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INVITED REVIEW

Temperature sensing across species

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Abstract The ability to detect changes in temperature is a fundamental sensory mechanism for every species and provides organisms with a detailed view of the environment. This review focuses on what is known of the neuronal and molecular substrates for thermosensation across species, focusing on the three robust model systems extensively used to study sensory signaling, the nematode *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster*, and the laboratory mouse. Nematodes migrate to thermal climes that are amenable to their survival, a behavior that is regulated primarily through a single sensory neuron. Additionally, nematodes "learn" to seek out this temperate zone based upon their prior experience, a robust model of learning and memory. *Drosophila* larvae also prefer select thermal zones that are optimal for growth and have also developed vigorous mechanisms to avoid unfavorable conditions. In mammals, the transduction mechanisms for thermosensation have been identified primarily due to the fact that naturally occurring plant products evoke distinct psychophysical sensation of temperature change. More remarkably, the elucidation of the molecular sensors in mammals, along with those in *Drosophila*, has demonstrated conservation in the molecular mediators of temperature sensation across diverse species.

Keywords Thermosensation · Nematode · Fruit fly · Mouse · TRP

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Introduction

The perception of external and internal temperature is a vital sensory mechanism that has implications in cellular and metabolic homeostasis, avoidance, and survival. Changes in environmental temperature are typically detected by select sensory neurons that can be crudely categorized into two populations, those that detect innocuous temperatures and those that sense noxious and potentially damaging thermal stimuli. The former are essential for many behavioral aspects that allow organisms to find food, locate a habitable climate zone, and maintain body temperature. The latter respond to stimulus intensities considered painful (nociceptive) and are essential in avoidance behaviors that work to limit exposure to environmental conditions that are harmful to the organism. Remarkably, the structural and anatomical makeup of the two types of thermosensory neurons is consistent across species. For example, the peripheral nerve endings of nociceptive neurons in the nematode and in fruit fly larvae terminate in naked nerve endings that are not associated with a specialized sensory structure, similar to nociceptive free nerve endings in mammals. In addition to structural similarities, in many cases, the molecular entities that are responsible for the detection and transduction of thermal stimuli are similar across species.

Nematode thermosensation

The relative simplicity of the *Caenorhabditis elegans* nervous system has made it an attractive and useful model organism for the study of the cellular and molecular basis for sensory signaling. A total of 959 somatic cells make up an adult hermaphrodite *C. elegans*, of which 302 comprise

the nervous system [1]. Despite this minimalism, *C. elegans* respond to complex environmental cues associated with the senses of smell, taste, and touch, all of which evoke stereotypical primary behavioral responses. More remarkably, nematodes also exhibit more multifaceted behaviors such as learning and memory [2]. For example, *C. elegans* display a strong preference for the cultivation temperature at which they are satiated, such that they migrate, or thermotax, towards this temperature when placed upon a thermal gradient [3]. Conversely, when deprived of food at a given temperature, they subsequently exhibit aversion to this temperature [4]. Therefore, the nematode has been extensively studied as a model system to genetically and molecularly tease out the substrates for thermosensation, in addition to neural plasticity and a relatively simplistic form of learning and memory.

Remarkably, a single sensory neuron, AFD, appears to mediate most aspects of temperature detection and normal thermotactic behavior [5]. The AFD is 1 of the 12 neurons that make up one of the bilaterally symmetrical pair of nematode sensory organs called the amphid sensilla, located near the tip of the nose. Amphid neurons detect an array of sensory stimuli in addition to temperature, including water-soluble and volatile attractants, pheromones, and chemical repellents [1]. The thermosensory necessity of AFD neurons was established when it was observed that animals are incapable of isothermal tracking to their cultivation temperature and display a preference for cold temperatures (a cryophilic response) when these cells are ablated with laser microbeams [5]. Electron microscopy reconstruction analyses of the nematode nervous system have shown that the interneuron AIY is the primary postsynaptic partner of AFD. AIY in turn makes a functional synapse with the interneuron AIZ and the major integrating interneuron RIA, which also receives synaptic input from AIZ [6]. When AIY and AIZ interneurons in animals are individually ablated, these animals are also deficient in isothermal tracking. However, AIY-ablated animals are cryophilic, whereas those lacking AIZ are thermophilic, preferring temperatures warmer than those at which they were cultivated. When both interneurons are ablated simultaneously, animals become so inactive on thermal gradients that an accurate phenotype is unattainable [5]. RIA-ablated animals are partially defective in thermotaxis, in addition to other sensory defects. Thus, a model for the neural circuitry controlling thermotaxis was proposed in which the AIY and AIZ interneurons integrate opposing drives from AFD and an, as of yet, unidentified sensory neuron, respectively (for review see [1]; Fig. 1). AFD activation of AIY drives animals towards warmer temperatures, relative to that at which they were cultivated, whereas AIZ activity induces them to seek out cooler temperatures.

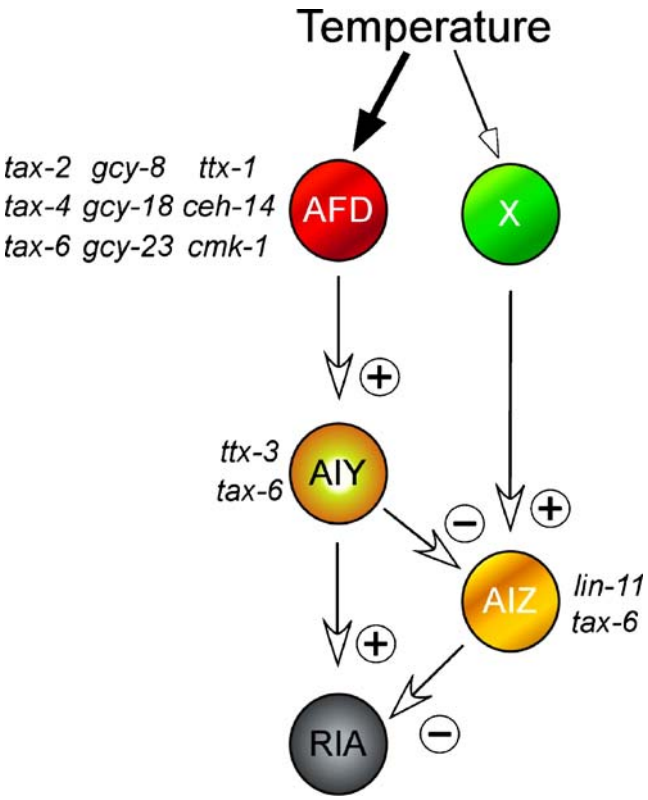

Fig. 1 The model for a *C. elegans* thermosensory neural circuit. Temperature-evoked activation of AFD stimulates the interneuron AIY, which in turn activates the integrating interneuron RIA. An unknown thermosensitive sensory neuron (*X*) activates AIZ, which in turn inhibits RIA. However, AIZ activity is reduced by AIY activation in this model circuit. The identity of genes known to be expressed in, and regulate the function of, the neurons in this circuit are *labeled*. Modified from Mori and Ohshima [5]

How then does the AFD respond to temperature? Recent elegant approaches using the genetically encoded intracellular calcium sensor cameleon have begun to elucidate how temperature change alters AFD activity. Kimura et al. demonstrated that warming ramps above, but not below, the cultivation temperature evoke transient increases in intracellular Ca^{2+} in AFD neurons in vivo. This result suggests that the neurons are not responding to absolute temperature per se but to a step change in temperature from a set point established by the cultivation temperature [7]. Clark et al. [8] later showed that AFD responds dynamically to temperature in that Ca^{2+} responses are bidirectional and are able to phase-lock to oscillations in temperature. Furthermore, calcium responses have also been observed in the dendrites in vivo, and AFD activity is directly coupled with the postsynaptic AIY interneuron. Thus, many of the aspects associated with the behavioral responses of *C. elegans* to temperature change may be controlled by this single sensory neuron.

