TRP Ion Channels and Temperature Sensation

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Abstract

The abilities to sense environmental and internal temperatures are required for survival, both for maintenance of homeostasis and for avoidance of tissue-damaging noxious temperatures. Vertebrates can sense external physical stimuli via specialized classes of neurons in the peripheral nervous system that project to the skin. Temperature-sensitive neurons can be divided into two classes: innocuous thermosensors (warm or cool) and noxious thermonociceptors (hot or cold). ThermoTRPs, a subset of the transient receptor potential family of ion channels, which are expressed in sensory nerve endings and in skin, respond to distinct thermal thresholds. In this review, we examine the extent to which thermoTRPs are responsible for providing a molecular basis for thermal sensation.

Contents

INTRODUCTION

DRG: dorsal root ganglion

GPCR: G protein–coupled receptor

Of the five senses—sight, hearing, taste, smell, and touch—touch is perhaps the most varied and least understood. It describes the ability to sense mechanical forces, chemical stimuli, and temperature, and the molecules that mediate this ability have been a long-standing mystery. Temperature and pressure is sensed at the tip of sensory neuron projections at the level of skin (Smith 2000). Temperature sensation in particular has received relatively little attention from biologists and yet is critical for interaction with the environment. Investigators have often assumed that structural specialization at the tip of sensory neurons is required for translating temperature information into an electrical signal. This assumption is based partially on data from genetic investigations of mechanosensation (Ernstrom & Chalfie 2002). In the worm, for example, diverse classes of proteins (extracellular, membrane-bound, and intracellular) are required for mechanosensation (Goodman & Schwarz 2003). This suggests that a mechanosensory cell is dependent on both extracellular and intracellular links for proper function. Similarly, mutations in various classes of proteins that cause deafness in mammals, and the complex anatomical structure of mammalian inner ear hair cells, also suggest the requirement for specialized structures for mechanosensation (Steel & Kros 2001, Hudspeth 2005). However, a hint that thermosensation may not require specialized structures at the tip of DRG sensory neurons came from studies in which cell bodies of DRG neurons, cultured after effective axotomy of nerve endings, could still respond to distinct thresholds of temperature (Cesare & McNaughton 1996).

TRP CHANNELS AS TEMPERATURE SENSORS

If DRG cell bodies can respond to heat in the absence of specialized nerve endings, can a single molecule (presumed temperature sensor) be sufficient to make a naïve cell responsive to heat or cold? What would this molecule be? Two families of membrane proteins have been implicated as receptors in other sensory systems: (*a*) Ion channels can be gated directly by sensory stimuli and cause an action potential—this is presumed to be the case for mechanosensation because no other known signaling mechanism is fast enough; and (*b*) G protein–coupled receptors (GPCR) can be activated by sensory stimuli and ultimately lead to action potentials via modulation of ion channels—this is the case for vision, taste, and olfaction (Hudspeth 1997, Mombaerts 2004). The identification and characterization of temperature-activated transient receptor potential ion channels represents the first illustration of a sensory ion channel gated by a physical stimulus.

Transient receptor potential (TRP) ion channels get their name from a *Drosophila* phototransduction mutant that shows a transient instead of a sustained response to bright light (Montell 2005). The mutation that caused this phenotype was identified as a putative ion channel and was named TRP (Montell & Rubin 1989). This was the first of many links between TRP channels and sensory transduction (Clapham 2003, Patapoutian et al. 2003). The *Drosophila* TRP, the founding member of the classical TRPC subclass, is activated by phospholipase C (GPCR) signaling. The cloning of heat-activated TRPV1 (vanilloid receptor-1) was first among a group of six temperature-activated TRP ion channels that we have dubbed thermoTRPs (Caterina et al. 1997, Patapoutian et al. 2003). There are four heat-activated channels (TRPV1-4) and two cold-activated channels (TRPM8 and TRPA1) (**Figure 1**) (Caterina & Julius 2001, Patapoutian et al. 2003). All six, when expressed in naïve cells (human embryonic kidney cells, chinese hamster ovary cells, Xenopus oocytes), have the amazing property of rendering the cells temperature sensitive. Each thermoTRP has unique characteristics, highlighted by distinct temperature thresholds of activation (**Table 1**). Sensitization or desensitization to repeated thermal stimuli (i.e., TRPV3 and TRPV2 are sensitized to repeated stimuli, whereas TRPV4 and TRPA1 are desensitized) and the ability to be modulated by distinct signaling mechanisms (see below) further distinguish thermoTRPs (Caterina et al. 1999; Guler et al. 2002; Peier et al. 2002; Smith et al. 2002; Watanabe et al. 2002; Xu et al. 2002; Story et al. 2003).

TRP CHANNELS AND CHEMESTHESIS

Languages throughout the world describe capsaicin (active ingredient in chili peppers) in the mouth as hot or burning. This makes sense since capsaicin activates TRPV1 in heterologous expression systems, and TRPV1 null mice do not respond to capsaicin (Caterina et al. 1997, 2000; Davis et al. 2000). The cooling effect of mint-derived menthol is also well recognized. The identification of TRPM8, a menthol- and cold-activated ion channel, demonstrated that various classes of TRP channels are involved in thermosensation and that these thermoTRPs are receptors for naturally occurring sensory compounds (McKemy et al. 2002, Peier et al. 2002). More thermoTRP-activating plant-derived sensory compounds have been recently discovered. Indeed, most natural compounds with oral sensory qualities distinct from olfaction and taste (chemesthesis) are now attributed to thermoTRP activation (Voets et al. 2005). Most of these compounds cause a burning sensation and activate TRPA1 and/or TRPV1, two thermoTRPs expressed in nociceptive sensory neurons (TRPA1 is in a subset of TRPV1-expressing neurons, see below). The list of such compounds includes the active ingredients in mustard oil (wasabe), wintergreen oil, cinnamon oil, garlic, etc.

DORSAL ROOT GANGLIA

The neurons of the peripheral nervous system that facilitate the detection of mechanical forces, chemical stimuli, and temperature originate in the DRG in the trunk and within the trigeminal ganglia in the head. The DRG are comprised of the cell bodies of sensory neurons and are located lateral to the spinal cord in the vertebral column. These sensory neurons are pseudounipolar with an axon that projects to peripheral tissues such as the skin and muscle, where stimuli are detected. This information is then transmitted to the brain via the spinal cord. Classified based on their conduction velocities, both innocuous- and noxious-temperature-sensing neurons have been identified as slow-velocity small-diameter unmyelinated C-fibers or intermediate-velocity medium-diameter lightly myelinated Aδ-fibers. Pain-sensing neurons (nociceptors) that respond to noxious temperatures can be divided further into those that are unimodal, which are activated by a unique thermal stimulus, and those that are polymodal, which detect painful chemical, mechanical, and thermal stimuli. Activation of polymodal nociceptors may elicit the burning pain sensation common to different noxious stimuli (Kandel et al. 2000).

TRP: transient receptor potential

ThermoTRP: a subset of transient receptor potential ion channels activated by distinct physiological temperatures

