approach is widely used due to its relative simplicity, and the ability to study functional responses of individual neurons or neuronal populations in a quantitative way and in precisely controlled experimental conditions.

A typical experimental protocol aimed at understanding the role of a particular ion channel in thermosensitivity consists of a comparison of the electrophysiological responses of the somatosensory neurons from wild-type and genetically modified strains, and of behavioural experiments, such as the temperature preference test. A good correlation between electrophysiological and behavioural data, coupled with biochemical confirmation of the expression of an ion channel in the nerve endings, provides solid ground for considering a molecular target as pertaining to thermosensitivity. This approach, which has been extensively used over the last 20 years, has identified a number of key ion channels. Even though the overall picture is far from complete, a general molecular framework that underlies thermosensitivity at the level of somatosensory neurons is now well accepted, and the molecular identity of receptors that respond to some temperature ranges have been widely recognized. These include transient receptor potential (TRP) cation channel types V1 (TRPV1) and M8 (TRPM8), the receptors of noxious heat and mild cold, respectively. Other temperature ranges remain molecular orphans, even though a number of candidates have been suggested over the years.

Most of our knowledge about the mechanism of cutaneous thermosensitivity comes from studies in mice and rats. Like many other mammals, including humans, mice perceive temperatures of 15–40°C as innocuous, and above or below this range as noxious. It is generally accepted that the mouse model provides a reliable ‘reference point’ for both molecular and physiological aspects of thermosensitivity, largely due to the availability of a standardized set of animal strains, genetic approaches, electrophysiological techniques and behavioural protocols.

With the advent of advanced and accessible experimental techniques, researchers have acquired the opportunity to look beyond the mouse model. Studies of birds, infrared-sensing snakes, bats and other organisms revealed a surprising versatility of the thermoreceptive apparatus, and at the same time confirmed the general molecular framework established in mouse and rat. Studying ‘non-standard’ animals, especially those with unique

![Figure 1. Temperature-gated ion channels known to contribute to thermal sensitivity in somatosensory neurons of vertebrates](image)
thermosensory specializations (Fig. 2) provides a unique perspective with which to understand the general principles of thermosensitivity. Below we discuss recent advances in the field of cutaneous thermosensitivity in both the standard and non-standard animal models, with a focus on vertebrates.

Sensing cold

**TRPM8.** The menthol- and cold-activated non-selective cation channel TRPM8 contributes to the detection of environmental cold in vivo and in somatosensory neurons. In heterologous systems, such as mammalian cell lines or *Xenopus* oocytes, mammalian TRPM8 exhibits detectable current at around $26^\circ\text{C}$, which steeply increases further upon cooling (McKemy et al. 2002; Peier et al. 2002). Similar to other temperature-gated ion channels, the temperature at which TRPM8 activity exceeds background is often referred to as ‘channel activation threshold’, even though the value of this parameter can change depending on expression level or other experimental conditions. TRPM8 is found in 15–20% of all somatosensory neurons, usually with small (<30 $\mu\text{m}$) soma diameter (Kobayashi et al. 2005). TRPM8-deficient mice show a significant loss of menthol- and cold-evoked responses at $26^\circ\text{C}$ at the behavioural, cellular and nerve fibre levels (Bautista et al. 2007; Colburn et al. 2007; Dhaka et al. 2007). In behavioural tests, temperature deficit is evident only at mildly cooling range, and becomes insignificant at temperatures below $10^\circ\text{C}$. At the same time, obliteration of the neuronal line which gives rise to TRPM8-positive neurons (Mishra et al. 2011) or targeted ablation of TRPM8 neurons in adult mice (Knowlton et al. 2013; Pogorzala et al. 2013) eliminates cold sensitivity in the full testable range. These data strongly suggest that TRPM8-positive neurons contain an unidentified TRPM8-independent molecular mechanism responsible for the detection of mildly and severely noxious cold.