Cyclic-nucleotide mediated transduction of thermal stimuli in AFD

What still remains a mystery is the actual temperature sensor in AFD neurons. Genetic studies suggest that thermal signals are transduced downstream of an unknown "thermosensor" in these neurons through a cGMP-gated ion channel encoded by the *tax-2* and *tax-4* genes [9, 10]. Mutants in both *tax-2* and *tax-4* exhibit identical thermotactic deficits that are akin to those observed in AFD-ablated animals. The *tax-4* and *tax-2* genes encode α- and β-subunits, respectively, of a cGMP-gated ion channel that is most similar to those expressed in rod photoreceptors. In addition to AFD neurons, Tax-2 and Tax-4 proteins are also expressed in other sensory neurons including ASE, AWB, and AWC and are localized to cilia and dendritic structures of these neurons [9, 10]. Mutants are also defective in many aspects of olfaction and chemosensation of salts, consistent with this neuronal localization. Interestingly, the behavioral phenotype of double mutants is not exaggerated in comparison to the single mutants, and expression of *tax-4* is able to rescue the *tax-2* mutant chemotactic phenotype [9]. However, the reciprocal is not true; expression of *tax-2* does not ameliorate the *tax-4* phenotype. Consistent with the results of the genetic rescue experiments, Tax-4 by itself forms a functional ion channel when expressed in HEK293 cells in vitro, whereas Tax-2 does not [10, 11]. Tax-4 is highly sensitive to cGMP versus cAMP (K_D values of 4×10^{-7} versus 1.4×10^{-4} M, respectively [10]). When co-expressed, Tax-4 and Tax-2 form a functional heteromeric channel that is ~25-fold less sensitive to cGMP in comparison to Tax-4 alone but still retains selectivity for cGMP over cAMP [11]. Additionally, Tax-4/Tax-2 channels are highly Ca^{2+}-permeable but much less sensitive to divalent ion block than Tax-4 channels. As described above, warming of animals above their cultivation temperature evokes an increase in intracellular Ca^{2+} in AFD, and this response is absent in a *tax-4* null mutant [7]. Thus, thermosensory signaling in AFD neurons is likely mediated by increased intracellular cGMP that promotes fluctuations in cytosolic Ca^{2+} concentrations as a part of the signal transduction pathway. Thus, the cyclic nucleotide-gated channel encoded by the *tax-2* and *tax-4* genes is likely a transducer of multiple modalities of sensory stimuli determined by the cell-type expressing the channels.

How then is a change in external temperature transduced into increased cGMP in nematode AFD neurons? Some clues to this process came from a recent work by Inada et al. [12] who used a reverse genetics approach to identify three members of a subfamily of guanylyl cyclase genes, *gcy-8*, *gcy-18*, and *gcy-23*, important for thermotaxis. Transgenic reporter constructs for each gene are expressed exclusively in AFD neurons and are localized to nerve endings. Interestingly, abnormal thermotactic behaviors are only observed in animals with double or triple gene mutations and not in any of the single mutants. Moreover, the abnormal phenotype of the triple mutants could be rescued by the expression of just one guanylyl cyclase, suggesting that these enzymes are functionally redundant. However, the mechanism for how thermal stimuli are converted into guanylyl cyclase activity remains uncertain. Two possible pathways have been proposed, one requiring temperature-dependent activation of an, as of yet, unidentified G-protein-coupled seven transmembrane receptor upstream of these enzymes, or, alternatively, that the guanylyl cyclases themselves act as temperature sensors [12]. Further biochemical and in vitro tests may soon shed light on these possibilities.

Regulatory mechanisms of *C. elegans* thermosensation

Analyses of several additional mutant nematode strains have identified a number of genes that likely serve important regulatory roles in *C. elegans* thermosensation. Many of these appear to function in cell fate determination, gene expression, and regulation of the signal transduction pathway described above. Early screens of sensory mutants identified a number of defects that lead to altered thermotaxis and were referred to as *ttx* mutations [3]. Of these, *ttx-1* mutants fail to track isothermally when cultivated at 20°C and are strongly cryophilic [3, 13]. AFD sensory nerve endings are severely defective in these mutants [14]. AFD neurons are distinguished by their elaborate microvillar finger-like structures that emanate from a single cilium [6, 14]. However, these microvilli are completely lost in *ttx-1* mutants, and the morphology of the cilia is grossly abnormal with its length approximately three times the norm [14], suggesting that Ttx-1 mediates the morphogenesis of AFD sensory nerve endings [13]. The *ttx-1* gene encodes a homologue of the OTD/OTX family of homeodomain proteins that have been implicated in patterning and development of sensory structures in both invertebrates and vertebrates [15]. Expression of GFP reporters from the *ttx-1* locus is exclusive to AFD neurons and is first observed after the establishment of cell fate and maintained throughout development. Interestingly, misexpression of *ttx-1* in olfactory neurons is sufficient to confer an AFD-like phenotype to these neurons [13]. Thus, like other members of the OTD/OTX gene family, *ttx-1* appears to mediate the development of the sensory nerve structure of AFD.

As the behavioral phenotype of *ttx-1* mutants is synonymous with that observed in AFD-ablated animals,

a similar correlation has been made with *ttx-3* mutants and animals in which the interneuron AIY is killed [5, 16]. Like *ttx-1*, the genetic basis for *ttx-3* mutants appears to involve disrupted gene expression as *ttx-3* encodes a LIM homeodomain protein, a class of highly conserved neural regulatory genes [16, 17]. Consistent with the behavioral phenotype, Ttx-3 is expressed exclusively in adult AIY interneurons and is likely involved in regulating expression of genes required for AIY differentiation as excessive neurite outgrowth is observed in axonal projections. Similarly, another LIM homeobox gene, *lin-11*, is expressed in the opposing AIZ interneuron, and morphology of these cells is defective in *lin-11* mutants with a phenotype remarkably similar to that observed in AIY interneurons in *ttx-3* mutants [17]. The *lin-11* mutants were first reported to be thermophilic, a phenotype that mimics that observed in AIZ-ablated animals [5, 17]. However, Satterlee et al. [13] subsequently found *lin-11*-null mutants to be only weakly thermophilic and suggested that *lin-11* does not specify all thermoregulatory functions of AIZ interneurons. In this study, double mutants of *ttx-1*, *ttx-3*, and *lin-11* were examined and, consistent with their observations, *ttx-1:lin-11* and *ttx-1:ttx-3* double mutants were mostly cryophilic. Lastly, a third LIM homeobox gene, *ceh-14*, is expressed in AFD neurons, and mutants in this transcription factor are largely athermotactic, a phenotype remarkably similar to AFD-ablated animals [18]. Only minor structural defects are observed in AFD neurons from *ceh-14* mutants with many markers of these sensory cells still intact. Thus, when taken in context of the behavioral phenotype of these animals, these data suggest that *ceh-14* does not play a role in the fate of AFD neurons but is required for differentiation [18].

