Rodolfo Madrid · Juan Bacigalupo
Editors

TRP Channels in Sensory Transduction

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Preface

In 1969, Cosens and Manning found a spontaneous mutation in *Drosophila melanogaster* that was revealed by a defect in the electroretinogram, in which the steady electrical response normally evoked by a sustained light pulse becomes transient. This impairment occurred in the receptor potential of the photoreceptor cells. For this reason, the mutant was termed Transient Receptor Potential, TRP. In 1989, Montell and Rubin cloned the gene and proposed that it encoded a novel ion channel, an idea that was confirmed in the following years by several laboratories. A second channel was also identified in the same cells by Phillips and coworkers in 1992, and was called TRP-like (TRPL), for its similarity with TRP. The first mammalian TRP channel sequence was reported independently in 1995 by the Montell and Birnbaumer laboratories, and it is known today as TRPC1, one of the seven members of the Canonical TRP (TRPC) channel subfamily, which also includes *Drosophila* TRP and TRPL. After the molecular cloning of TRPV1, the first member of the Vanilloid subfamily of TRP channels by David Julius and his group in 1997, our understanding of the molecular and cellular mechanisms underlying sensory transduction has made dramatic progress. Since then, a vast number of publications have accumulated in the literature regarding this remarkable ion channel superfamily.

The TRP superfamily is divided into seven subfamilies: TRPC (seven members in mammals with the two closely related fly channels, TRP and TRPL), TRPV (Vanillod, six members), TRPM (Melastatin, eight members), TRPN (name derived from the fly mutant no mechanoreceptor potential C, one member), TRPA (Ankyrin, one member), TRPP (polycystic, three members) and TRPML (Mucophilin, three members) (See the phylogenetic tree of TRP channels in Chap. 1 of this book, Fig. 1.2). TRP channels are key molecular components of many physiological processes. One of the most salient features of the TRP channels is the paramount role that they play in a wide variety of sensory modalities, underlying the receptor potential in the corresponding primary sensory neurons. They participate in photo-, chemo-, thermo-, mechano- and osmoreception, pain and itching perception, and other sensory reception modalities. It is also noteworthy that, contrary to channels belonging to other superfamilies, TRP channels exhibit an impressive structural diversity, a characteristic that has contributed to complicate their classification; nev-

ertheless, the large majority of these channels possess six transmembrane domains, reminiscent of many voltage-dependent channels. Although some of these channels appear to respond mainly to a single stimulus type, many of them are polymodal, capable of being gated by stimuli of entirely unrelated nature, such as natural and artificial compounds, voltage and temperature, as is the case of the eleven thermoTRP channels that has been described. All TRPs are cation selective, many of them for monovalents, several additionally let Ca^{2+} through, while a few are strictly Ca^{2+} selective.

So far, only a small number of TRPs have been studied in some detail in intact sensory structures. An important factor for this is that they are usually confined to small and often inaccessible cellular compartments specialized in sensory transduction, such as microvilli, cilia and nerve terminals. Heterologous expression has been a powerful strategy for the study of TRP channels, mainly because it circumvents the difficulty of accessing their native location in their respective sensory cells. However, this approach must be taken with caution, because the functional properties of the channels may be largely modified when expressed in a foreign membrane, as observed in several cases (as for example *Drosophila* TRP and TRPL; Chap. 4 of this book).

This book comprises ten chapters written by experienced researchers in the field of sensory transduction, and particularly TRP channels. The origin of the present book was a symposium on TRP channels and sensory transduction, organized by the Editors for the First Meeting of FALAN (Federation of Neuroscience Societies in Latin America, the Caribbean and the Iberian Peninsula) in Cancún, México, in 2012. This symposium interested Springer NY, which kindly offered us to carry out this book.