A growing body of evidence supports the notion that in addition to its role sensing environmental cold, TRPM8 is involved in body temperature regulation (e.g. see Almeida et al. 2012; Gavva et al. 2012). Correlating with this function are data showing that cold activation parameters for TRPM8 orthologues differ significantly between warm- and cold-blooded animals, and appear to tie in with the value of normal core body temperature. For example, the half-maximal temperature activation value for the TRPM8 of chickens ($29^\circ\text{C}$) is higher than that from rats ($24^\circ\text{C}$), which possibly reflects the difference in core body temperature between these species ($37^\circ\text{C}$ and $40–42^\circ\text{C}$, respectively). In accordance with this trend, the half-maximal temperature activation of two species of frogs, *Xenopus laevis* and *Xenopus tropicalis*, is $14^\circ\text{C}$ (Fig. 2) (Myers et al. 2009). Cold sensitivity of TRPM8 thus appears to be coupled to body temperature, agreeing with a role for TRPM8 in body temperature regulation, and highlighting evolutionary flexibility of the temperature receptor.

**Na,1.8.** The tetrodotoxin-resistant voltage-gated sodium channel Na,1.8 (Akopian et al. 1996) is expressed in the majority of somatosensory neurons, including

![Figure 2. Molecular adaptations to temperature sensitivity in vertebrate TRP channels](image-url)
virtually all (90%) nociceptors (Shields et al. 2012). In comparison with tetrodotoxin-sensitive voltage-gated sodium channels from the somatosensory system, such as Na$_{+}\text{,1.7}$, Na$_{+}\text{,1.8}$ is more resistant to inhibition by cold (Zimmermann et al. 2007). Pharmacological and genetic studies have shown that Na$_{+}\text{,1.8}^{-/-}$ animals are almost completely insensitive to painful cold (below 10°C), suggesting a key role of this ion channel in cold sensing and/or propagation of action potentials at low temperatures (Zimmermann et al. 2007; Abrahamsen et al. 2008; Minett et al. 2014).

Recently, a gain of function mutation, T790A, has been identified in the mouse Na$_{+}\text{,1.8}$ (Blasius et al. 2011). Animals carrying the mutation exhibit a severe neurobehavioural phenotype referred to as 'Possum'. Electrophysiological studies showed that the Possum mutation causes a dramatic increase in Na$_{+}\text{,1.8}$-mediated current density, leading to enhancement of excitability of DRG neurons and peripheral afferent nerve fibres. Notably, Possum mice are hypersensitive to noxious cold, but not to other types of painful stimuli, which supports the notion that Na$_{+}\text{,1.8}$ is a key regulator of cold sensitivity (Blasius et al. 2011; Garrison et al. 2014).

A recent study reported cold-induced regulation of Na$_{+}\text{,1.8}$ expression in neurons of gastropod molluscs. It has been demonstrated that hibernating snails have a reduced Na$_{+}\text{,1.8}$-like current density and significant down-regulation of Na$_{+}\text{,1.8}$ protein expression in buccal and cerebral ganglia – the regions responsible for feeding and olfaction, respectively – when compared to the active state (Kiss et al. 2014) (Fig. 2). Thus the involvement of Na$_{+}\text{,1.8}$ in cold-induced regulation of neuronal excitability may have deep evolutionary origin. Whether or not a similar molecular mechanism takes place in mammalian hibernators, is unknown. We surmise that a systematic investigation of molecular adaptations to cold tolerance in the somatosensory system of hibernators from different clades has the potential to reveal new molecular pathways of cold sensitivity, but such an analysis has yet to be performed.