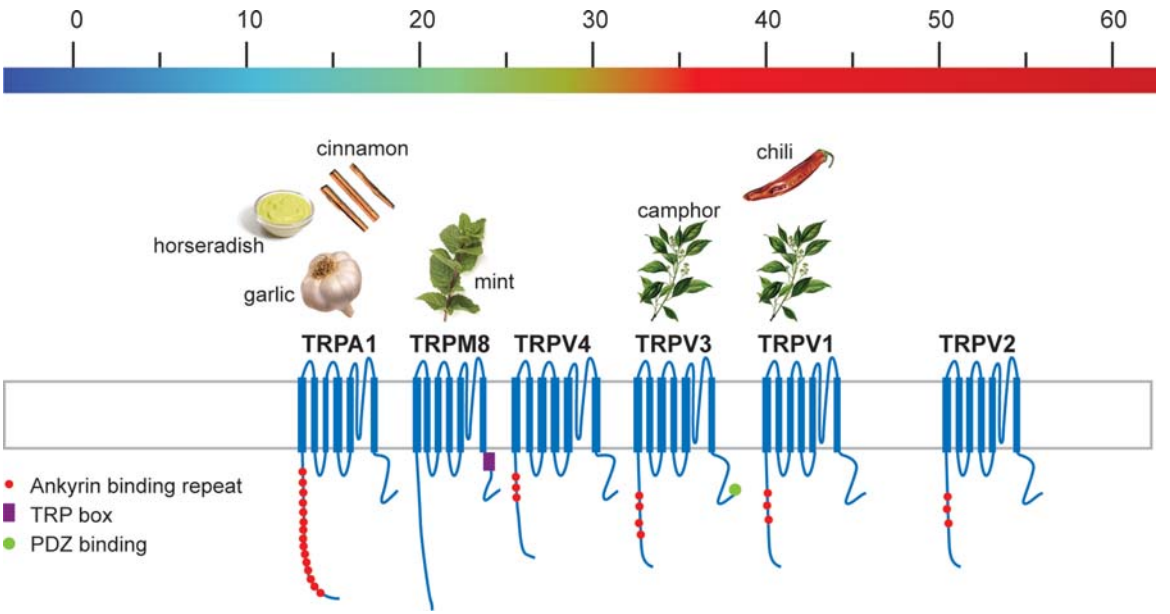

Figure 1

Schematic representation of the thermoTRPs that function in temperatures ranging from noxious heat to noxious cold. Proposed membrane topology and functionally important domains are represented. They include six putative transmembrane units with a proposed pore region between transmembrane domains 5 and 6. The amino and carboxy termini are cytoplasmic and contain various interaction domains like variable numbers of ankyrin repeats, TRP box or PDZ binding domains. Various botanical compounds activate the thermoTRPs. TRPV1 is activated by capsaicin, the pungent ingredient in chilly peppers, whereas TRPM8 is activated by menthol, the cooling compound from mint. TRPA1 is activated by various pungent compounds like allicin, the active ingredient in garlic, cinnamaldehyde, the pungent component of cinnamon, and isothiocyanates, the pungent ingredients found in wasabe.

(Bandell et al. 2004, Bautista et al. 2005, Jordt et al. 2004, Macpherson et al. 2005). Through capsaicin and allicin, plants such as chili peppers and garlic, respectively, deter mammalian predators from consuming the plant. TRPV1 from avian species respond to heat but not to capsaicin (Jordt & Julius 2002). This lack of response is convenient because it allows birds to consume peppers and serves an important role in dispersing seeds. Paradoxically, some humans enjoy peppers and raw garlic despite its potent ability to activate pain neurons. There are a few theories of why humans may enjoy the burning of capsaicin and allicin. One theory is that it causes pleasant pain, a predictable pain that is nondamaging. Another theory is that activation of these pain neurons causes hypersalivation and hypersensitivity in the mouth so that other sensory/taste stimuli are enjoyed at more intense levels (Rozin et al. 1982).

Not all natural compounds that activate thermoTRPs have a pungent quality. Camphor, known best for its distinctive odor, activates TRPV3, a warm-activated thermoTRP, and TRPV1 (Moqrich et al. 2005; Xu et al. 2005). Camphor is often recognized to cause a cooling sensation, although no published work supports this theory. Instead, camphor modulates warm sensation, consistent with its activity on TRPV3 (Green 1990). Chemical modulation of thermoTRPs is not limited to natural compounds. Many endogenous and synthetic compounds (mostly small hydrophobic molecules) activate thermoTRPs (Voets et al. 2005).

Table 1 Properties of TRP channels involved in thermal transduction[1]

Channel	Temperature sensitivity	Nonthermal agonists	Blockers	Tissue distribution	Null mutants
TRPV1	≥42°C	capsaicin, lipoxygenase, acidic pH, resiniferatoxin, NADA, anandamide, EtOH allicin, camphor	ruthenium red, capsazepine	PNS, brain, spinal cord, skin, tongue, bladder	impaired thermal avoidance and hyperalgesia
TRPV2	≥52°C	growth factors (mouse)	ruthenium red	PNS, brain, spinal cord, widely expressed	not reported
TRPV3	≥33°C	camphor, 2-APB	ruthenium red	PNS (human)?, skin	impaired thermotaxis and thermal avoidance
TRPV4	~27 °C –42°C	hypotonic, phorbol esters	ruthenium red, gadolinium	kidney, PNS, skin, inner ear, brain, liver, trachea, heart, skin, hypothalamus, fat	impaired thermotaxis, thermal avoidance, and hyperalgesia, osmotic regulation, pressure sensation
TRPM8	≤25°C	menthol, icilin, eucalyptol		PNS, prostate (human)	not reported
TRPA1	≤17°C	cinnamaldehyde, mustard oil, allicin, icilin, etc. (see Bandell et al. 2004)	ruthenium red, camphor	PNS, hair cells	not reported
Non-TRP proteins that may be involved in thermosensation					
TREK-1	cold	membrane stretch, polyunsaturated fatty acids, intracellular pH		PNS, brain	not reported
P2X3	warmth	ATP		PNS	enhanced thermal avoidance
Na/K ATPase	cold?		ouabain	PNS?	not reported
BNC1, ASIC DRASIC	cold (potentiated)	acidic pH	amiloride	PNS	not reported

[1]PNS: peripheral nervous system.

THERMOTRPs AND VOLTAGE

How is an ion channel activated by heat or cold? This is a fundamental but mainly unanswered question. There are many possibilities. Because gating of these channels in artificial membranes has not yet been possible, one cannot rule out that thermoTRPs are indirectly activated by temperature. Indeed, heat can activate TRPV4 in whole-cell configuration but not in excised membrane patches (Watanabe et al. 2002). This finding suggests that a soluble intracellular factor is required for heat activation of TRPV4. TRPM8 and TRPV1, in contrast, can be activated by temperature changes in excised patches, arguing that at least soluble intracellular factors are not required (Reid & Flonta 2002, Tominaga et al. 1998). Temperature activation could also occur indirectly via membrane-bound factors or phase transition of the plasma membrane or directly via conformational change of the channel. Recently, two studies proposed a thermodynamic model for a direct effect of temperature on thermoTRP channels (Brauchi et al. 2004, Voets et al. 2004). Nilius and colleagues showed that TRPM8

Q_{10}: the change in the rate of activity resulting from a 10°C increase in temperature

PIP_2: phosphatidylinositol 4,5-bisphosphate

and TRPV1 are voltage activated and that temperature shifts the voltage-dependent activation curve of these channels to more physiological ranges (Voets et al. 2004). Why is TRPV1 activated by heat, and TRPM8, by cold? Voets et al. (2004) used a simple two-state (closed-open) model and found that the opening rate of TRPV1 is steeply temperature dependent (Q_{10} of 14.8), whereas its closing rate has shallow temperature dependence (Q_{10} of 1.3) (i.e., TRPV1 opens more frequently in hot temperatures). In contrast, the opening rate of TRPM8 has shallow temperature dependence (Q_{10} of 1.2), whereas its closing rate is steeply temperature dependent (Q_{10} of 9.4) (i.e., TRPM8 closes infrequently at cold temperatures). Therefore, this simple model proposes that thermosensitivity of TRPM8 and TRPV1 arise from a difference in activation energies associated with voltage-dependent opening and closing. Latorre and colleagues observed similar thermodynamic properties for TRPM8; however, they claim that the fast component of TRPM8 cold activation is not voltage dependent (Brauchi et al. 2004). Instead, Latorre and colleagues assert that TRPM8 can be separately gated by cold and voltage (through separate sensors) and that channel gating can be explained by three separate two-state equilibriums, which interact allosterically with each other. The two studies argue that intrinsic thermodynamic properties of these two thermoTRPs are directly responsible for temperature gating. This is a fundamental finding and will open the door for mechanistic investigations on how temperature gates these ion channels. Specifically, future studies will focus on which domains and residues are responsible for the sensitivity of these ion channels to chemicals, temperature, and voltage. S2–S3 transmembrane regions of TRPV1 and TRPM8 are important for activation by capsaicin and icilin, respectively; however, these domains do not seem to influence temperature or menthol activation (voltage sensitivity is not yet explored) (Chuang et al. 2004, Jordt & Julius 2002). A tyrosine residue in S3 is critical for heat activation of TRPV4, possibly involving binding a phorbol ester released by heat (Vriens et al. 2004).

The voltage dependence of TRPM8 and TRPV1 also challenges the validity of assigning a single temperature "threshold" for each channel because this varies according to voltage. Therefore, thresholds are an approximation, and the cellular environment can shift these thresholds considerably. Is activation by voltage a fundamental requirement for a thermoTRP? Investigators have not yet proved that all six thermoTRPs are voltage activated, and not all voltage-sensitive ion channels are temperature activated. In the case of TRPM8 and TRPV1, a combination of large differences in enthalpy for channel opening and closing, large changes in entropy (which scales the voltage shift), and small gating charge seems to contribute to temperature activation (Nilius et al. 2005). In another study, 2-aminoethyl diphenylborinate (2-APB) and diphenylboronic anhydride (DPBA) activated TRPV3 by shifting the voltage dependence of activation, leading researchers to propose that these compounds act as modulators of voltage-dependent gating, perhaps reminiscent of TRPM8 and TRPV1 (Chung et al. 2005). As we learn more about these channels, a more quantitative thermodynamic definition may better define these channels. For now, thermoTRPs are defined as ion channels that are activated (not just modulated) by temperature, as temperature undoubtedly has profound modulatory effect on numerous ion channels.