The aim of this book is to offer up-to-date discussions regarding some iconic members of this highly interesting ion channel superfamily, which additionally are among those that are best characterized. Rather than extensive and exhaustive, the chapters attempt to be comprehensive to a non-specialized audience, as well as informative to those in the field. Inevitably, for instructive purposes, a small number of technical issues treated in a formal manner were included in some chapters. We felt that these formalisms were necessary for the comprehension of the thermodynamics and dynamic modeling of thermoTRPs.

We would like to thank the authors for their excellent contributions that made this book possible. The editors also thank Simina Calin and Portia Wong, Editors of Springer NY, for efficient guidance, support and excellent editorial advice.

We hope that the book will be found useful for Neurobiology students, teachers and specialist in sensory transduction. Enjoy to this TRiP to the Senses.

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Chapter 1
Biophysical and Molecular Features of Thermosensitive TRP Channels Involved in Sensory Transduction

Gonzalo Ferreira, Natalia Raddatz, Yenisleidy Lorenzo, Carlos González and Ramón Latorre

Abstract Temperature is one of the physical variables that cells and biological organisms constantly monitor to achieve homeostasis and maintain chemical reactions at a suitable speed for the living environment to which they are adapted. In order to monitor and maintain temperature on a constant basis, thermosensitive molecules were selected during evolution. One of the most remarkable sets of molecules acting as sensors is constituted by thermosensitive transient receptor potential channels (thermoTRP channels). TRP channels are a superfamily of non-selective tetrameric cation channels closely related to the classic superfamily of voltage-gated channels, having a set of distinctive sequence elements in common, while acting as polymodal receptors. This latter ability is what makes them suitable for integrating many kinds of signals in different cells, ranging from chemical to physical stimulation (*i.e.*: temperature-, mechano- and chemo-sensitivity). These channels act as allosteric proteins modifying sensitivity to one stimulus in the presence of another, and thus allowing the integration of many different signaling processes that are critical for sensing the extracellular and intracellular environment and for maintaining homeostasis. This ability has made them vital for life support. Several subfamilies of TRP channels have been described. From these subfamilies, some types of channels have been distinguished as being temperature-sensitive, such as TRPV1–4, TRPM 2–5/8, TRPA1 and TRPC5. In this chapter, thermosensitivity will be defined. Then, we will describe the thermosensitive molecules identified so far, focusing our analysis on ion channels, particularly on thermosensitive TRP channels involved in sensory transduction. Their gating and permeation properties and

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gating modifiers shall be at the center of the discussion so as to place them in the context of ion channels and life evolution.

Keywords ThermoTRP channels · Biophysics of TRP channels · Sensory transduction

1.1 Thermosensitivity in Organisms and Across Species

Thermosensitivity is the property of inorganic and organic materials to respond to changes in heat or temperature faster than the average speed of response observed in comparable kinds of substances found in nature. In this chapter, we will focus on the thermosensitivity found in living organic materials, particularly in organisms, cells and molecules.

Thermal variation is among the oldest types of stress that biological beings have been subjected to, and adaptation responses to it usually involve certain pathways conserved through evolution. Regarding organisms, thermosensitivity can be found in eukaryotes and prokaryotes. In complex eukaryotes, total and local body temperature control has been measured in birds and mammals, where it has been found that total body thermosensitivity generally varies between −4 and −12 W/(kg°C) (Mercer and Simon 1984). In reptiles and fish, it has been reported that sexual determination is especially thermosensitive (Baroiller and D'Cotta 2001; Pieau et al. 2001). Whole body temperature adjustment, in particular, has been related to neurons of the central nervous system and sensing in peripheral receptors (Hori and Katafuchi 1998). Phenomena related to cold and heat shock genes that are expressed with sudden changes in temperature have been extensively reported and studied in prokaryotes (Hurme and Rhen 1998). It is interesting that, in general, such responses seen in prokaryotes are conserved among more complex organisms such as eukaryotes (Al-Fageeh and Smales 2006).