In addition to transcriptional regulation of nematode thermosensation, post-translational modifications, such as phosphorylation, regulate thermotaxis and memory of cultivation temperature in *C. elegans*. Mutations in *tax-4* and *cmk-1*, the latter coding for a Ca^{2+}/calmodulin-dependent protein kinase I (CaMKI), result in temperature-dependent defects in gene expression in AFD neurons, in addition to the thermotactic deficits already discussed in *tax-4* mutants [19]. Tax-4 appears to regulate AFD gene expression upstream of Cmk-1, and this enzyme functions in AFD neurons in a manner distinct from those previously described for this class of kinase [19]. Therefore, it was proposed that thermotactic behavior, including memory of cultivation temperature, occurs as a result of Tax-4- and Cmk-1-mediated gene expression. Similarly, mutations in the *tax-6* gene, which encodes for the Ca^{2+}/calmodulin-dependent phosphatase calcineurin A subunit, result in a number of sensory abnormalities, including impaired chemotaxis and thermotaxis [3, 20, 21]. Tax-6 is expressed in a number of tissues, including sensory neurons, interneurons, and muscle cells and, more specifically, in all three neurons of the putative thermosensory circuit, AFD, AIY, and AIZ. Phenotypically, *tax-6* mutants tend to migrate towards temperatures above that at which they were cultivated (thermophilic). When a *tax-6* cDNA was specifically expressed in AFD neurons, via the *gcy-8* promoter, in *tax-6* mutants, these animals showed normal thermotactic responses, thereby suggesting that calcineurin works cell-autonomously in AFD neurons [21]. However, AFD-specific expression of a constitutively active form of Tax-6 resulted in athermotactic or cryophilic behavior, as if the AFD neuron was inactive. In addition to AFD function, *tax-6* is also essential for thermal signaling in AIZ interneurons. Kuhara and Mori [22] recently monitored temperature-evoked changes in intracellular Ca^{2+} in AIZ interneurons and found that starvation down-regulated AIZ activity in wild type animals, a phenomenon not observed in *tax-6* mutants. Taken together, these data support a model in which neuronal activity activates calcineurin, presumably through Tax-2/Tax-4 mediated Ca^{2+} entry, which in turn negatively regulates thermal signaling in a manner that is related to associative learning.

Thermal nociception in *C. elegans*

In stark contrast to the detailed descriptions of the neural circuitry and essential genes for nematode thermotaxis, little is known of the neural substrates involved in behavioral responses to noxious temperatures [23]. Other noxious modalities, such as mechanical and chemical, are primarily detected by the two ASH neurons located at the tip of the nose, but perturbation of ASH cell signaling has no affect on temperature responses [23]. Elegant behavioral analyses performed by Wittenburg and Baumeister [24] demonstrated that *C. elegans* are able to generate nocifensive, or pain-like, responses to temperatures above the range of fertile temperatures (13 to 26°C). Adult animals exhibit a stereotyped withdrawal response to temperatures near 33°C that manifests as a stop in forward movement, followed by reversing for one to two body lengths, then a reorientation away from the heat source. Ablation of AFD, AIY, or AIZ does not alter this response nor is this behavior altered in many of the known thermotactic mutants, such as *tax-2*, *tax-4*, *ttx-1*, *lin-11*, *unc-86*, and *ttx-3* [24]. Surprisingly, the pungent component of hot chili peppers capsaicin, which activates the mammalian thermosensor TRPV1 (see below) [25], sensitized withdrawal behaviors [24]. Capsaicin itself does not evoke an acute nocifensive response, but this effect is attenuated by the competitive capsaicin antagonist capsazepine [24, 26]. The *C. elegans* genome contains several capsaicin receptor-related genes of which *osm-9* and *ocr-2*

are required for nociceptive responses mediated by the ASH nociceptor [23]. However, these related proteins do not appear to be receptors for capsaicin. Moreover, thermal avoidance behaviors are still intact in *osm-9* mutants [23]. Thus, the cellular and molecular substrates mediating acute thermal avoidance in the nematode remain unclear.

Drosophila thermosensation

Many developmental and behavioral aspects of the fruit fly *Drosophila melanogaster* parallel those of higher organisms. Moreover, like the nematode *C. elegans*, a sophisticated array of genetic and molecular tools have been developed that makes analysis of gene function in this multi-cellular organism especially feasible. *Drosophila* have been used as a robust model system to gain insights into many of the cellular and molecular aspects of vision, gustation, and olfaction (for review see [27–29]). However, it has only recently been employed to elucidate the basis of thermosensation.

Thermotactic and thermosensory behaviors in *Drosophila*

Like *C. elegans*, *Drosophila* prefer to reside at specific temperatures. Sayeed and Benzer [30] found that when adult flies are placed upon a linear thermal gradient (18 to 31.5°C), these animals show a strong preference for ~24° C. However, unlike nematodes, flies cultivated or acclimatized at temperatures either above or below 24°C (29 or 18° C) still retain this partiality and thus do not reset their preferences based upon experience. Moreover, in a two-temperature choice paradigm, adult flies prefer 22 over 30° C. Similarly, Liu et al. [31] showed that *Drosophila* larvae prefer to reside at 18°C when given the choice of this temperature versus either 11 or 30°C. In another experimental paradigm, larvae placed upon a thermal gradient from 27 to 41°C were shown to thermotax to the coolest temperatures, avoiding those above their optimal growth temperature [32]. What about temperature extremes? As in many models of nociception in mammals, noxious heat evokes a stereotypical nocifensive-like response in *Drosophila*. Tracey et al. [33] developed a simplistic yet elegant paradigm to assess the behavioral response of *Drosophila* larvae to noxious heat. Similar to freely moving nematodes, *Drosophila* larvae will pause and move away when lightly touched with a probe set at ambient temperatures [34]. However, when the probe is heated to temperatures above 39°C, this same touch stimulus will evoke a nocifensive response in which the larva vigorously roll away from the probe in a cork-screw like motion [33]. Thus, *Drosophila* possess innate and stereotypical behavioral responses to temperature and are able to thermotax to an environment that is suitable for survival.

Neuronal substrates for thermosensation

Like in the nematode, genetic or surgical ablation of specific types or subsets of neurons has identified those likely to be involved in *Drosophila* temperature sensation. In adult flies, Sayeed and Benzer [30] set out to establish the anatomical location of thermoreceptors responsible for temperature preference in their linear temperature gradient assays and found that if the third antennal segments are removed, flies no longer exhibit a thermal preference and distribute evenly across the gradient. Moreover, the *Drosophila* mutant ss^a, in which the distal regions of the third antennal segment are mutated into leg-like structures, exhibits no temperature preference when placed on the linear gradient or when presented with temperature steps [30, 35]. However, both control and antennae-less flies avoid temperatures above 31°C, leading the authors to suggest the existence of two "thermosensors," one in the fly antennae that mediates the preference for 24°C and a second that drives flies away from higher temperatures that may be perceived as noxious [30].