THERMOTRPs AND PIP_2

In addition to voltage, ion channels of the TRP superfamily are particularly amenable to modulation by phosphatidylinositol 4,5-bisphosphate (PIP_2). The mechanisms whereby PIP_2 modulates these channels, however, seem to be disparate. Two studies recently showed that activation of TRPM8 by cold or menthol requires the presence of PIP_2 (Liu & Qin 2005, Rohacs et al.

2005). The cool/menthol receptor fails to adapt and the activation temperature is strongly shifted downward when stimulated in excised patches from sensory neurons. This suggests that additional intracellular components are necessary for full activation of TRPM8. The two studies found that PIP_2 can prevent the rundown of TRPM8 in excised patches and is essential for TRPM8 activation. This finding is consistent with several reports demonstrating activation of TRPM channels by PIP_2 (Kraft & Harteneck 2005). Furthermore, Rohacs et al. mapped residues in TRP domain of TRPM8 required for PIP_2-mediated activation. Moreover, in agreement with McKemy et al. (2002), who found that desensitization of TRPM8 requires extracellular calcium, this group demonstrated that, in a negative feedback loop, flow of extracellular calcium through TRPM8 activation could activate phospholipase C (PLC), causing PIP_2 depletion and the subsequent inhibition of TRPM8 activation. The regulation of TRP channels likely requires complex interactions of a variety of stimuli—temperature, compounds, voltage, and PIP_2—with each affecting sensitivity to the others. In contrast to TRPM8, which requires the presence of PIP_2 to support its function, PIP_2 exerts a constitutive inhibition of TRPV1 through interaction at the distal c-terminus of the channel (Prescott & Julius 2003). A reduction of cellular PIP_2 levels, as occurs upon the activation of GPCRs, increases the heat sensitivity of TRPV1. The reduction of PIP_2 levels seen may reflect conditions during inflammation, leading to a heightened perception of pain from increased activation of TRPV1 and a concurrent inhibition of the cool-sensing receptor TRPM8. A recent study showed that depletion of PIP_2 occurs concomitantly with the activation of TRPV1, and its subsequent replenishment in the membrane determines recovery of the channel from desensitization (Liu et al. 2005). These studies indicate that PIP_2 regulation may be a common but complex feature of many TRP channels.

TRPVs AND LIFE WITHOUT POTENTIAL HEAT SENSORS

Although warm temperatures (32°C–42°C) can be pleasant in some conditions, most animals find high temperatures (≥43°C) to be noxious. Using electrophysiological recordings, distinct sensory afferents have been identified that respond to warm temperatures or noxious heat. In primates, warm-sensitive fibers fire action potentials continuously at a low rate at normal skin temperature (34°C). As temperature rises, the rate of action potentials increases, with one type of warm fiber having a maximum response at 41°C and a second type continuing to increase firing at greater temperatures (Hensel & Iggo 1971). Investigators have identified two types of noxious heat-sensory neurons on the basis of the temperature required for activation. In cultured sensory neurons, ~45% of small- to medium-diameter neurons have an activation threshold of 43°C, and a smaller second group comprised of medium- to large-diameter neurons respond to temperatures above 52°C (Nagy & Rang 1999).

Four members of the TRPV family of nonselective cation channels can be activated by warm to hot temperatures. TRPV1 (43°C) and TRPV2 (52°C) have properties consistent with noxious heat sensors. Both TRPV3 (33°C) and TRPV4 (25°C–34°C) respond to warm temperatures. Of these TRPV1, TRPV3, and TRPV4 have been genetically ablated in mouse models. In this section we review the characterization of these knockouts and what they tell us about sensing heat.

TRPV1

TRPV1, first identified by its responsiveness to the pungent compound capsaicin from hot chili peppers, is activated by temperatures in the noxious range (>43°C). In the mouse, TRPV1 is expressed primarily in small-to-medium-diameter peptidergic sensory neurons, characteristic of nociceptive Aδ- and C-fibers (Caterina et al. 1997, Tominaga et al.

Nociception: the response of the peripheral nervous system to damaging stimuli

ATP: adenosine triphosphate

WT: wildtype

Hyperalgesia: a sensitized response to a painful stimulus due to inflammation or injury

Rectification: property of a channel wherein the current does not flow with the same ease from the inside as from the outside

1998). Consistent with a role in nociception, TRPV1 is also stimulated by low pH and can be potentiated by a number of factors known to participate in inflammation, including nerve growth factor, bradykinin, lipids, prostaglandins, protein kinases A and C, and ATP (Tominaga & Caterina 2004).

Two independent groups carried out studies on TRPV1$^{-/-}$ mice (Caterina et al. 2000, Davis et al. 2000). Both groups found a complete loss of capsaicin and thermal (42°C–52°C) sensitivity in cultured sensory neurons. In addition, low pH failed to elicit TRPV1-like currents. Behavioral responses to capsaicin were also absent in TRPV1$^{-/-}$ mice. However, using skin-nerve preparations, Caterina et al. (2000) recorded heat responses with a peak temperature of 47°C; 13/24 C-fibers responded to heat in wild-type (WT) animals but only 4/24 C-fibers responded to heat in null mice. The TRPV1$^{-/-}$ fibers, however, displayed a substantially reduced heat-evoked discharge compared with WT. Nevertheless, this data suggests that TRPV1 alone cannot account for all perception of noxious heat below the 52°C threshold of TRPV2. In a follow-up study using ex vivo preparations (skin stimulated with a temperature ramp of 31°C–51°C) from the same strain of TRPV1$^{-/-}$ mice, 19/33 of WT and 32/42 of TRPV1$^{-/-}$ nociceptive C-fibers responded to the stimulus with an identical coding frequency. Consistent with this result, a majority of the WT heat-sensitive C-fibers in this study did not express TRPV1. This group also showed that TRPV2 could not account for this finding because it was not expressed in these heat-activated fibers as determined by immunohistological staining. Differences in recording preparations (skin-nerve vs. ex vivo) may explain why the numbers and properties of heat-activated C-fibers observed in TRPV1$^{-/-}$ mice were not the same in the two studies (Woodbury et al. 2004). However, a new study using skin-nerve preparations also failed to detect differences in the heat responses of C-fibers between WT and TRPV1$^{-/-}$ mice (Zimmermann et al. 2005). Regardless, noxious heat sensation in TRPV1$^{-/-}$ mice seems to be mostly intact. Indeed, Caterina et al. (2000) observed behavioral deficits only in acute thermal sensation at temperatures ≥50°C, and Davis et al. (2000) could not detect any acute thermal differences. Because sensitivity to moderate noxious stimuli is present in skin nerve preps from TRPV1$^{-/-}$ mice but not present in dissociated DRG cultures, this suggests that perhaps the skin plays a role in communicating thermal stimuli to neurons in a TRPV1-independent manner (see section on keratinocytes). Both studies found a virtual absence of inflammation-induced [complete Freund's adjuvant (CFA), mustard oil, carrageenan] heat hyperalgesia in TRPV1$^{-/-}$ mice, which is consistent with the sensitizing effect inflammatory soup molecules have on TRPV1. These data clearly validate the importance of TRPV1 in the establishment of inflammatory pain and perhaps as a therapeutic target for pain management.

TRPV3

TRPV3 is activated at the warm temperature threshold of 33°C and exhibits increasing responses at higher noxious temperatures (Peier et al. 2002, Smith et al. 2002, Xu et al. 2002). As noted, TRPV3 is activated by the botanical compound camphor as well as the chemical compound 2-aminoethoxyphenyl borate and strongly sensitizes to repeated heat or chemical stimulation. Prolonged activation of TRPV3 also evokes a biphasic current in which an initial sensitizing phase is followed by a second phase during which outward rectification is lost and higher current amplitudes are generated (Chung et al. 2005). In mice, TRPV3 is expressed in the keratinocytes of the skin and not in sensory neurons as measured by northern blot and in situ hybridization (Peier et al. 2002). Other studies looking at expression in monkey and human tissue found TRPV3 mRNA and protein in sensory neurons, respectively (Smith et al. 2002, Xu et al. 2002). In human DRGs, TRPV1 and

TRPV3 were coexpressed. These two proteins also interacted in a heterologous expression system (Smith et al. 2002). However this assembly of TRPV1 and TRPV3 was not observed in a recent study using fluorescent resonance energy transfer and coimmunoprecipitation experiments (Hellwig et al. 2005). Interspecies differences may account for this difference in expression patterns. However, a recent study noted expression of TRPV3 in keratinocytes but failed to detect it in skin fibers that innervate the human breast (Gopinath et al. 2005).