1.1.1 A Biophysical Description of Thermosensitivity

Thermosensitivity for whole organisms or materials is physically expressed by specific heat units. Usually, the thermosensitivity of molecules or biological processes is measured by its Q_{10} (or temperature coefficient), which is a measure of the rate of change in biological or chemical systems resulting from increasing the temperature by 10 °C. In general, it is calculated as follows:

$$Q_{10} = \left(\frac{k_1}{k_2}\right)^{\frac{10}{(T_1 - T_2)}} \tag{1.1}$$

where k_1 and k_2 are the rate constants of the reaction proportional to the rates of the process at two different temperatures (T_1 and T_2), normally using the Kelvin

scale. Q_{10} for most biological systems and molecules measured in physiological conditions is generally between 2 and 3, whereas for thermosensitive molecules or biological processes it is usually more than 5 (Nelson et al. 2008). However, Q_{10} has several limitations in measuring thermosensitivity since it is quite dependent on the ratio of temperatures used to measure it.

A more accurate description of thermosensitivity and the dependence of reaction rates on temperature are related to Arrhenius energy of activation, which gives us a relationship between the kinetic constants of chemical reactions and the energy activation:

$$k_x = Ae\frac{-Ea}{RT} \tag{1.2}$$

where k_x is the velocity constant, A is the prefactor, being a constant related to the frequency of molecular collisions, Ea is the energy of activation, R is the gas constant and T is absolute temperature.

From Eqs. (1.1) and (1.2), we have:

$$Ea = -RT_1\left(\frac{T_1 + 10}{10}\right)\ln Q_{10} \tag{1.3}$$

Thus,a thermosensitive molecule with a $Q_{10} \sim 10$ implies an $Ea \sim 40$ cal/mol at a temperature of 298 K.

In transition state theory, activation energy by Arrhenius can be somehow related to the following equation:

$$k = -\kappa\frac{k_B T}{h}e - \frac{\Delta G^*}{RT} \tag{1.4}$$

where κ is the transmission factor, $\frac{k_B T}{h}$ is the frequency factor (also called the pre-exponential factor) which has the units of s^{-1} and ΔG^* is the standard Gibbs energy of activation. As it can be seen, it is similar to Eq. 1.2 derived from Arrhenius statements for the whole reaction, with $A = \kappa\frac{k_B T}{h}$. Taking into account that:

$$\Delta G^* = \Delta H^* - T\Delta S^* \tag{1.5}$$

where ΔH^* is the enthalpy change, T is the absolute temperature and ΔS^* is the entropy change, we reach the following relationship between the energy of activation Ea from Arrhenius, the transition state theory (Eq. 1.5):

$$Ea = \Delta H^* - T\Delta S^* \tag{1.6}$$

Therefore, a molecule will be more thermosensitive when the enthalpy differences to reach the active state at two given temperatures are higher. Keeping in mind that enthalpy and entropy are functions of temperature as well, a general expression

for Gibbs free energy at a given condition would be as follows (Digel et al. 2008; Nelson et al. 2008):

$$\Delta G = \Delta H_{(TR)} - T\Delta S_{(TR)} + \Delta C_p (T - TR) + \Delta\Delta C_p \ln\left(\frac{T}{TR}\right) \quad (1.7)$$

where TR is an arbitrary reference temperature and ΔC_p is the heat capacity change for a particular protein or molecule between temperature T and the reference temperature TR. This equation allows us to know the limits of protein stability when there are temperature changes (heat or cold).

The temperature dependence of ΔH and ΔS is usually parabolic. This will be quite evident when C_p is high, meaning that in those cases we might find denaturation by hot and cold temperatures as extreme examples of variations in molecule conformation due to temperature. Most interactions that yield particular conformations at physiological temperatures and that stabilize proteins in their native environments are weak non-covalent bonds (Petsko and Ringe 2004).

Thermosensitive molecules could also react to other physical parameters (i.e., voltage, mechanics, degrees of hydration, etc.). Allosteric interactions between the variables that control protein conformations would arise quite easily. Hence, any attempt to construct gating kinetic models for thermosensitive channels should be allosteric (Matta and Ahern 2007; Latorre et al. 2007a, b; Baez-Nieto et al. 2011; Jara-Oseguera and Islas 2013).