Whereas these seminal observations were conducted in adult flies, recent approaches to address the cellular and molecular basis for *Drosophila* thermosensation have used fly larvae, as this has been a more tractable model system. Like in *C. elegans*, peripheral sensory neurons in larvae have been mapped anatomically and structurally and can be observed optically as they lie at the surface of the semi-transparent cuticular layer [31]. Sensory neurons fall into two classes: type I, which terminates in a single ciliated dendrite, and type II, which lack sensory cilia and extend multiple dendrites [23]. In the adult, type I neurons are organized in complex structures, such as the sensory bristles that cover the fly and chordotonal organs, and function in various aspects of mechanosensation [36]. Type II neurons, on the other hand, spread their dendrites throughout the epidermis and terminate as free nerve endings, very much like vertebrate nociceptors [23, 37].

In larvae, distinct thermosensory responses have been observed in type I neurons in the terminal sensory organ and in type II multidendritic (md) neurons in the abdominal body wall [31, 33, 35]. Using transgenic larvae expressing the Ca^{2+} reporter cameleon, Liu et al. [31] observed increased intracellular Ca^{2+} in only the terminal organ upon cooling from 18 to 10°C but not when temperatures were raised to 40°C. Extracellular electrical recordings in the terminal organ also uncovered substantial basal activity at room temperature, which increased with cooling and

decreased when the larvae were warmed. In contrast, intracellular Ca^{2+} largely decreased in type II md neurons upon cooling, whereas heating produced a mixed result with some cells exhibiting increased intracellular Ca^{2+}, whereas others had no changes at all [31]. Using suction-electrode recordings from sectioned abdominal nerves, Tracey et al. [33] observed increased spiking at temperature thresholds of ~28 and 38°C, suggesting the existence of at least two functionally distinct md thermoceptors in the abdominal wall.

Lastly, genetic silencing of either type I or type II neurons has demonstrated segregated behavioral responses between the two sensory neuron types. When synaptic transmission was blocked in the terminal organ by expression of the tetanus toxin light chain (TeTxLC), transgenic larvae no longer preferred 18 over 11°C [31]. However, the larvae still avoided warmer temperatures in that they preferred 18 to 30°C comparable to wild type larvae. Similarly, expression of TeTxLC in md neurons largely prevented the nocifensive rolling-escape behavior exhibited by wild type larvae when touched with a probe heated to 46°C [33]. Thus, these data suggest that type I neurons may in fact be cold sensors and mediate thermotactic responses that drive *Drosophila* larvae away from temperatures below 18°C, whereas type II neurons detect noxious, tissue-damaging heat and trigger an escape response.

TRP ion channels mediate thermosensory responses

What then mediates the response to temperature in *Drosophila*? Remarkably, evidence to date suggests that members of the transient receptor potential (TRP) family of ion channels mediate thermosensation in the fly, demonstrating that there is a high degree of similarity between the molecular entities mediating thermosensation in mammals and insects (see below) [29]. This link was first observed in an elegant forward genetic screen conducted by Tracey et al. [33] in which they looked for mutations that prevent the nocifensive rolling response observed when larvae came in contact with a heated probe. A mutant, called *painless*, was isolated, which exhibits significantly reduced response to stimulation with a probe heated between 42 and 48°C, although temperatures above 52°C still evoke a nocifensive response similar to that observed in wild types. This observation is intriguing in light of the fact that similar temperature ranges are observed for mammalian nociceptive afferents, with C- and type II Aδ-fibers responding to a thermal threshold of ~43°C and type I Aδ-fibers exhibiting activation above 52°C [37]. In addition to deficits in thermosensation, *painless* mutants are also less responsive to mechanical stimuli. Wild type larvae stimulated with a 45-mN von Frey filament will also roll away from the stimulus, but this response is absent in *painless* mutants [33]. Higher threshold mechanical stimuli (100 mN) will evoke a nocifensive response, suggesting that *painless* mediates both thermal and mechanical nociception to moderate intensity noxious stimuli.

The *painless* gene codes for a member of the TRPA subfamily and is expressed in a subset of multidendritic neurons. Transcripts are observed in md neuron precursors and localized to the dendrites in advanced embryos [33]. Consistent with mRNA expression, anti-Painless antisera and a transgenic strain that drives GFP expression from the *painless* promoter, observed expression in the chordotonal organs, multidendritic neurons, the antennal-maxillary complex, and a subset of cells in the central nervous system. Immunoreactivity was strongly localized to puncta in the dendritic arbor found beneath the embryonic epidermis. As described above, extracellular recordings of md neuronal activity demonstrated that heating above 38°C evokes an increase in spike frequency, which is absent in *painless* mutants [33]. Interestingly, a biphasic increase in activity is observed between 25 and 30°C in *painless* mutants but not in wild type recordings. However, recordings were not performed at the temperature extremes near 52°C, stimulus intensities that evoke a nocifensive response from *painless* mutants. Thus, Painless expression in md neurons suggests that these sensory neurons may be akin to moderate heat threshold mammalian nociceptors [37].

Transgenic lines expressing reporter constructs from the *painless* locus also revealed expression in subsets of sensory neurons involved in gustation [38]. Expression in gustatory bristles in the labial palpus, leg tarsus, and the anterior wing margin was observed and, in many cases, overlapped with neurons expressing markers via the gustatory neuron reporter *Gr66a*, which have been implicated in aversive responses to tastants [38, 39]. The only TRPA channel in mammals is a receptor for many pungent compounds, including allyl isothiocyanate (AITC), the pungent ingredient in wasabi (see below) [40, 41]. In a two-choice preference test, wild type flies exhibit a strong aversion to AITC, whereas *painless* mutants do not [38]. This aversion was further shown to be gustatory. When ravenous flies encounter sweet substances, they extend their proboscis, a behavior that declines in the presence of aversive substances such as AITC. The *painless* mutants do not exhibit aversion to isothiocyanates in this assay, and the wild type phenotype is rescued by transgenic expression of a wild type *painless* cDNA, as is also the case in the two-choice preference assay [38]. This gustatory defect in *painless* mutants is not due to a general inhibition of gustation, as mutants still respond to other tastants such as quinine and NaCl. Therefore, these genetic and behavioral data demonstrate that the *Drosophila painless* is likely the functional orthologue of the mammalian wasabi receptor

TRPA1. However, this hypothesis needs to be solidified by a demonstration that isothiocyanates can directly activate Painless in heterologous expression systems, as is the case for the mammalian channel [40]. Similarly, genetic and behavioral evidence clearly shows that *painless* mediates thermosensory and mechanosensory signaling in vivo. However, it has yet to be shown experimentally that Painless is the primary "detector" of these stimulus modalities or if it is downstream of an unknown thermosensor or mechanosensor.