Moqrich et al. (2005) have reported that thermotaxis is impaired in TRPV3$^{-/-}$ mice. After establishing that there is no difference in exploratory behavior between TRPV3$^{-/-}$ and WT mice, a two-temperature choice assay was employed in which the mice were presented with a choice of occupying a warm surface (35°C) or room temperature. WT mice overwhelmingly chose the warm surface, spending 92% of the time at 35°C. TRPV3$^{-/-}$ mice spent significantly less time on the warm side (64%) than did WT mice. In a second thermotaxis assay, mice were placed on a linear thermal gradient in which the temperature varied from 15°C to 55°C (Moqrich et al. 2005). After an exploratory phase (25 min), WT mice spent the most time between 30°C to 38°C. TRPV3$^{-/-}$ mice, however, did not display a thermal preference for 60 min, after which time they also chose the zones between 30°C and 38°C. Taken together, these data clearly demonstrates impairment in thermotaxis behavior in TRPV3$^{-/-}$ mice. Both assays also showed that warm preference is not lost in TRPV3$^{-/-}$ mice, which suggested that other genes including TRPV4 are required for innocuous warm sensation. As reported for TRPV1$^{-/-}$ mice, the authors also observed nociceptive behavioral deficits to acute thermal stimulation at temperatures $\geq$50°C in TRPV3$^{-/-}$ mice, which indicated that TRPV3 and TRPV1 have overlapping functions in noxious heat sensation. In contrast with TRPV1$^{-/-}$ mice, no deficits in heat hyperalgesia were observed in the TRPV3$^{-/-}$ mouse. The authors also provided strong evidence that TRPV3 transmits its signal through keratinocytes, establishing for the first time a role for the skin in the conductance of thermal sensation (see below). Perhaps the sensitizing effects of increasing heat on TRPV3 can explain its role in both innocuous warm and noxious heat perception. At low levels of activation a warm signal is induced, whereas at high levels of activation a nociceptive signal is generated, possibly by the graded release of a factor from keratinocytes to sensory endings.

Thermotaxis: movement of an organism according to temperature

TRPV4

TRPV4, originally identified as an osmosensory ion channel, is also activated by warm temperatures (25°C–34°C) (Guler et al. 2002, Liedtke et al. 2000, Strotmann et al. 2000, Watanabe et al. 2002). Other nonthermal activators of TRPV4 include anandamide, its metabolite arachidonic acid (AA), and the cytochrome P-450 metabolites of AA such as epoxyeicosatienoic acid (Watanabe et al. 2003). TRPV4 also responds to phorbol esters such as 4 α-phorbol 12, 13-didecanoate (4 α-PDD) (Watanabe et al. 2002). Activation of TRPV4 at room temperature by hypotonic cell swelling is moderate; however, this response is augmented by an increase in temperature to 37°C (Guler et al. 2002). Temperature is a critical modulator of TRPV4 because warmer temperatures cause a more rapid activation of TRPV4 by all stimuli. Unlike TRPV3, the characteristic response profile of TRPV4 is desensitization upon sustained or repeated heat stimulation of temperatures >42°C (Guler et al. 2002). TRPV4 expression has been reported in many thermosensory tissues including DRG neurons, skin, and the hypothalamus, although expression in the DRG is controversial; some groups have reported expression and others could not detect it (Alessandri-Haber et al. 2003, Delany et al. 2001, Guler et al. 2002, Liedtke et al. 2000, Suzuki et al. 2003). However, TRPV4 immunoreactivity is lost

in TRPV4$^{-/-}$ DRG, which provides compelling evidence for the expression of TRPV4 in mouse DRG (Liedtke & Friedman 2003). Studies of mouse and rat skin immunohistochemical staining have revealed a high level of cutaneous TRPV4 expression. In particular, TRPV4 immunoreactivity is observed within the suprabasal keratinocytes (Guler et al. 2002). In addition, TRPV4 is present in sensory nerve fibers innervating the human breast, which suggests that TRPV4 may signal warm thermosensation directly through sensory endings or through indirect communication from keratinocytes (Gopinath et al. 2005).

Original studies on TRPV4$^{-/-}$ mice reported lowered behavioral responses to intense noxious mechanical stimuli and impaired osmotic regulation. However, these mice display no changes in escape latency from acute painful heat stimuli on the hot plate (35°C–50°C) or upon radiant paw heating (Liedtke & Friedman 2003, Suzuki et al. 2003. Todaka et al. 2004). TRPV4$^{-/-}$ mice unexpectedly showed longer escape latencies on the hot plate when compared with WT animals after subcutaneous injection of carrageenan, implying a role for TRPV4 in thermal hyperalgesia (Todaka et al. 2004). Because the activation profile of TRPV4 suggests a role in warm-temperature sensation, Lee et al. (2005) conducted a detailed analysis of responses to warm temperatures in TRPV4$^{-/-}$ mice. On a thermal gradient, the TRPV4$^{-/-}$ mice selected warmer floor temperatures than did WT littermates (Lee et al. 2005). In a two-temperature choice test, TRPV4$^{-/-}$ mice showed a strong preference for 34°C, whereas the WT mice failed to discriminate between 30°C and 34°C. Both these assays consistently showed that the absence of TRPV4 results in a tolerance of higher temperatures. However, these assays do not clearly demonstrate if the role of TRPV4 in thermosensation is in avoidance of noxious hot temperatures or selection of preferred temperature. In contrast with earlier reports in which alterations in acute thermal behavior were not observed, TRPV4$^{-/-}$ mice had longer withdrawal latencies during acute tail heating by immersion in a water bath of 45°C–46°C, suggesting some role of TRPV4 for heat nociception. This apparent inconsistency could be due to differences in the sensitivity of hot plate (paws) versus tail flick assays and/or how these assays are scored. This group failed to see differences in behavior between WT and TRPV4$^{-/-}$ mice owing to CFA-induced inflammation when placed on the thermal gradient, questioning a role for TRPV4 in heat hyperalgesia. TRPV4 is also expressed in thermoregulatory hypothalamic regions. However, circadian body temperature fluctuation and core body temperature after warm stress is normal in TRPV4$^{-/-}$ mice. This finding indicates that TRPV4 is not essential for thermoregulation and suggests that the alterations in thermal behavior in TRPV4$^{-/-}$ mice are due to deficits in peripheral sensation.

Taken together, these knockout studies suggest that, in mice, TRPV1, TRPV3, and TRPV4 have overlapping functions in warm-hot thermal sensation. TRPV3 and TRPV4 are both required for proper thermotaxis in response to warm temperatures and may compensate for each other. TRPV3 mediates thermal sensation via its expression in keratinocytes, whereas TRPV4 may function in both keratinocytes and sensory neurons. All three channels seem to have a role in noxious heat perception. TRPV1$^{-/-}$ and TRPV3$^{-/-}$ mice have prolonged tail withdrawal latencies at temperatures above 50°C, and TRPV4$^{-/-}$ mice have greater latencies at 45°C–46°C. Although it may be appealing to infer from this data that TRPV4 is responsible for signaling noxious stimuli at thermal thresholds below TRPV1 and TRPV3, genetic background differences could likely account for these results. It is difficult to determine where true overlap in function between these channels exists until these mutant strains are studied in parallel on the same genetic background. In support of a role for these channels in nociception, Gopinath et al.

(2005) found expression levels of all three to be upregulated in painful human breast tissue: TRPV3 and TRPV4, in keratinocytes, and TRPV1, in sensory nerve endings. In addition, TRPV1 is necessary for thermal hyperalgesia, whereas TRPV3 is dispensable for this function. Until further studies are conducted, the role of TRPV4 in thermal hyperalgesia remains unclear. These data makes it clear that combinatorial knockouts of these channels (TRPV1/TRPV3, TRPV3/TRPV4, and TRPV1/TRPV3/TRPV4) are necessary to understand fully the role of TRPV channels in heat sensation because heat thermosensation is largely intact in each of the individual null mice. A null mutant for TRPV2, the high-heat receptor (>52°C), has not been reported, and its role in noxious heat perception remains to be evaluated.

In addition, investigators have recently shown TRPM4 and TRPM5 to be modulated by heat similar to TRPV1 (Talavera et al. 2005). TRPM5 is known to signal downstream of taste receptors and may contribute to the effects of temperature on taste (Cruz & Green 2000, Perez et al. 2002, Zhang et al. 2003). We do not know whether these channels may also play a role in thermosensation.

THERMOSENSATION IN KERATINOCYTES?