1.1.2 Thermosensitive Molecules and Sensory Transduction

Temperature can promote dramatic changes in cells ranging from membrane fluidity, DNA-RNA processes and structure-catalysis from enzymes (Digel et al. 2008). All these elements and their modifications due to heat/temperature can serve as molecular sensors for thermosensitivity. Regarding *membranes*, phase-transition changes occur with sudden transitions in temperature, which might influence gene expression (Vigh et al. 1998). Changes in membrane fluidity due to temperature can trigger the expression of enzymes that would modify molecular composition.

Alterations in *nucleic acids* seem to constitute a more efficient biomolecular sensor for temperature changes and gene expression. The 3D architecture of RNA has been recently described as a critical sensor event, being part of what is known as *riboswitches* (Mandal and Breaker 2004). There are two regions in this complex ribosomic RNA constituent, namely the aptamer and expression platforms. The aptamer platform has been described to sense changes in molecules promoted by heat/temperature and, hence, to regulate gene expression at the translation level. Regarding DNA, the supercoiling level and topoisomerases appear to be critically sensitive to temperature (Tse-Dinh et al. 1997).

Ultimately, the critical biomolecular targets for temperature control and sensing are *proteins*. In *E. coli*, chemoreceptor protein systems such as *Tar, Tsr, Trg and Tap*

have been described. What is interesting is that they share a significant degree of homology in spite of the different ranges in thermal sensitivity they all exhibit (Nara et al. 1991). In complex organisms, specialized ion channels are able to transduce heat/cold into channel opening. Sensory cells and several tissues express thermosensitive molecules, from which thermoTRP channels are a clear example and are directly or indirectly involved in the homeostasis of temperature as a physiological phenomenon (Digel et al. 2008).

Here, we will discuss these concepts in molecules, with a particular emphasis on ion channels as specialized sensors and effectors that have evolved to sense and control heat and temperature in living organisms. In particular, we will center our discussion on thermosensitive TRP channels, which play a critical role in sensory thermotransduction-

1.2 Thermosensitive Channels and Thermal Transduction

Our skin is a physical barrier that, among other functions, enables us to discriminate temperatures ranging from extreme cold (about −10 °C) to extreme heat (about 60 °C). The neurons that allow us to sense this wide range of temperatures are located in the trigeminal ganglia (Walder et al. 2002) innervating the face and head and in the dorsal root ganglia (DRG) for the rest of the body. The process of thermosensation begins with specialized proteins which are expressed in the free nerve endings of the afferent fibers innervating the skin and respond to external temperature changes.

All proteins including ion channels and indeed all biological or chemical processes are sensitive to temperature with a Q_{10} value of ~2 (Hille 2001). However, thermo-TRP receptor channels present an exceptionally high Q_{10} value ≥20. To date, at least 10 TRP channels have been observed to exhibit steep temperatures, namely TRPV1, TRPV2, TRPV3, TRPV4, TRPM2, TRPM3, TRPM4 and TRPM5, which are activated upon warming, and TRPM8, TRPA1 and TRPC5, which are activated upon cooling (Vriens et al.; Caterina et al. 1997, 1999; McKemy 2002; Peier 2002a, b; Smith et al. 2002; Watanabe et al. 2002b; Xu et al. 2002; Chung et al. 2003; Story et al. 2003; Talavera et al. 2005; Togashi et al. 2006; Karashima et al. 2009; Zimmermann et al. 2011). They show different activation thresholds covering the range of thermal oscillations to which living organisms are usually exposed. For example, TRPV1 is activated by temperatures in the noxious range (>42 °C), whereas TRPM8 in neurons is mainly closed at temperatures >33 °C and is primarily open at lower temperatures, allowing sodium and calcium ions to flow into the nerve terminals leading to depolarization, which in turn triggers action potential firing (Babes 2009).