In addition to Painless, the *Drosophila* genome encodes three other members of the TRPA subfamily: dTRPA1, dTRPA2, and dTRPA3 [42]. Of these, dTRPA1 and dTRPA2, also known as Pyrexia, have been implicated in thermosensation [32, 43]. dTRPA1 was first shown to be temperature sensitive in vitro, as threshold temperatures in the warm range (~27°C) activate transient ionic currents when the channel is expressed in heterologous systems [44]. Moreover, dTRPA1 is essential for larval thermotaxis, as knockdown of channel expression by RNAi prevents these animals from avoiding temperatures above the optimum [32]. This phenotype is specific for dTRPA1 as dsRNAs for the other TRPA family members, as well as the two *Drosophila* TRPV channels and the lone TRPM, have no affect on this phenotype. Whereas knockdown of dTRPA1 influences thermotactic behaviors, nocifensive responses like those absent in *painless* mutants are unaffected. Thus, dTRPA1 likely plays a role in *Drosophila* thermotaxis but is not required for avoidance and nocifensive responses to noxious heat. Antisera raised against dTRPA1 label primarily a small subset of central brain neurons, neuroendocrine cells of the corpus cardiacum, and unidentified cells adjacent to the mouthhooks and in the digestive system [32]. Surprisingly, protein expression is not observed in the two regions previously known to contain thermosensitive sensory neurons, multidendritic neurons, and the chordotonal organs [31, 33]. In larvae, in which md neurons were silenced by expression of TeTxLC, or in animals lacking chordotonal neurons, no deficits were observed in the thermotactic assay used (thermal gradient from 27 to 41°C) [32]. However, when TeTxLC, or the cell death-promoting gene *Hid*, is expressed under the putative dTRPA1 promoter, larvae do not fully migrate to the cooler zone (27°C), a partial phenotype of that exhibited after RNAi knockdown of dTRPA1. Thus, when taken as a whole, these data demonstrate that dTRPA1 mediates thermal avoidance of innocuous temperatures above the optimal growth temperature of *Drosophila* larvae and, moreover, unmasks a previously unknown subset of thermosensory cells.

In further support of the importance of TRPA channels in *Drosophila* thermosensation, a temperature preference screen of P-element insertion mutants isolated the *pyrexia* mutant, which codes for dTRPA2 [43]. In vitro, temperatures above 40°C evoke ionic currents in cells expressing dTRPA2 that primarily conducts potassium ions over sodium, suggesting that thermal activation of the channel does not lead to depolarization but will in fact hyperpolarize the neuron. The channel is expressed in a number of sensory neurons that innervate bristles located around the eyes and on the dorsal part of the thorax and in the proboscis and antennae. Interestingly, dTRPA2 is expressed not only in multidendritic neurons, as is Painless, but also in non-multidendritic neurons that line the epidermis. dTRPA2-specific antibodies detect robust neurite expression but, surprisingly, not in the axonal tips as would be expected for a sensor. The most robust phenotype of dTRPA2-null flies is a remarkable paralysis evoked when the animals are exposed to temperatures above 40°C. Thus, the cellular localization, high permeability to potassium, and paralytic phenotype to high temperatures suggest that this channel serves to protect flies from high temperature strain [43].

Other thermosensory mechanisms

The preponderance of evidence suggests that members of the TRPA subfamily mediate thermosensory responses in *Drosophila*. However, a recent study has uncovered a new potential player in thermosensation, histamine. Hong et al. performed a P-element screen very much like that used to identify the *pyrexia* mutants [43], and isolated several mutants which exhibit deficits in thermotactic behaviors [45]. The genes identified encode several proteins that serve in histamine signaling, including a subunit of a histamine-

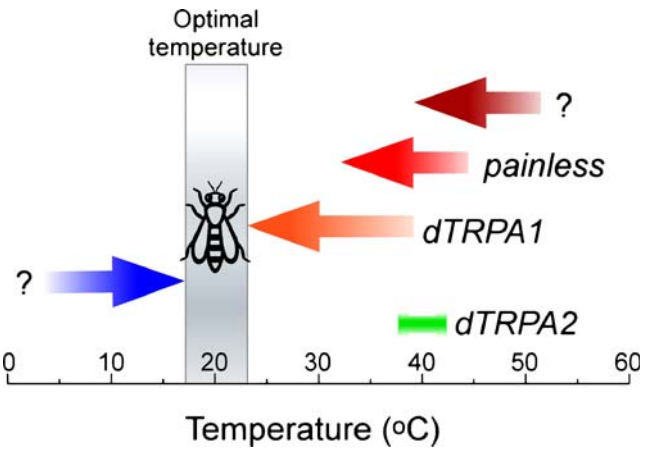

Fig. 2 *Drosophila* TRP ion channels drive both larvae and adult flies to thermotax towards the optimum growth temperature or provide aversive signals from noxious stimuli. dTRPA1 and an unknown thermosensor drive animals towards the optimum clime, whereas Painless allows them to avoid noxious heat. dTRPA2 provides thermotolerance for temperatures near the noxious range. Aversive responses to high intensity noxious heat (>50°C) is mediated by an unknown thermosensor

gated ion channel and histamine decarboxylase. Expression of these proteins is found throughout the central nervous system but not co-expressed with dTRPA1. These mutants display a preference for warmer temperatures near 27°C, whereas wild type and rescued animals prefer to congregate near 24°C [45]. Interestingly, pharmacological antagonists of histamine receptors evoke a similar phenotype as the histaminergic mutants.

From these studies and those previously discussed, a model for *Drosophila* thermosensation can be posed, which includes at least five separate pathways (Fig. 2). First, type I sensory neurons of the chordotonal organs serve as cold thermoceptors and drive larvae from cold temperatures (~10–18°C) towards the optimal growth temperature. Second, thermosensory cells expressing dTRPA1 reciprocally drive larvae to thermotax toward the growth temperature from warm temperatures (~38–24°C). Third, type II multidendritic sensory neurons expressing Painless mediate nocifensive responses to moderately noxious heat greater than 38°C. Fourth, dTRPA2 provides flies a mechanism to establish thermal tolerance. Last, an as yet unidentified sensory pathway mediates nocifensive response to highly noxious heat (~52°C) and, like the Painless pathway, evokes an escape response to harmful stimuli. All told, it is clear that TRP ion channels are crucial for at least three of these pathways, and it remains to be seen if other members are involved in determining the remaining thermosensory responses or if other molecular players, such as those involved in histamine signaling, are required for some portions of *Drosophila* thermosensation.

Vertebrate thermosensory mechanisms

Although many aspects still remain enigmatic, the relative minimalism of the nematode nervous system was crucial in the discovery of the cast of neuronal players in *C. elegans* thermosensation. As evidenced in *Drosophila*, the circumstances are exceedingly more complex in higher organisms. However, the robust molecular tools available in *Drosophila* have enabled many researchers to begin to unravel the genetic and molecular logic for thermosensation in the fruit fly in a relatively short period of time. However, mammals are neither simplistic in their neural networks nor are the same molecular and genetic approaches available in *C. elegans* and *Drosophila* practical in vertebrate model systems, such as the mouse. Nonetheless, the last decade has been replete with fundamental and seminal discoveries into the biological processes that mediate temperature sensation in mammals. Remarkably, the key to these great strides was not the advent of a new genetic or cellular manipulation but was in fact due to the properties of plant extracts that have been employed medicinally and in culinary fare for centuries [46].