### Sensing heat

**TRPV1.** The capsaicin- and heat-activated non-selective cation channel TRPV1 exhibits robust temperature activation above 42°C in heterologous systems and primary neurons (Caterina et al. 1997; Cao et al. 2013a). TRPV1 is expressed in 40–60% of somatosensory neurons with small (<30 μm) and medium (30–40 μm) soma diameter (Kobayashi et al. 2005). TRPV1-deficient mice show complete loss of capsaicin sensitivity and significant reduction in the detection of noxious (≥50°C) heat stimuli and/or thermal hyperalgesia (Tominaga et al. 1998; Guo et al. 1999; Caterina et al. 2000; Davis et al. 2000; Cavanaugh et al. 2011; Park et al. 2011). The 10°C difference between the detectable TRPV1 activity *in vitro* (42°C) and a clear physiological effect of its deletion on thermosensitivity *in vivo* (≥50°C) is puzzling. Even after taking into consideration the difference between the test object temperature and afferent ending in the skin, the gap appears significant, while the underlying mechanism remains elusive. At the neuronal level, the deletion of TRPV1 significantly attenuates heat-evoked discharges in C-fibres (however, see Woodbury et al. 2004) and inhibits heat-stimulated excitation in dissociated DRG neurons (Caterina et al. 2000; Davis et al. 2000; Zimmermann et al. 2005; Vriens et al. 2011). It appears likely that even though TRPV1 marks almost all heat-sensitive neurons it is not the only molecular thermosensor in these cells. Indeed, whereas the deletion of TRPV1 does not affect thermosensitivity below 50°C, selective ablation of TRPV1-positive neurons in adult mice obliterates temperature discrimination in the whole testable range of temperatures above 37°C (Pogorzala et al. 2013), suggesting the existence of a TRPV1-independent mechanism of heat sensation. Over the years, a number of temperature-sensitive ion channels, mostly of the TRP group, have been suggested as novel heat sensors. Some of them, like TRPV2, TRPV3 and TRPV4, were later shown to have a minimal role in heat sensation (Huang et al. 2011; Park et al. 2011), while others, such as TRPM3 remain interesting and in need of further study (Vriens et al. 2011; Straub et al. 2013). As of this writing, temperatures in the non-TRPV1 range do not have a commonly accepted molecular sensor.

In mice, neither the deletion of TRPV1 nor complete elimination of TRPV1-expressing neurons affects core body temperature (Mishra et al. 2011). However, pharmacological blockade of TRPV1 causes hypothermia (Gavva et al. 2007), suggesting a role for TRPV1 in a cross-talk between environmental and body temperature. A capsaicin-insensitive splice variant of TRPV1 is expressed in the neurons of the supraoptic area of hypothalamus (Sharif Naeini et al. 2006) (however, see Cavanaugh et al. 2011). These neurons exhibit temperature-activated firing and vasopressin release and are tuned to detect minute variations in the normal 37°C body temperature. Both these properties become significantly attenuated upon pharmacological inhibition or genetic ablation of TRPV1 (Sharif-Naeini et al. 2008; Sudbury et al. 2010; Sudbury & Bourque, 2013). Even though the capsaicin-insensitive splicing isoform of TRPV1 from hypothalamus still awaits cloning and analysis in heterologous systems, these studies support the notion of a key role of TRPV1 in the regulation of physiological responses to changes in core body temperature.

Similar to TRPM8, TRPV1 function is modified in birds, whose body temperature (40–42°C) is significantly higher than in most mammals. The chicken orthologue of TRPV1 is heat sensitive, but the apparent activation...
threshold is shifted to around 46°C (Jordt & Julius, 2002), which possibly reflects the need to ‘adjust’ sensitivity in accordance with body temperature. It would be interesting to see if this trend continues in other animals with ‘non-standard’ body temperatures. Such an analysis would not only reveal evolutionary flexibility of temperature sensors, but would also help elucidate molecular determinants in the TRPV1 structure (Cao et al. 2013b; Liao et al. 2013) which fine-tune the temperature sensitivity of the channel.