Madrid and coworkers (Madrid et al. 2009), found that different expression ratios between TRPM8 and Kv1 tune cold sensitivity in cold thermoreceptor neurons. They pointed out that low cold thresholds can be produced by high TRPM8 expression levels and low or absent expression of I_{KD} a (a current dependent on Shaker-like Kv1.1–1.2 channels that act as an excitability brake). In contrast, specific high-

threshold cold neurons would result in low TRPM8 levels and high levels of I_{KD} expression (Madrid et al. 2009). Thus, apparently TRPM8 and I_{KD} play a critical role in determining the thermal excitability of cold-sensitive neurons (See Chap. 9 by Gonzalez et al. in this book).

In addition to TRPs, other ion channels participate in thermal transduction. Leak or background potassium channels, namely TREK-1, TREK-2 and TRAAK, members of the two-pore domain K^+ (K2P) channel family, have been shown to be sensitive to temperature in different expression systems such as *Xenopus oocytes* and COS cells (Kang 2005). TREK-1 has shown low activity at room temperature, which increases with rising temperatures and reaches maximum activation at 37 °C (Maingret et al. 2000). This suggests that TREK-1 is active at body temperature, thus contributing significantly to background K^+ conductance in physiological conditions. Moreover, TREK-2 and TRAAK whole-cell currents show a ~20-fold increase when bath temperature rises from 24 to 42 °C, with activation thresholds of 25 and 31 °C, respectively (Kang et al. 2005). These channels tend to hyperpolarize neurons, closing rapidly and reversibly with small temperature reductions.

Finally, hyperpolarization-activated nonselective cationic channels of the HCN family, specifically HCN1, play an important role in determining the firing patterns of cold receptors in mammals, hence contributing to the ability of neurons to respond to stimuli with high frequency bursts (Babes 2009). Although it does not seem to be involved in responses to acute cooling, mice lacking the *hcn1* gene show altered cold perceptions (Orio et al. 2009). Accordingly, an ample group of thermosensitive ion channels has been discovered but further studies are still required to better understand the global phenomenon of thermo-transduction.

1.3 TRP Channels

1.3.1 A Brief History of TRP Channels

*T*ransient *R*eceptor *P*otential genes (*trp*) were first described in the late 1960s in the fruit fly *Drosophila melanogaster* (Cosens and Manning 1969). The name is derived from a spontaneously occurring mutation in the *Drosophila* fly, resulting in visual transduction defects. The eyes of these mutant flies elicit a transient electric response to continuous light instead of a continuous response, as it is the case of the wild type flies. Twenty years later, it was reported that the *trp* gene encodes a large protein, namely the TRP channel, consisting of 1275 amino acid residues displaying eight putative transmembrane (TM) segments (Montell and Rubin 1989). In addition, the amino acid sequence did not present clear homologies with any other known ion channel.

Just 3 years later, Phillips et al. (1992) found a new gene that was named TRP-like (*trpl*). The protein encoded by *trpl* showed structural homologies with *Drosophila TRP* and also with the superfamily of voltage-gated channel genes (Phillips et al. 1992). In the same year, Hardie and Minke demonstrated that the *TRP* gene

coded for a highly Ca^{2+} permeable channel (Hardie and Minke 1992). In 1995, Petersen revealed that TRP related proteins were also present in vertebrates, such as *Xenopus laevis* and mice cDNA libraries (Petersen et al. 1995), while Wes and Zhu reported the full sequence of the first human homologue, TRPC1, which shares 37 % identity with the *Drosophila trp* gene (Wes et al. 1995; Zhu et al. 1995). Today, we can list more than 100 channels within this family in both invertebrates and vertebrates (Nilius and Owsianik 2011).