Sensory nerves densely innervate skin and directly sense temperature shifts in the skin. The conventional role assigned to skin is as a barrier crucial for the maintenance of temperature and of electrolyte and fluid balance. However, some recent studies raise the possibility that skin epithelial cells can also directly function as thermosensory cells. These cells are proposed to then relay the information to thermosensitive afferents. The expression of the thermoTRPs TRPV3 and TRPV4 in keratinocytes, and the abnormal thermosensory behavior of TRPV3$^{-/-}$ and TRPV4$^{-/-}$ mice as discussed above, has raised the possibility that skin epithelial cells can mediate the perception of warm temperatures directly. In addition to TRPV3 and TRPV4, TRPV1 immunoreactivity has been reported in human skin and in human keratinocytes (Denda et al. 2001). Activation of epidermal TRPV1 by capsaicin caused increases in intracellular calcium levels (Inoue et al. 2002). This action was blocked by the TRPV1 receptor antagonist capsazepine. Capsaicin-induced activation of TRPV1 resulted in the release of proinflammatory mediators like prostaglandin E2 and interlukin-8 (Southall et al. 2003). However, similar expression or activation by capsaicin is not seen in primary mouse keratinocytes (Chung et al. 2003). It remains to be seen if the TRPV1 expression in humans has a physiological role in thermosensation.

In vitro studies in keratinocytes further solidified the evidence that these cells contribute to temperature sensation. In the mouse 308 keratinocyte cell line, mild warming evoked calcium influx and transmembrane currents (Chung et al. 2003). Although expression analysis revealed the presence of both TRPV3 and TRPV4 in these cells, the warm-evoked response in most cells resembled those observed for TRPV4 in heterologous systems. The activation threshold for the response was 31°C–33°C, which was inhibited by ruthenium red. The temperature response was potentiated by hypo-osmolarity and 4αPDD, a selective agonist of TRPV4. This stimulus interdependence during activation of the channel is interesting given that osmotic and thermo stimuli appear to activate the channel via distinct mechanisms. Vriens et al. (2004) have shown that TRPV4 activation by osmotic cell swelling involves PLA2-dependent formation of AA, whereas phorbol esters and heat act via a PLA2-independent pathway. The lack of TRPV3-like responses in this study may be due to lower cell-surface expression of this channel in these cells. Two studies in primary mouse keratinocytes have demonstrated two distinct types of heat-evoked responses (Chung et al. 2004, Moqrich et al. 2005). Responses attributed to TRPV4 exhibit an activation threshold of 32°C and are desensitized by prolonged repeated heat

PLA2: phospholipase A2

CGRP: calcitonin gene-related peptide

stimuli. This response was absent in keratinocytes from TRPV4$^{-/-}$ mice, which confirms that this type of response is mediated by TRPV4 (Chung et al. 2004). In this study the rarer type of response was attributed to TRPV3 with currents exhibiting sensitization during repetitive heat stimuli. However, coapplication of the TRPV3 agonist 2-APB resulted in almost 80% of the cells showing a TRPV3-like response. In the study by Moqrich et al. (2005) almost 80% of cells respond to heat with a characteristic TRPV3-like profile, and ~30% of the cells show a TRPV4-like profile. Most cells were also responsive to 2–10 mM camphor, and the response to warm temperatures was robustly potentiated by camphor. This difference in TRPV3-like currents observed in the two studies may reflect different culture conditions used and varying surface-level expression of TRPV3. TRPV3-like currents persisted in keratinocytes derived from TRPV4$^{-/-}$ mice and were absent from TRPV3$^{-/-}$ mice. In keeping with the observation of lack of TRPV3 expression in DRG neurons in rodents, no significant camphor responses were observed in the DRG. These studies suggest that keratinocytes participate in mammalian thermosensation mediated by TRPV3 and TRPV4. Whether the two ion channels function in an overlapping manner remains to be established. A recent in vitro study failed to show formation of heteromultimers of TRPV3 and TRPV4, although they seemed to favor homo-oligomeric assembly (Hellwig et al. 2005).

These recent findings suggest that keratinocytes can participate in mammalian thermosensation. Given this idea, some questions arise: How do the keratinocytes communicate with sensory neurons? Do labeled lines exist in this communication between keratinocytes and sensory neurons, with TRPV3 and TRPV4 expressing keratinocytes communicating to distinct thermosensitive afferents? Experiments by Zylka et al. (2005) using genetically coded axonal tracers have revealed that peptidergic and nonpeptidergic epidermal innervations of sensory neurons are spatially segregated within the skin and also in the spinal cord. It is thus tempting to speculate the existence of molecularly distinct and parallel neuronal circuits for innocuous warm sensation from skin to sensory neurons. Although numerous free nerve endings innervate the skin, researchers have not demonstrated that these neuro-epidermal connections are synapses. Electron microscopic observations of typical synapse-like features on neurons innervating the skin, such as invaginations with dense mitochondria, endoplasmic reticulum and the presence of dense core vesicles, and plasma membrane thickening on adjacent epidermal cells, has led to proposals that communication between skin and nerves may involve synaptic and nonsynaptic means (Chateau & Misery 2004, Kaidoh & Inoue 2000). One nonsynaptic mode of communication between the two cells is that skin keratinocytes may release specific signaling molecules upon thermal stimulation, which leads to the activation of receptors on sensory neurons (Lee & Caterina 2005). Skin cells can produce cytokines, neuronal growth factors and neurotransmitters that modulate neuronal function. For example, noxious heat releases immunoreactive calcitonin gene-related peptide (CGRP) from isolated rat skin in a TRPV1-independent manner (Petho et al. 2004). Activation of the endothelin B-receptor results in release of β-endorphins from cultured human keratinocytes (Khodorova et al. 2003). This opioid binds to its receptor on sensory neurons to inhibit nociception. Innocuous heat-activation of TRPV3 and TRPV4 in keratinocytes may result in the release of a factor(s), as yet unidentified, that leads to activation of a subset of sensory neurons innervating the skin. Mechanical stimulation of human epidermal keratinocytes releases ATP, and this may be a communicating molecule (Koizumi et al. 2004). A subpopulation of DRG neurons expresses the ATP-gated ion channel P2X3, and aberrant thermal selection behavior is observed in P2X3−/− mice (Shimizu et al. 2005).

Another possibility is that heat activation of TRPV3 and TRPV4 in the skin could affect thermotaxis without directly activating afferents. Skin warming results in local vasodilation (Charkoudian 2003). TRPV3 and TRPV4 may regulate thermal homeostasis in skin by regulation of local blood flow. Calcium homeostasis is also very important for normal keratinocyte growth, differentiation, and function (Hennings et al. 1980). A transient hair follicle abnormality is observed in juvenile TRPV3$^{-/-}$ mice (Moqrich et al. 2005). However, gross abnormalities are not observed in either the TRPV4$^{-/-}$ or the TRPV3$^{-/-}$ mice. TRPV3 and TRPV4 may play no role in regulating the calcium homeostasis required for normal growth and differentiation of keratinocytes. Alternatively, they may compensate for each other in this function. Studies in double knockout mice may resolve this issue.

TRPs AND COLD: CONTRIBUTION OF TRPM8 AND TRPA1

Similar to heat, the perception of cold can range in quality from innocuous (cool) to painful (noxious cold). Our ability to discriminate between cold temperatures has been studied by controlled studies on human subjects such that cooling the skin by as little as 1°C evoked a cooling sensation, indicative of a highly sensitive system (Campero et al. 2001). In general, innocuous cool is defined as temperatures between 30°C and 15°C, whereas noxious cold is generally perceived as temperatures below 15°C (Davis & Pope 2002, Hensel 1981, Spray 1986).

The sensation of cold arises from the specific excitation of cutaneous receptors when the skin is exposed to decreased temperatures. Both Aδ-fibers and C-fibers respond within the temperature range of innocuous cool perception (Darian-Smith et al. 1973, Hensel et al. 1974, Hensel & Iggo 1971, Hensel & Zotterman 1951b, Iggo 1969). At normal skin temperature, cold fibers fire continuously; and the rate of firing increases as the skin is cooled. In contrast, warming the skin leads to the shutdown of these fibers (Campero et al. 2001, Hensel 1981, Spray 1986). Cold fibers also decrease their firing frequency over time or adapt when held at constant temperature (Campero et al. 2001, Darian-Smith et al. 1973). This characteristic is consistent with our ability to adapt to modest decreases in temperature.

Painful cold perception is associated with feelings of burning, cold aching, and pricking (Chery-Croze 1983a,b; Yarnitsky & Ochoa 1990). Noxious cold stimulates activity in 10%–100% of polynociceptive Aδ- and C-fibers, depending on species and maximal cooling stimulus. Temperatures between 0°C–15°C elicit responses in 10%–30% of these nociceptors (Georgopoulos 1977, LaMotte & Thalhammer 1982, Leem et al. 1993, Simone & Kajander 1996, Simone & Kajander 1997). Activity elicited in nociceptors with temperatures <0°C may also be an indicator of tissue damage due to freezing rather than a direct response to cold.