As the active ingredients in hot chili peppers and mint leaves mimic the distinct psychophysical sensations of hot and cold, respectively, it was reasoned that if the molecular targets of these compounds were identified, these receptor proteins were likely to be involved in mediating the detection of temperature [46]. This hypothesis was first validated when Caterina et al. [25] cloned the capsaicin receptor, TRPV1, from DRG neurons and established that this ion channel is activated by noxious heat in vitro. Subsequently, we identified a cold and menthol receptor, TRPM8, from TG neurons using a similar paradigm, thereby establishing that members of the TRP family of ion channels play an important role in thermosensation [47]. Subsequently, four additional TRP channels have been implicated in temperature sensation both in vitro and in vivo, and their properties, along with TRPV1 and TRPM8, can conceivably account for the entire spectrum of perceived temperatures [48–55] (Fig. 3).

The hot of capsaicin and the cool of menthol

Over a half century ago, Nicolas Jancsó demonstrated that, when applied to the skin, capsaicin produces a robust burning sensation, which is followed by vasodilation and neurogenic inflammation (reviewed in [56]). Furthermore, high doses of capsaicin produce analgesia, referred to as capsaicin desensitization, such that ensuing heat and chemical stimulation are less likely to be perceived [57]. Capsaicin also has central nervous system effects exhibited as a marked fall in body temperature when capsaicin is given systemically. This hypothermic effect is due, in part, to vasodilation and a fall in metabolic activity at cool

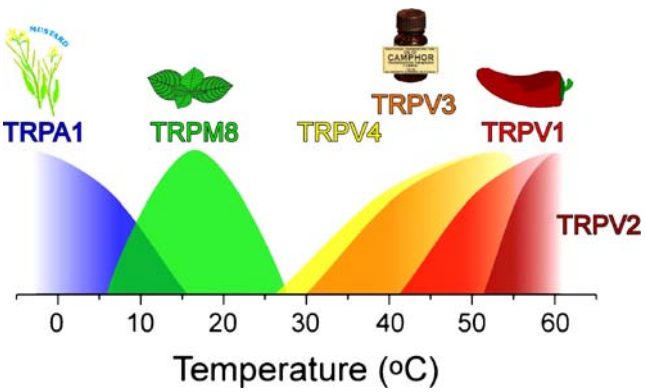

Fig. 3 Mammalian TRP ion channels detect a broad range of temperatures and their in vitro properties predict the thermal zones each channel potentially mediates. Moreover, many of these channels are activated by plant derivatives, which provide distinct sensations of temperature. Mouse models have shown that TRPV1, TRPV3, and TRPV4 are involved in thermosensing in vivo, whereas analyses of cold response in TRPA1-null mice are controversial

temperatures [56]. However, when given systemically to neonatal rats, capsaicin induces the selective degeneration of small-diameter sensory neurons, leading to reduced vasodilation and the absence of systemic capsaicin-mediated reductions in body temperature [58]. Likewise, as in adults, capsaicin-treated rats are less responsive to stimulation with chemical irritants and noxious heat.

Psychophysically, we perceive the shift between innocuous warmth and noxious heat near 43°C. Temperatures above this 'moderate' threshold evoke responses in approximately half of rodent primary afferent fibers, whereas a smaller proportion respond at 'high' threshold temperatures above 52°C [59, 60]. The former population is made up by C- and type II Aδ-fibers, whereas the latter are composed of type I Aδ-fibers [37]. Interestingly, the majority of heat-sensitive fibers with a "moderate" thermal threshold of ~43°C are activated by capsaicin, a functional feature of nociceptive sensory neurons [59]. Capsaicin, like noxious heat, depolarizes select subsets of sensory afferents by increasing the permeability to Na^+ and Ca^{2+} ions [59, 61–63]. Thus, capsaicin and capsaicin-sensitive afferents mediate many of the elements associated with noxious heat stimulation.

As with capsaicin and the perception of heat, the cooling compound menthol evokes a sensation of cold. Menthol, a cyclic terpene alcohol found in leaves of plants of the *Mentha* species, is used in a wide range of products, such as confectionary, candy, toothpastes, vapo-rubs, and aromatherapy inhalations [64]. Moderate concentrations of menthol induce a pleasant cool sensation, whereas higher doses can be noxious, causing burning, irritation, and pain [65–69]. From seminal studies conducted by Hensel and Zotterman [70] in the 1950s, it came to be appreciated that menthol elicits its "cool" sensation by decreasing the threshold temperature for activation of cold receptors. Indeed, they hypothesized that menthol exerts its actions upon "an enzyme" that is involved in the activation of these nerves [70]. Similar responses are observed in cultured neurons with both menthol and cold evoking rapidly activating, nonselective cation conductances in a temperature-dependent manner [47, 71]. Both menthol- and cold-evoked currents also adapt to prolonged stimulation at a rate that is similar to what is observed in primates and humans [72].

In contrast to the definitive thermal thresholds of noxious heat-sensitive nerves, similar distinctions for noxious cold-sensitive fibers are not as easily resolved. In most cases, the perception of cold initiates when the skin is cooled as little as 1°C from normal body temperature [73]. In electrophysiological recordings, both C- and Aδ-cold fibers fire continuously at body temperature with cooling in the range of 30–15°C increasing the rate of firing, whereas warmer temperatures decrease firing [73–76]. The perception of cold pain is felt as temperature approach 15°C with qualities perceived as burning, aching, or pricking [77]. As described previously, studies into cold-sensitive fibers were inconclusive in defining the biological basis for cold signaling. However, a number of laboratories interested in cold transduction began to use primary cultures of either DRG or TG neurons as in vitro models of sensory afferents. Approximately 10% respond to cold temperatures, with thresholds for activation below 30°C, and almost all of these sensory neurons are menthol-sensitive as well [47, 71, 78, 79]. Thus, these in vitro data support the hypotheses of Hensel and Zotterman in that it seemed likely that cold and menthol work through a similar mechanism, leading to the search for their common molecular site of action.

Cloning of the capsaicin and menthol receptors

In the nematode, the milestone in thermosensory research was the discovery of the requirement of AFD in appropriate temperature response [5]. In vertebrates, the same significance can be placed upon the cloning of the capsaicin receptor, TRPV1, in 1997 [25]. TRPV1, when expressed in heterologous expression systems, produces a large cationic current in response to not only capsaicin but also to noxious heat at a temperature threshold near 43°C. Additionally, TRPV1 currents are pH-sensitive, demonstrating that the channel functions as a polymodal detector of noxious stimuli in that it is sensitive to heat, capsaicin, and protons [46, 80]. Hence, the cloning and functional analysis of TRPV1 revealed for the first time how sensory neurons detect "moderate" threshold heat responses at the molecular level. Furthermore, several additional TRPV channels are also activated by warmth and heat, suggesting that thermosensory responses to heat are largely mediated by one class of proteins (see below) [48–50, 52–54].