1.3.2 The Superfamily of TRP Channels and Thermosensitive TRP Channels

The TRP superfamily comprises a large number of ion channels with diverse functions, serving as cellular sensors of physical and chemical stimuli such as light, temperature, voltage, touch, pain, osmolarity, pheromones, phosphatidylinositol 4,5-bisphosphate (PIP_2), Ca^{2+}, cyclic nucleotides, among others (Clapham et al. 2001; Clapham 2003; Ramsey et al. 2006). For example, TRPV1 responds to heat, capsaicin and low pH, while TRPM8 is activated by menthol and cold. Hence, TRP channels are involved in the classic sensory transduction processes of multicellular organisms, such as vision, hearing, smell, taste, touch, temperature, and pain sensation. The division into subfamilies on the basis of amino acid sequences and structural similarities does not provide a functional classification for TRP proteins. For example, some members of the TRPV subfamily are involved in thermal and nociceptive sensing (TRPV1–4), whereas others are insensible to temperature but are highly selective to Ca^{2+}, thus participating in the epithelial Ca^{2+} transport (TRPV5–6).

Structurally, all members of the TRP family include six putative transmembrane domains, cytosolic N- and C-terminal tails and an ion pore between transmembrane domains 5 and 6 (Montell 2005) (Fig. 1.1). According to their amino acid sequence homology, TRP channels are grouped into seven subfamilies which, in turn, are classified into groups 1 (TRPC, TRPV, TRPM, TRPA, and TRPN) and 2 (TRPPP and TRPML) based on sequence and topological differences (Montell et al. 2002a). Group 1 of TRPs shows the strongest sequence homology with the founding member of the superfamily, *Drosophila* TRP (Montell and Rubin 1989). Group 2, on the other hand, includes distant relatives that share sequence homologies over the transmembrane segments and contain a large loop separating the first two transmembrane domains. An eighth subfamily (TRPY, where Y stands for *yeast* TRPs) is distantly related to Group 1 and Group 2 (Palmer et al. 2001; Denis and Cyert 2002).