Menthol derived from mint elicits a sensation of cold when applied to the skin or mucous membranes. Remarkably, menthol modulates the activity of cool-induced currents in individual free nerve endings. In recordings of cold-sensitive afferents, menthol stimulates these fibers at subthreshold activation temperatures, which suggests that menthol acts directly on the molecule(s) responsible for cold transduction (Hensel & Zotterman 1951a, Schafer et al. 1986). In analogous studies using cultured rodent sensory neurons, electrophysiological and calcium-imaging experiments identified a population of neurons that responded to both innocuous cool and menthol, corresponding to ~10% of total neurons (Reid & Flonta 2001b, Viana et al. 2002). An important step forward came when Reid & Flonta (2001b) reported that the stimulation of cultured DRGs with cool and/or menthol leads to the activation of a nonselective cation channel.

An intense search to identify a cold-activated ion channel led to the identification of a cool/menthol receptor TRPM8 (CMR1) by two independent groups (McKemy et al. 2002, Peier et al. 2002). One group used a genomics-based approach, reasoning that TRP channels, which encode a family of nonselective cation channels that are involved in thermosensation, may encode additional thermoreceptors. TRPM8 was identified by its expression in sensory neurons and its ability to be activated by cold and menthol (Peier et al. 2002). Using menthol as a stimulus, the same group that first identified TRPV1 used expression cloning to isolate TRPM8 from a rat trigeminal neuron cDNA library (McKemy et al. 2002). In heterologous expression systems, TRPM8 was activated with a threshold of ~25°C–28°C similar to the threshold temperature observed in cold/menthol-sensitive sensory neurons (27°C–33°C). Additionally, TRPM8 has many of the same characteristics of the native cool/menthol channel, including outward rectification, ion selectivity, adaptation, and the ability of subthreshold levels of menthol to shift the activation temperature (McKemy et al. 2002, Peier et al. 2002).

In addition to menthol, a number of cooling compounds, including eucalyptol, spearmint, WS-3, and icilin, activate TRPM8 (McKemy et al. 2002; Bandell et al. 2004; Behrendt et al. 2004). Investigating the mechanism of activation of TRPM8 by various cooling compounds has improved our understanding of how the TRPM8 ion channel is gated. For instance, icilin, a more potent stimulus than menthol, activates TRPM8 in a manner distinct from menthol and cold. Initially researchers found that icilin could activate TRPM8 only in the presence of extracellular calcium (McKemy et al. 2002). A subsequent study found that a rise in intracellular calcium, either from intracellular stores or from influx through TRPM8, is required for full channel activation (Chuang et al. 2004). Residues in the third transmembrane domain of TRPM8 are required for icilin activity (Chuang et al. 2004). This same domain is involved in capsaicin binding to TRPV1 and is critical for activation of TRPV4 by 4αPDD, indicating at least a partial conservation of ligand activation of thermoTRPs (Jordt & Julius 2002, Vriens et al. 2004). In further support of this observation, the TRPV1 antagonists capsazepine, BCTC, and thio-BCTC, which act at the capsaicin-binding pocket, also inhibit TRPM8 activation. Lowering external pH also inhibited TRPM8 activation by icilin and menthol (Behrendt et al. 2004). Another study found that low intracellular pH inhibited both cold- and icilin-mediated activation of TRPM8, whereas menthol-mediated activation was not affected (Andersson et al. 2004). This supports earlier findings that the ligand-activation determinants of TRPM8 are separable (Chuang et al. 2004). As may be the case with differential PIP_2 gating of TRPV1 and TRPM8 (see above), it is interesting to speculate that low pH, which sensitizes TRPV1, could also suppress the pleasant cool sensations elicited by TRPM8 and thereby heighten pain perception.

Although investigators generally agree that TRPM8 is a cool-temperature receptor, the characterization of the expression pattern of TRPM8 in rodents has generated some controversy. Using in situ hybridization analysis, Peier et al. (2002) originally reported that TRPM8 is expressed in ~10% of the trigeminal ganglion and the DRG. This result is consistent with the percentage of neurons activated by cool/menthol in sensory neuron culture studies (Reid & Flonta 2001b, Viana et al. 2002). TRPM8 is specifically expressed in small-diameter neurons, suggesting C-fibers or possibly thinly myelinated Aδ-fibers. In accordance with the proposed role of TRPM8 as an innocuous cool receptor, the channel does not colocalize with known markers of nociceptive fibers such as CGRP, isolectin B4 (IB4), substance P, and TRPV1 (Peier et al. 2002). However, a number of groups have used capsaicin and menthol response profiles of cultured sensory neurons to propose that TRPV1 and TRPM8

expressions overlap. This idea has led investigators to suggest that these presumed coexpressing neurons could explain the phenomena of paradoxical cold and/or that TRPM8 may have a role in the perception of painful cold (Babes et al. 2004, Dodt & Zoterman 1952, McKemy et al. 2002, Reid et al. 2002, Viana et al. 2002). A subsequent report found that this coexpression of TRPM8 and TRPV1 could be due to culturing conditions (Story et al. 2003). Although in vitro culture systems are a valuable tool, we doubt that they fully reflect the naive state of these neurons. Culture conditions inflict substantial trauma and change the local environment of the cells considerably, even when neurons are cultured for a very short time. One in vivo study used overlapping in situ hybridization with TRPM8 and immunostaining with TRPV1 and observed colocalization of these two genes (Okazawa et al. 2004). However, subsequent reports, using three independent antibodies raised against TRPM8, show that there is little or no coexpression of TRPV1 and TRPM8 in sensory neurons (Abe et al. 2005, McKemy 2004). It therefore seems unlikely that a population of TRPV1/TRPM8 neurons could explain the phenomena of paradoxical cold, even if such a condition exists in rodents. Some have suggested that TRPV1 could be expressed in TRPM8 neurons or vice versa after a stimulatory event such as inflammation. In the study by Obata et al. (2005), TRPM8 expression levels do not change following peripheral inflammation, and coexpression with TRPV1 does not occur. The argument remains that menthol response is a more sensitive, albeit indirect, measure of TRPM8 expression than is immunostaining, and thus coexpression with TRPV1 is still a possibility.

Whether TRPM8 has a role in cold nociception remains unknown. A number of reports have demonstrated that at very high concentrations menthol can elicit pain (Cliff & Green 1994, Green 1992, Wasner et al. 2004). It is unclear if under these conditions menthol is acting solely on TRPM8 or perhaps stimulating other independent signaling pathways because lower concentrations that should be sufficient for TRPM8 activation do not cause pain (Eccles 1994). In humans, innocuous cold elicits pain when Aδ-fibers are blocked. The painful signal is thought to be conducted by innocuous cool-activated nociceptive C-fibers, but this sensation is normally suppressed by cool-activated Aδ-fibers (Fruhstorfer 1984, Wahren et al. 1989, Yarnitsky & Ochoa 1990). However, the pain evoked by Aδ-block may be due to errors in central processing. Because TRPM8 may be expressed in both Aδ- and C-fibers, both the painful and nonpainful signals could be transmitted through TRPM8. That TRPM8 is not coexpressed with any known nociceptive markers (at least in the mouse) questions this supposition; a second caveat could be that Aδ-block-induced cool pain does not occur in rodents. Additionally, TRPM8 does not seem to be required for inflammation-induced cold hyperalgesia (Obata et al. 2005). Ultimately, the true role of TRPM8 in the sensations of cool and cold pain will not be resolved until knockout studies are performed. The data does suggest that a receptor(s) other than TRPM8 may be involved in the perception of noxious cold.

Using the genomics-based approach that identified TRPM8, Story et al. (2003) reported the cloning and characterization of a noxious cold-activated ion channel TRPA1 (ANKTM1). TRPA1 is a distantly related TRP channel expressed in DRG neurons and in the inner ear (Corey et al. 2004, Story et al. 2003). In heterologous expression systems, TRPA1 is activated at a broad range of temperatures (12°C–24°C) with an average threshold of ~17°C, which approximates the threshold of noxious cold for humans (Story et al. 2003). Similar to TRPM8, TRPA1 is expressed in either Aδ- and/or C-fibers owing to a lack of coexpression with neurofilament 150. Consistent with a role in cold nociception, TRPA1 is coexpressed with the nociceptive markers CGRP, Substance P, and with TRPV1. TRPA1 is not coexpressed with TRPM8, suggesting distinct

CA: cinnamaldehyde

BK: bradykinin

functions in cold perception for these channels. Coexpression with the noxious heat receptor TRPV1 suggests that TRPA1 marks a class of polymodal nociceptors that respond to both noxious heat and cold. These neurons may code for the burning sensation associated with pain, whereas other neurons would code for the contextual thermal modifiers. For instance at noxious cold temperatures, TRPM8 activation could provide the cool/cold signal, whereas TRPA1 activation would add the painful burning component. Also because the range of threshold for TRPA1 activation in vitro includes innocuous temperatures, TRPA1-expressing C-fibers could mediate the cool-activated pain perception elicited during Aδ-block.