Similar to the process where a sensor for heat was found with capsaicin, we and others identified a cold sensor on sensory afferents by cloning a menthol receptor, TRPM8 [47, 51]. When the channel is expressed in heterologous expression systems, currents are activated by a number of cooling compounds in addition to menthol, such as eucalyptol (the active ingredient in eucalyptus oil) and the super-cooling compound AG-3-5 [47, 81]. Biophysically, TRPM8 has surprisingly similar properties to those recorded in both cultured DRG and TG neurons using similar experimental paradigms [47, 71]. TRPM8 currents are evoked by cold with an activation threshold temperature of ~26°C, with activity increasing in magnitude down to 8°C, spanning what are considered both innocuous cool (~30–15°C) and noxious cold temperatures (<15°C) [47, 51]. TRPM8 was the first cold-activated ion channel to be identified and established the general role for TRP ion channels in thermosensation.

Mouse models deficient in temperature sensing

The cloning of TRPV1 and TRPM8 called attention to the essential role of TRP ion channels in temperature sensing in vertebrates. This has been further validated with the identification of several additional TRPs as likely thermosensors, including TRPV2, TRPV3, TRPV4, and, perhaps, TRPA1 [46]. These channels were identified as such based upon either their sequence or structural similarity to TRPV1, expression in sensory afferents, or in vitro biophysical properties [46]. The first of these, TRPV2, is a candidate transducer of high threshold heat responses observed in medium diameter, lightly myelinated type I Aδ fibers that respond to temperatures >50°C [48, 59]. In vitro, TRPV2-mediated currents are activated at a threshold temperature of 52°C, and TRPV2 expression in sensory ganglia is suggestive of expression in type I Aδ fibers [48, 82]. However, behavioral responses of TRPV2-null mice have not been reported. Similarly, TRPM8-deficient mice have also not been produced; thus, the putative in vivo roles for these two channels have not been addressed experimentally. In contrast of the other TRP channels implicated in mammalian thermosensation, knockout mice for TRPV1, TRPV3, TRPV4, and TRPA1 have been generated and characterized [41, 83–85].

TRPV1 was the first thermosensor to be critically tested in vivo [86, 87]. Isolated sensory neurons obtained from TRPV1-null mice do not respond to capsaicin, lack heat-gated responses in the moderate threshold range, and have visibly diminished sustained acid-evoked currents in vitro. In vivo, TRPV1$^{-/-}$ mice no longer exhibit nocifensive responses to vanilloids and are impervious to capsaicin-mediated hypothermia. Capsaicin also affects urinary function, stimulating bladder afferent neurons, which results in a voiding reflex that is absent in TRPV1$^{-/-}$ mice [88, 89]. Surprisingly, nocifensive behaviors to temperatures in the moderate threshold range (43 to 48°C) were similar to wild types, but TRPV1-null mice do exhibit reduced responsiveness to temperatures above 50°C [86, 87]. More strikingly, TRPV1-null mice fail to develop hypersensitivity, or hyperalgesia, to thermal stimuli after inflammatory peripheral tissue injury [86, 87]. Inflammation creates a local tissue acidosis, along with the production of several inflammatory mediators that induce thermal and mechanical hyperalgesia [37]. Hypersensitivity to thermal stimuli associated with inflammation are completely absent in TRPV1-null mice, although mechanical hypersensitivity remains intact. Thus, TRPV1-null mice lack essentially all of the physiological and pathological responses associated with capsaicin stimulation and do not develop inflammatory thermal hyperalgesia but are still sensitive to moderately noxious heat.

In vitro, TRPV3 and TRPV4 are activated at temperatures considered warm [49, 50, 52–54]. TRPV4, a functional orthologue of the *C. elegans* TRP channel Osm-9 [90, 91], was first identified as an osmosensitive ion channel and is activated by decreased osmolarity [92–94]. However, subsequent studies suggest that TRPV4 is a polymodal receptor, as it can also be triggered at temperatures >25°C and protons [49, 50]. The channel is expressed in several tissues, including the preoptic/anterior hypothalamus, keratinocytes, and primary sensory neurons, all regions that serve in various aspects of thermosensation and thermoregulation [50, 94, 95]. TRPV3, alternatively, is activated by a number of natural plant products that produce a sensation of warmth, including camphor and the active components of oregano, thyme, and cloves [83, 96]. Remarkably, TRPV3 appears to be exclusively expressed in keratinocytes, and ionic currents are reported to be evoked at threshold temperatures of either 31 or 39°C in heterologous cells expressing the channel [52–54]. Thus, these two TRPV channels were hypothesized to mediate the signaling of warm temperatures in ranges below that detected by either TRPV1 or TRPV2.

Indeed, behavioral analyses of TRPV3- and TRPV4-null mice partially bears the above hypothesis out, with some subtle differences in thermosensory responses [83, 84]. When placed upon a thermal gradient (0.9 to 48.8°C), TRPV4-null mice prefer warmer temperatures in comparison to their wild type littermates (28.1°C for wild type and 32°C for TRPV4$^{-/-}$) [84]. Additionally, when given the choice between two innocuously warm temperatures, they are partial for 34 over 30°C, a phenotype not observed in wild type mice. However, when asked to choose between 34 and 36°C, both wild type and TRPV4 nulls show a strong preference for the cooler temperature. This behavioral phenotype suggests that TRPV4 activation drives mice towards temperatures near 30°C, but that mice lacking this channel still retain the ability to avoid higher temperatures [84]. Surprisingly, TRPV4-null mice also present with a prolonged withdrawal latency to noxious temperatures (near 47°C) in a tail water-immersion assay, suggesting that the channel mediates some aspects of acute avoidance of noxious heat in addition to thermotactic behaviors [84].

When given the choice between floor plate zones held at either room temperature or 35°C, wild type mice spend >90% of their time in the warmer zone [83]. However, mice lacking TRPV3 fail to migrate to 35°C when given this choice but strongly avoid colder temperatures (15°C) versus room temperature, similar to wild type mice [83]. In a temperature gradient assay similar to that used to test TRPV4 nulls (15 to 55°C), wild type mice, after 30 min of exploring the experimental chamber, spend the majority of the time between 30 and 38°C, whereas TRPV3 nulls display no significant preference for this restricted zone

during this timeframe [83]. Interestingly, this inability to identify the preferred thermal zone is transitory, as TRPV3$^{-/-}$ mice will eventually migrate to 35°C after approximately 1 h in the chamber. Thus, TRPV3 appears to be partially necessary in vivo for the ability to thermotax towards warmth, but mice lacking this channel will eventually acquire normal thermal preference with time using alternative mechanisms. Additionally, similar to TRPV4-null mice, there is a subtle behavioral deficiency in high threshold noxious heat responses (>50°C) in TRPV3$^{-/-}$ mice, a phenotype similar to TRPV1 nulls [87]. Thus, mice deficient in any of the three heat sensitive TRPV channels display similar behavioral phenotypes in their acute nocifensive responses to extreme heat but exhibit no behavioral deficits in responses to moderate noxious heat. Moreover, as both TRPV3 and TRPV4 are expressed in keratinocytes, the former exclusively, it is unclear how thermal activation of these channels in non-neuronal cells is transmitted to sensory neurons to communicate thermal stimuli.