**TRPA1.** The non-selective cation channel TRPA1, found in a subset (20–25%) of TRPV1-positive neurons (Story et al. 2003), is perhaps the most versatile of all polymodal sensory ion channels. Different TRPA1 orthologues were reported to mediate the detection of cold, heat and noxious chemicals, such as allyl isothiocyanate (AITC), the pungent agent from wasabi and other mustard plants (Jordt et al. 2004). In rodents, TRPA1 appears to contribute to noxious cold detection in pathological conditions (Bautista et al. 2006; Karashima et al. 2009; del Camino et al. 2010; Knowlton et al. 2010). Somewhat similarly, TRPA1 acts as a cold sensor in *Caenorhabditis elegans* (Chatzigeorgiou et al. 2010). In striking contrast, TRPA1 serves as a heat sensor in birds (Saito et al. 2014) and in ancestral vertebrates such as snakes (Gracheva et al. 2010), frogs and lizards (Saito et al. 2012; Kurganov et al. 2014). In invertebrates, TRPA1 mediates heat sensitivity in silkworm (Saito et al. 2014), mosquito and fly (Viswanath et al. 2003; Rosenzweig et al. 2005; Hamada et al. 2008; Kang et al. 2012; Zhong et al. 2012), exhibiting robust activation at around 21°C, 25°C and 28°C, respectively. The biophysical origin of temperature responses in TRPA1 is poorly understood and the location of temperature-sensing and -gating elements in the channel structure are obscure. Several reports pointed at the ankyrin repeats in the N-terminal domain of TRPA1 as major regulators, which can either dramatically affect or even reverse the directionality of temperature responses (Cordero-Morales et al. 2011; Wang et al. 2013; Jabba et al. 2014). A recent study, however, suggested that the N-terminal domain could play only a regulatory role, because purified recombinant human TRPA1 without the N-terminus is activated by cold and chemicals, including AITC (Moparthi et al. 2014). Together, these observations suggest that TRPA1 evolved as a temperature sensor several times in different vertebrate and invertebrate species, and that its functional fine-tuning often proceeds through the process of convergent evolution.

**Leak potassium channels: possible regulators of cold and warm perception**

The ‘two-pore’ (K_{2P}) potassium channels mediate voltage-independent potassium ‘leak’, a key factor that contributes to establishing the resting potential of the plasma membrane (Fig. 1). The K_{2P}s are expressed in various cell types, including somatosensory neurons, where they are thought to regulate excitation (Kang & Kim, 2006; Dobler et al. 2007; Bautista et al. 2008; Acosta et al. 2014; Guo & Cao, 2014). K_{2P}s of the TREK group, which includes TREK-1, TREK-2 and TRAAK, are activated by temperature. In heterologous systems, the channels are silent at around 14°C and maximally active at around 40–45°C (Maingret et al. 2000; Kang et al. 2005; Bagriantsev et al. 2011, 2012). The expression pattern of the heat-activated K_{2P}s overlaps with both TRPV1 and TRPM8 (Maingret et al. 2000; Alloui et al. 2006; Yamamoto et al. 2009), suggesting a role in the regulation of temperature sensitivity. Accordingly, genomic deletion of TREK-1 and/or TRAAK increases the firing rate of heat-sensing C-fibres and stimulates heat and cold avoidance in behavioural tests (Alloui et al. 2006; Noel et al. 2009; Descoeùr et al. 2011). It should be noted that the deletion of TREK-1 and TRAAK produces a number of neurological phenotypes, including altered mechanosensation, anaesthetic responses and others (e.g. see Laigle et al. 2012), suggesting that the effects of the channel deletions on mechanosensitivity can be indirect. Moreover, it remains unclear to what extent the temperature-regulated dynamics of the background K^+ leak, and not the background leak itself, are responsible for the deletion phenotypes. In support of the latter hypothesis, it was shown that the deletion of TASK-3, a K_{2P} channel known to be expressed in a limited subset of DRG neurons (Talley et al. 2001; Marsh et al. 2012), potentiates cold sensitivity at the level of somatosensory neurons and in behavioural tests (Morenilla-Palao et al. 2014), even though the channel itself lacks robust temperature sensitivity (Maingret et al. 2000; Bagriantsev et al. 2011). All things considered, the temperature phenotypes of the K_{2P} knockout strains are most consistent with a hypothesis that K_{2P} activity counterbalances temperature-evoked depolarization caused by TRPV1 or TRPM8, and that the deletion of the K_{2P}s favours depolarization and thus potentiates sensitivity to both cold and warmth.