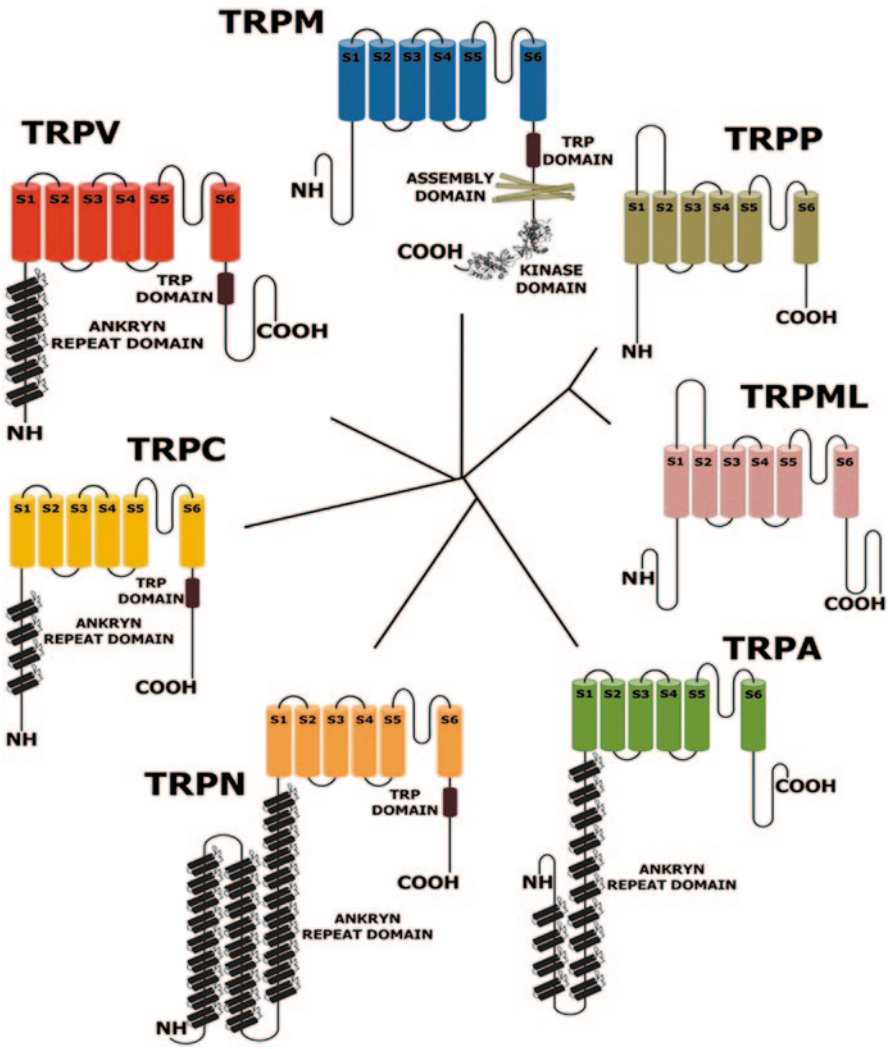

Fig. 1.1 TRP channel subfamilies and schematic structure of their major subunits. TRP channels present four subunits containing six transmembrane (*TM*) segments (S1–S6). Ankyrin domains (number variable) are present in the N-terminal region of TRP channels in Group 1 subfamilies. The TRP domain (well-conserved region) and protein kinase domain are present in the C terminus region

1.3.2.1 Group Subfamilies

Group 1 Subfamilies

TRPC (classic) TRPC channels are ubiquitously expressed among cell types and mediate signals in response to phospholipase C (PLC)-coupled receptors (Clapham 2003; Montell 2005). They have four N-terminal ankyrin-like repeat domains

(ARD), a TRP box, a homer-binding region, a calmodulin IP_3-receptor binding site (CIRB) and a PDZ domain in TRPC4-5. The first mammalian homologues of *Drosophila* TRPs were referred to as TRPC1, TRPC2, and TRPC3, which share 40 % homology (Wes et al. 1995; Zhu et al. 1995). Since then, seven mammalian TRPC proteins (TRPC1-7) have been described (Montell et al. 2002b). Their subunits can assemble into homomeric and heteromeric channels (Goel et al. 2002; Hofmann et al. 2002; Strubing et al. 2003; Schaefer 2005), and they have been proposed to be activated by a variety of signals including Ca^{2+} store depletion, membrane lipids and vesicular insertion into the plasma membrane.

TRPV (vanilloid) The TRPV subfamily of ion channels contains three ankyrin repeats, a TRP box and calmodulin binding site. It consists of six members, of which TRPV1-4 are temperature-sensitive non-selective cation channels, and TRPV5–6 are channels insensitive to temperature and the most Ca^{2+} selective ($P_{Ca2+}/P_{Na+} > 100$) channels among mammalian TRPs (Montell 2005). Mammalian channels TRPV1, TRPV2 and TRPV4 are also involved in sensing osmolarity changes and mechanical stimuli (Kim et al. 2003; Gong et al. 2004).

TRPM (melastatin-related) TRPM channels share four regions of high homology (TRPM homology regions—MHRs) in their N-terminal cytoplasmic segment. The TRPM subfamily contains eight members (TRPM1–8) that share a cytoplasmic coiled-coil domain and a well-conserved TRP box, but have diverse C-terminal domains with structures that are important in controlling the ion channel activation mechanism. TRPM6/7 and TRPM2 are designated as chanzymes, since their C-terminal contains catalytic domains that show protein kinase activities and ADP-ribose pyrophosphatase activities, respectively (Nadler et al. 2001; Perraud et al. 2001; Runnels et al. 2001; Sano et al. 2001; Schlingmann et al. 2002; Walder et al. 2002). TRPM2–5 are heat activated, while TRPM8 is activated by cold temperature. In contrast to all other TRPs, both TRPM4 and 5 form ion channels permeable to monovalent cations but not to Ca^{2+} (Launay et al. 2002; Hofmann et al. 2003; Nilius et al. 2003). TRPM3 has been reported to be activated by noxious heat (Vriens et al. 2011) and by reductions in extracellular osmolarity in HEK-293 cells (Grimm et al. 2003).