The thermosensitive TRP ion channels described above perform functionally in mediating heat both in vivo and in vitro. However, a non-TRP-related mouse model has been described in which thermosensation is impaired, namely, mice deficient in the ionotropic purinergic receptor $P2X_3$ [97]. This is an ATP-gated ion channel that is expressed in a subpopulation of small-diameter nociceptive neurons. ATP released upon tissue damage produces a sensation of pain, and mice null in $P2X_3$ lack rapidly desensitizing ATP-gated cation currents. However, an unexpected observation in $P2X_3$-null mice is that, in electrophysiological recordings from spinal cord wide dynamic range neurons, they are unable to code the intensity of innocuous warm stimuli but retain sensitivity to noxious heat [98]. Thus, these results suggest that $P2X_3$, although it has not been reported to be activated by warm temperatures in vitro, has a role in warm thermosensation in vivo. However, behavioral studies of $P2X_3$-null mice subsequently found that these mice unexpectedly exhibit enhanced avoidance of both hot and cold temperatures when studied in the linear thermal gradient paradigm [99]. Moreover, they also show signs of enhanced avoidance in the water-immersion tail flick assay to both hot and cold temperatures generally considered noxious. Thus, whereas in vitro recordings suggest that warm thermal signaling is absent in $P2X_3$-nulls, behavioral experiments have shown that $P2X_3^{-/-}$-deficient mice are unexpectedly better able to avoid unpleasant temperatures than wild types.

Whereas the in vitro and in vivo discrepancies observed with $P2X_3$-null mice have perplexed many in the thermosensory field, two independent reports on TRPA1-null mice have further complicated an already confusing story [72, 100]. TRPA1 was first reported by Story et al. [55] to be activated in vitro by cold temperatures near that considered noxious (<17°C). However, the hypothesis that TRPA1 mediates perceptual responses to noxious cold was contradicted by at least two laboratories that could not reproduce cold activation of the channel in vitro [40, 101]. Furthermore, TRPA1 was shown to be a receptor for several pungent compounds, including the active ingredients in wasabi, cinnamon, and uncooked garlic [40, 102–104]. Several laboratories have examined the correlation between cold responses and these agonists, with mixed and inconclusive results [40, 72, 102–105]. Thus, a chilly controversy arose regarding the involvement of TRPA1 in thermosensation, one that would presumably be resolved by the study of TRPA1-null mice.

Unfortunately, this turned out not to be the case. Working independently, the laboratories of David Julius and David Corey generated TRPA1-null mice and tested their sensitivity to cold [41, 85]. In a remarkable similarity to the discrepancies found with TRPA1 responses to cold in vitro, one group observed no cold sensation deficits in the knockout mice, whereas the second group did. Bautista et al. [41] found that the percentage of cold-sensitive sensory neurons in culture, number of nocifensive responses to acetone-evoked cooling, paw-withdrawal latencies, and induction of shivering in a cold-plate paradigm (20 to −10°C) were indistinguishable in wild type and TRPA1$^{-/-}$ mice. In the contrary, Kwan et al. [85] did report a decrease number of paw lifts when TRPA1$^{-/-}$ mice were placed upon a surface cooled to 0°C, as well as in the duration of paw shakes during acetone evaporative cooling. However, this significant difference in thermal sensitivity was only observed in female mice and not in males. When aggregate data obtained from male and female mice are considered, the differences in cold-evoked responses are significantly dissimilar. Conversely, when the data are analyzed by gender, female TRPA1$^{-/-}$ mice exhibit markedly reduced cold sensitivity, whereas males do not (see supplemental Fig. S1 of Kwan et al. [85]). Gender differences in pain sensitivity are well established [106], but the underlying mechanisms for the observed cold sensation differences between male and female mice deficient in TRPA1 in the Kwan et al. study remains enigmatic. Furthermore, it is difficult to compare the results of these behavioral studies of TRPA1$^{-/-}$ mice, as they were performed in subtly different ways (i.e. measurements of paw-withdrawal latency versus duration) [41, 107].

Other thermosensory processes

Besides the known thermosensitive TRP ion channels, several additional thermosensory mechanisms have been suggested through in vitro studies. It is a biochemical axiom that cold temperatures hamper protein function, and

this inhibitory property of cold has been suggested to be a mechanism for neuronal depolarization. A number of studies have suggested that cold inhibition of background K^+ conductances or the Na^+/K^+-ATPase leads to membrane depolarization of cold-sensitive afferents [78, 108–110]. In the former example, the K^+-channel TREK-1 is strongly inhibited by cooling in vitro and expressed very highly in sensory neurons [111]. Thus, it has been proposed that thermosensory neurons depolarize by cold-evoked inhibition of TREK-1 [111]. However, similar to the hypothesized role of $P2X_3$ based upon the in vitro data, TREK-1-null mice do not exhibit any significant differences in response to cold in isolated skin-saphenous nerve preparations [112]. Indeed, C-fibers recorded from TREK-$1^{-/-}$ mice are more sensitive to heat than wild types, firing more action potentials and have a lowered firing threshold in response to noxious heat. This observation is consistent with cellular localization of this K^+ channel, which is extensively co-localized with TRPV1 [112]. Thus, TREK-1 likely plays an important role in membrane excitability in sensory afferents, and its absence makes nociceptors more readily depolarized.

Members of the degenerin family of epithelial sodium channels (DEG/ENaC) are involved in many aspects of sensory signaling, including mechanosensory responses in the touch cells of *C. elegans*, responses in mammalian mechanoreceptors, salt and sour taste in mice, and proton responses in sensory afferents [113–116]. In regard to thermosensory signaling, in vitro recordings find that many of these channels are potentiated by cold temperatures [117]. The constitutively active epithelial ENaC channel is markedly potentiated by cold temperatures with a half-maximal response near 25°C. Three other channels of this family, which are acid-gated, including the DRG-specific DRASIC, were potentiated by cooling by slowing channel inactivation [117]. However, thermosensation deficits in mice null for these channels have yet to be reported [118, 119].

Conclusions

Although tremendous strides have been made in our understanding of the neurons and molecules involved in thermosensation, many of the processes used by various organisms to detect thermal change remain enigmatic. In the nematode, two key thermosensor neurons have yet to be identified, namely, the sensory neuron presynaptic to AIZ, and the nociceptor(s) that is(are) responsible for the response to noxious heat. The identification of these neurons will undoubtedly lead to the discovery of new molecular players in *C. elegans* thermosensation. In the fruit fly, it is still not clear if Painless is an obligatory detector of thermal and mechanical stimuli or if it is a crucial player downstream of an unknown "sensor" in the signal transduction cascade of moderately noxious heat and force. Moreover, the identities of the molecules that mediate the response to high threshold noxious heat in *Drosophila* larvae remain unclear. In mammalian systems, the roles of TRPV2 and TRPM8 in thermosensation still need to be evaluated in vivo, as well as how TRPV3 or TRPV4-mediated changes in keratinocyte activity are transduced to the central nervous system. These are only a few examples of the outstanding questions in the field and many others can be posed. However, if the pace of research that has led to the seminal and exciting discoveries described in this paper continues, answers to these and other fundamental questions will undoubtedly arise shortly.

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