**Tuning temperature sensitivity of vertebrates to the extreme**

Several vertebrate classes (amphibians, reptiles and birds) evolved TRPA1 as a heat sensor (Saito et al. 2012, 2014; Kurganov et al. 2014). In functionally specialized species of snakes, such as rattlesnakes, pythons and boas, TRPA1 was proposed to serve as a primary infrared sensor playing an essential role in the detection of warm-blooded prey (Gracheva et al. 2010). Interestingly, pythons and boas belong to an ancient group of snakes, whereas rattlesnakes are relatively young in evolutionary terms.

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Nevertheless, despite the huge evolutionary distance – more than 30 million years – infrared-sensing snakes from the three different families co-opted the same molecular strategy to sense minute amounts of heat emanating from their prey: through functional tuning of TRPA1 (but not TRPV1, for example). Indeed, snake TRPA1 has one of the lowest apparent thermal thresholds identified so far in vertebrates, with a detectable temperature activation in vitro and in somatosensory neurons at around 28°C (Gracheva et al. 2010) (Fig. 2). This fascinating molecular adaptation allows the snakes to detect their warm-blooded prey not only using visual or olfactory clues, but also though the perception of heat emitted from the prey.

Intriguingly, in comparison with heat-insensitive TRPA1 orthologues, the snake TRPA1 is poorly sensitive to activation by AITC, suggesting that the enhanced thermal sensitivity of the snake channel comes at the expense of chemical activation by electrophilic compounds (Gracheva et al. 2010; Cordero-Morales et al. 2011). This molecular adaptation makes sense from a physiological point of view. Indeed, fine-tuning of a molecular receptor to the extreme may require cancellation or reduction of ‘noise’ from a non-essential modality, such as sensitivity to electrophiles.

Another group of animals that have the capability to detect infrared radiation emitted by the prey are vampire bats. Vampire bats have unique feeding habits as they consume only blood, which implies the ability to efficiently detect infrared radiation emitted by the prey. At the molecular level, evolutionary specialization involved the modification of the already existing heat sensor, TRPV1, but in an unexpected way: through the generation of a new isoform through alternative RNA splicing. Trigeminal neurons of vampire bats express two splicing isoforms of TRPV1: a long isoform, which is functionally similar to the human, rat and mouse TRPV1 orthologues, and which is activated at around 40°C, and a short isoform, that differs by 62 amino acids in the C-terminus, and is activated at around 30°C. The short TRPV1 isoform is expressed entirely in the trigeminal somatosensory neurons that innervate the pit organ, head and face, whereas the long isoform is localized primarily in dorsal root ganglia and is probably responsible for ‘normal’ heat sensitivity throughout the body (Gracheva et al. 2011).

Molecular tuning of TRPV1 has also been discovered in species evolutionarily distant from bats. Zebrafish have a truncation in exon 15 of the TRPV1 gene (Gracheva et al. 2011). Thus, the two species present an example of a convergent molecular evolution, whereby the same gene is independently targeted to tune temperature sensitivity according to the specific behavioural needs.

The somatosensory system evolves rapidly to support different lifestyles and molecular adaptations in the animal kingdom. Different orthologues of TRPM8, TRPV1 and TRPA1 exemplify functional diversifications that help animals adapt to the environmental temperature variations as well as to develop and occupy new ecological niches. Changing the biophysical properties of the receptor channels is probably not the only mechanism of somatosensory fine-tuning. Alternative strategies could involve alterations in the expression level of thermo-TRPs and/or the number of thermosensitive neurons. This strategy is illustrated by the recent findings in the TG of star-nosed moles (Gerhold et al. 2013) and tactile foraging ducks (Schneider et al. 2014), where TRPV1 and TRPM8 expression is significantly down-regulated. The down-regulation of thermoreceptors could be a trade-off for the exceptional mechanosensory ability in these animals, which supports the notion that the development of sensitivity to different modalities is interconnected. These findings underscore evolutionary flexibility of the somatosensory system in vertebrates and highlight the need to look beyond the rodent model to explore general and specialized principles of somatosensory system organization, including the ability to sense temperature.

References