Lending credence to the idea that TRPA1 is a nociceptor, the pungent compounds cinnamaldehyde (CA), allyl isothiocyanates (mustard oil), wintergreen oil, ginger oil, and clove oil all activate TRPA1 (Bandell et al. 2004, Jordt et al. 2004). Allicin, the pungent component of garlic, activates both TRPA1 and, to a lesser extent, TRPV1. [A subsequent study of allicin questioned the physiological relevance of TRPV1 activation by allicin (Bautista et al. 2005, Macpherson et al. 2005)]. In humans, CA is perceived as a burning, tingling sensation and induces nociceptive behavior in mice (Bandell et al. 2004, Cliff & Heymann 1992, Prescott & Swain-Campbell 2000). The inflammatory peptide bradykinin (BK) also activates TRPA1 when coexpressed with its GPCR (Bandell et al. 2004). BK directly excites nociceptive DRG neurons and causes hyperalgesia (Couture et al. 2001, Levine et al. 1993). These data support the hypothesis that cold activation of TRPA1-expressing polymodal nociceptors would elicit painful sensations. BK likely activates TRPA1 in part through the phospholipase C (PLC) pathway. The study by Jordt et al. (2004) also provided evidence that PLC signaling can stimulate TRPA1 activation. They showed that, when TRPA1 is coexpressed with the PLC-coupled M1 muscarinic acetylcholine receptor (mAChR), activation by the mAChR agonist carbachol causes a TRPA1-like response. This same group also showed that a rapid rise in intracellular calcium caused by the drug thapsigargin is sufficient to activate TRPA1. CA or cold do not likely activate TRPA1 through independent stimulation of PLC because application of either stimulus does not lead to the release of calcium, a known consequence of PLC activation. Taken together these data strongly suggest that TRPA1 itself is a polymodal nociceptor that activates neurons, which in turn transmit a burning pain signal. TRPA1 has also been identified as a candidate for the mechanosensitive transduction channel in vertebrate hair cells (Corey et al. 2004). This raises the intriguing possibility that TRPA1 may play an additional role in mechanical nociception.

Lending support to the idea that TRPA1 plays a role in cold perception, a population of DRG-cultured neurons with TRPA1-like properties was identified. These neurons are activated by noxious cold (~14°C) and/or CA or capsaicin but are not activated by menthol and innocuous cool. In contrast with CA, mustard oil activates this population in addition to a larger, cold-insensitive population (Bandell et al. 2004, Story et al. 2003). This suggests that although mustard oil is an activator of TRPA1, it may also activate sensory neurons in a TRPA1-independent manner. Mustard oil may also be a more potent activator of TRPA1 than either CA or cold, and thus it may activate sensory neurons that express low levels of TRPA1.

The finding that TRPA1 is a noxious cold receptor, however, is not without controversy. Two independent groups, while reporting activation of TRPA1 by mustard oil, did not see cold-mediated activation of TRPA1 in heterologous expression systems (Jordt et al. 2004, Nagata et al. 2005). However, four other laboratories have observed TRPA1 activation by cold [Reid 2005; H. Hosokawa & S. Kobayashi (Kyoto University), personal communication; H. Hu & M. Zhu (Ohio State University), B. Nilius (Ku Leuven), personal

communication]. These results suggest that discrepancies in experimental design may account for the inability of some groups to observe cold activation of TRPA1. For instance, the length, temperature, and rate of temperature change of the cold stimulus differ in these studies. Jordt et al. (2004) and Babes et al. (2004) also failed to detect a mustard oil/menthol insensitive/cold population in cultures of trigeminal ganglion neurons and DRG neurons, respectively. A number of studies have attempted to identify distinct cold-sensitive populations in cultured sensory neurons, and all have reported differing results in the overall percentages, temperature thresholds, and ligand activation profiles of these populations (Babes et al. 2004, Bandell et al. 2004, Nealen et al. 2003, Jordt et al. 2004, Story et al. 2003). These results strongly suggest that the preparation of cultured sensory neurons, as well as how stimuli are applied, may vary markedly between laboratories, and these discrepencies could explain in part the divergence in results from these studies.

A strong support for a role for TRPA1 in noxious cold perception comes from a recent report by Obata et al. (2005). These authors find that TRPA1 expression, similar to TRPV1, is increased after peripheral inflammation owing to CFA or nerve injury. In addition, they report that cold hyperalgesia due to inflammation/nerve injury is dependent on signaling through nerve growth factor and activation of the MAP kinase p38 in TRPA1-expressing neurons. Inhibitors of NGF and p38 block increases in TRPA1 expression owing to inflammation/nerve injury. Knockdown of TRPA1 expression in sensory neurons with antisense TRPA1 specifically blocks inflammation/nerve injury–induced cold hyperalgesia. Providing further evidence for a role for TRPA1 in cold sensation, noxious cold induces p38 phosphorylation mainly in TRPA1-expressing neurons, and this phosphorylation is blocked by antisense TRPA1. These data, together with studies showing that TRPV1 is required for heat hyperalgesia, indicate that TRPV1/TRPA1-expressing sensory neurons are involved in coding for thermal pain sensitization after an inflammatory event. As with TRPM8, the function of TRPA1 as a noxious cold receptor will not be ascertained until further studies including the analysis of TRPA1 knockout mice are performed.

TEMPERATURE SENSATION IN INVERTEBRATES

Distinct members of the thermoTRP families are present in the invertebrates *Caenorhabditis elegans* and *Drosophila melanogaster* as illustrated in **Figure 2**. At least some of the invertebrate TRPs appear to be playing a role in thermosensation as well.

In the mammalian TRPV family it is clear that a single TRPV ion channel gave rise to all six TRPV members because they are more related to each other than to any invertebrate TRPVs. There are no sequence orthologues in *D. melanogaster* and *C. elegans* for the 4 mammalian TRPVs (TRPV1–4), which are activated by varying thresholds of heat. In agreement with the lack of orthology, none of the invertebrate TRPVs are activated by heat, although a general sensory function seems to be conserved. For example, in *C. elegans* the TRPV members OSM-9 and OCR-2 are required for chemo- and mechano-sensation as evidenced by defects in osmotic avoidance, nose touch, and response to noxious chemicals in the *osm-9/ocr-2* mutants (Tobin et al. 2002). They are expressed in the polymodal nociceptor neuron ASH. The expression of the mammalian TRPV4 in ASH neurons in *osm-9* mutants restores the avoidance response to hypertonicity and nose touch, which implies that mammalian TRPV4 can be integrated into the normal ASH signal-transduction pathway (Liedtke et al. 2003). TRPV4-expressing mammalian cells were responsive to hypoosmotic stimuli; however, in the ASH neuron, TRPV4 probably interacts with additional cellular factors to respond to hyperosmotic stimuli. In contrast, TRPV1 expression did not rescue the specific

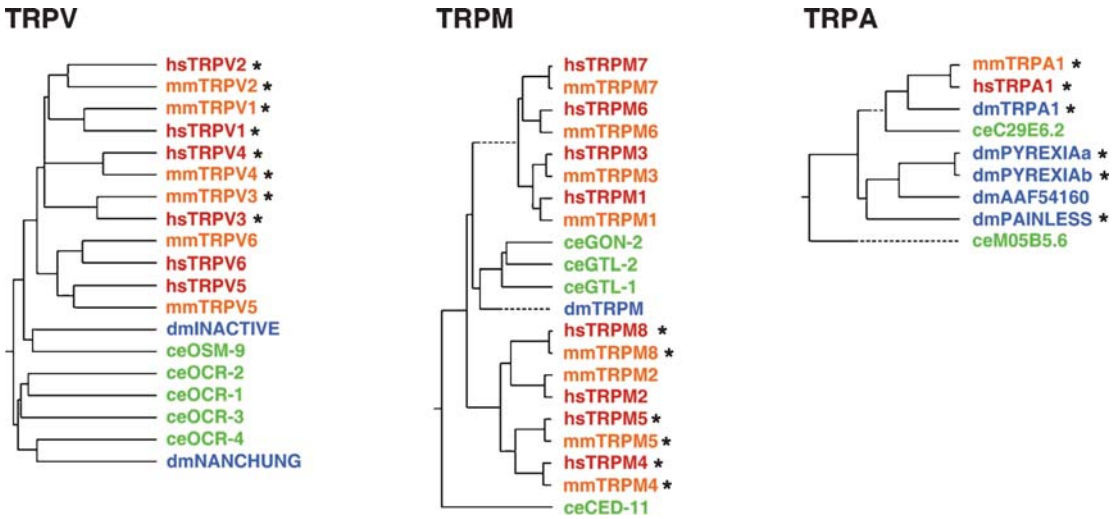

Figure 2

Phylogenies are shown for three families of TRP cation channels: TRPV, TRPM, and TRPA. *H. sapiens*, *M. musculus*, *D. melanogaster*, and *C. elegans* members of these families are color coded. Channels implicated in thermosensation are labeled with an asterisk.

nociceptive behavioral defects in the mutants, although expression of TRPV1 in polymodal neurons in *C. elegans* induced avoidance of capsaicin in *osm-9* mutants (Tobin et al. 2002). This finding indicates that activation of the ASH neuron results in an avoidance behavior; however, TRPV1 may not respond to the pathway that normally activates OSM-9. Reminiscent of TRPV4, *nanchung* and *inactive*, two *D. melanogaster* TRPV members, are activated by osmotic stress and mediate hearing in flies (Gong et al. 2004, Kim et al. 2003). Similar to the TRPV family members, none of the eight mammalian TRPMs have a 1:1 orthologous relationship with their invertebrate counterparts.

TRPA1 is the single mammalian member of the TRPA subfamily. This family includes four *D. melanogaster* and two *C. elegans* members. Unlike the other two thermoTRP subfamilies, mammalian TRPA1 has an orthologue in *D. melanogaster*: dTRPA1 (formerly called dANKTM1). dTRPA1 is activated by a warming rather than a cooling stimulus when expressed in Xenopus oocytes (Viswanath et al. 2003). Consistent with this activity, dTRPA1 is required for normal thermotaxis behavior in *D. melanogaster* larvae (Rosenzweig et al. 2005). dTRPA1 is expressed in two groups of CNS neurons, and these neurons are required for normal thermotaxis by genetic ablation studies. Body wall neurons that show an increase in intracellular calcium concentrations in response to a warming and cooling stimulus do not express TRPA1 (Liu et al. 2003). Some of these body wall neurons known as the multidendritic neurons do express a more distant dTRPA family member named *painless* (Tracey et al. 2003). A reverse genetic screen of fly mutants identified *painless* to be required for noxious heat and mechanical responses, although direct activation of the channel in heterologous systems has not yet been demonstrated. Knockdown of dTRPA1 had normal withdrawal responses to noxious temperature probe, and *painless* mutation had no impact on thermotaxis. Hence these two behaviors must be mediated by different molecular mechanisms. Genetic screens for mutants defective for heat response led to the identification of another *D. melanogaster* TRPA called *pyrexia*

(Lee et al. 2005). pyrexia is activated by temperatures above 40°C when expressed in xenopus oocytes or HEK (human embryonic kidney) cells. It appears that the *D. melanogaster* TRPA family members are heat activated, unlike the mammalian TRPA1, which is activated by cold. The mechanistic basis for this opposite thermosensitivity is yet to be explored.

NON-TRP PROTEINS THERMOSENSATION

The studies of thermoTRPs have advanced our understanding of the molecular mechanisms of thermal transduction. Given the sophistication of mammalian thermosensitivity this process most likely involves complex interactions of ion channels, receptors, and proteins in addition to TRPs. For example $P2X_3$, an ATP-gated cation-selective ion channel, was proposed to have a role in innocuous warm coding (Souslova et al. 2000). $P2X_3$ is expressed by a subset of sensory neurons, and $P2X_3^{-/-}$ mice show attenuated dorsal horn neuronal activity in response to warm temperatures. Recently Shimizu et al. (2005) showed that $P2X_3^{-/-}$ mice have enhanced avoidance of both hot and cold temperatures on a thermal gradient. These mice also showed significantly higher acute thermal nociception in a tail immersion assay at temperatures >42°C and at temperatures <15°C. It is unclear, however, if the abnormal thermosensory phenotype of the $P2X_3^{-/-}$ mice is the result of direct involvement of $P2X_3$ in temperature sensing. Activation or potentiation of this channel by warm temperatures has not been observed in heterologous systems. Injection of $P2X_3$ antagonists A317491 and TNP-ATP failed to display any changes in thermal preference assays. One explanation given is that the long-term absence of $P2X_3$ and the resulting compensatory changes may contribute to the thermal-avoidance phenotype observed in $P2X_3^{-/-}$ mice.

In addition to TRPM8 and TRPA1, other membrane proteins are proposed to play a role in cold transduction. One mechanism proposed is the decrease in activity of many proteins at cold temperatures that will depolarize neurons. For example, researchers suggest that the closing of a background K+ conductance is involved in causing depolarization and firing of action potentials in cold-sensitive neurons (Reid & Flonta 2001a, Viana et al. 2002). The molecule involved may be TREK-1, a K+ channel strongly inhibited by cooling (Maingret et al. 2000). TREK-1 is a member of the two-pore domain K+ channel expressed at high levels in peripheral DRG neurons, as well as in central hypothalamus. It reversibly increases its activity with increases in temperature. Hence Maingret et al. (2003) proposed that at physiological temperatures TREK-1 is open, keeping the neuron near its resting potential. At lower temperatures, TREK-1 would close and depolarize the neurons. However, single-cell reverse transcription polymerase chain reaction (RT-PCR) experiments in DRG neurons expressing TRPM8 or TRPA1 did not show any coexpression of TREK-1 with the two cold-activated TRP channels (Nealen et al. 2003). Studies in TREK-1 knockout animals will be needed to establish the physiological role of TREK-1 as a temperature sensor. In addition to K+ channels, inhibition of a Na+/K+ ATPase has been proposed to play a role in cold transduction (Pierau et al. 1974). Lower temperatures also potentiate the activity of some membrane proteins. For example, members of the degenerin (epithelial sodium channels (DEG/EnaC) family of sodium channels are potentiated by cold (Askwith et al. 2001). A member of this family, DRASIC, is expressed in DRG, and modulation of the channel by cold temperatures was examined in dissociated cultures. Cold temperatures enhanced DRASIC currents in the presence of a specific agonist, although cold by itself could not activate the channel. How these proteins are mechanistically involved in temperature sensation remains to be established.

RT-PCR: reverse transcription polymerase chain reaction

Although some neurons and genes required for thermotaxis in *C. elegans* have been described, there is no evidence as yet of the involvement of TRP channels in this behavior. Thermoresponse in *C. elegans* is complex, as it exhibits plasticity: After normal cultivation on a plate at temperatures ranging from 15°C–25°C, WT worms migrate toward cultivation temperature on a temperature gradient (Mori 1999). Laser ablation studies have revealed that temperature in this 15°C–25°C range is perceived by a pair of sensory neurons, AFD in addition to two pairs of interneurons, AIY and AIZ. Utilizing the cameleon sensor, AFD neurons respond to warming but not to cooling, implying that another neuron plays some role in the cryophilic drive during thermotaxis (Kimura et al. 2004). In addition to thermotaxis, *C. elegans* also shows reflexive withdrawal reaction to acute heat stimuli; however, the cellular and molecular components involved are not known (Wittenburg & Baumeister 1999). Two cyclic nucleotide-gated (CNG) ion channels tax-2 and tax-4 acting as heteromers are critical for thermotaxis and sensory neuronal outgrowth (Komatsu et al. 1996). Another CNG channel cng-3 plays a role in response to thermal stress in worms (Cho et al. 2004). Genetic studies have revealed several members of the homeobox protein family (ceh-14, ttx-3, lin-11, ttx-1) to be essential for normal thermotaxis (Mori et al. 2004). Expression profiling of embryonic neuronal cultures resulted in a group of genes that show AFD-specific expression; however, the functional significance of these genes in relation to temperature responses has not yet been fully studied (Colosimo et al. 2004).

CONCLUSIONS/FUTURE DIRECTIONS

Great progress has been made in our understanding of the mechanisms of temperature sensation since the cloning of TRPV1. Through electrophysiological, molecular, and loss-of-function studies, it is now clear that thermoTRP channels play an integral role in thermal sensation over the entire range of temperature perception. However, many questions remain unresolved. It is still largely unclear how temperature gates these channels. Is temperature gating an intrinsic property of all these channels? How do chemical agonists activate thermoTRPs? Both direct and indirect signaling mechanisms may be involved. How is activation of thermoTRPs coded to provide an accurate representation of our thermal environment? Is it through specific labeled lines? Do action-potential firing rates provide specific information? For instance, how can a thermoTRP channel be involved in the perception of both innocuous and noxious stimuli? Of the thermoTRPs studied with genetic ablation studies (TRPV1, TRPV3, and TRPV4), all have a role in thermal nociception. Will the same be true for the cold receptors TRPM8 and TRPA1? What is the role of central processing in how external temperature is interpreted? Will thermoTRPs prove to be effective targets for pain management? Finally, how many thermoTRPs are there, and are they the only major players in thermosensation? These questions and others will undoubtedly be addressed in the coming years.

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