TRPA (ankyrin) This family includes only one member present in humans and mammals, namely TRPA1. The N-terminal region of the TRPA1 protein comprises 17 ankyrin repeats, which are common structural motifs that mediate protein–protein interactions (Gaudet 2008). It is expressed in primary somatosensory neurons (30 % co-expressed with TRPV1) , keratinocytes, astrocytes, vascular smooth muscle and endothelium of the cardiovascular system, gastrointestinal tract, respiratory system, pancreas, inner ear and odontoblasts. It is activated by different chemical compounds, including the psychoactive components from marijuana (i.e., delta-9-tetrahydrocannabinol), environmental irritants, products from inflammatory responses and pungent compounds of wasabi, garlic (allicin), cinnamon oil (cinnamaldehyde), mustard oils (allyl isothiocyanate) and tear gas (acrolein) (Jordt et al. 2004). TRPA1 has been reported to be a cold receptor involved in the detection of noxious cold and hypersensitivity to cold (Story et al. 2003; del Camino et al. 2010) (see below).

TRPN (no mechanoreceptor potential C [NOMP-C homologues]) This family has a single member that can be found in worms, flies, and zebrafish (Littleton and Ganetzky 2000; Walker et al. 2000; Sidi et al. 2003). Mammals do not encode any TRPN homologues. TRPNs lack the TRP domain, but they do have the conserved TRP-box. Apparently they have a role in mechanotransduction (Walker et al. 2000).

Group 2 Subfamilies

TRPP (polycystin) The TRPP subfamily is formed by polycystic kidney disease (PKD) proteins or polycystins. It is formed by three members, namely TRPP2 (also known as PKD2), TRPP3 (or PKD2L1), and TRPP5 (or PKD2L2). TRPP channels carry an EF-hand motif (structural domain in proteins that bind Ca^{2+}) and an ER retention signal. TRPP channels are present in both motile and primary cilia, and may function to sense fluid flow, osmolarity, and mechanical stretch (Stayner and Zhou 2001; Nauli et al. 2003; Venkatachalam and Montell 2007).

TRPML (mucolipin) The TRPML subfamily is defined by a human protein (i.e., TRPML1) that forms a non-selective Ca^{2+} channel (Bargal et al. 2000; Bassi et al. 2000; Sun et al. 2000). Mutations in this protein are responsible for the lysosomal storage disorder *mucolipidosis IV*, which is characterized by severe neurodegeneration. In addition to TRPML1, mammals encode two other closely related proteins referred to as TRPML2 and TRPML3. TRPML1 and TRPML2 are expressed in the lysosomal membrane, while TRPML3 resides predominantly in the endoplasmic reticulum (ER) membrane in cultured cells (Manzoni et al. 2004; Kiselyov et al. 2005; Venkatachalam et al. 2006).

1.3.2.2 ThermoTRP Channels

Cloning and characterizations of a temperature-sensitive subclass of the TRP channels (i.e., thermoTRPs) have shed light on our understanding of the molecular basis of thermal sensation. As we mentioned before, to date, ten TRP channels have been reported to be directly activated at specific temperature ranges covering from noxious heat to painful cold. TRPV1, TRPV2, TRPV3 and TRPV4 are heat-activated, whereas TRPM8, TRPA1 and TRPC5 are activated by cold (McKemy et al. 2002; Peier et al. 2002a; Story et al. 2003; Talavera et al. 2005; Dhaka et al. 2006; Huang et al. 2006; Togashi et al. 2006; Zimmermann et al. 2011) (see Fig. 1.2). TRPM2, TRPM3, TRPM4 and TRPM5 also show temperature sensitivity but are not usually included in the thermo-TRP family because they are not expressed in primary somatosensory neurons and need adequate concentrations of intracellular Ca^{2+} to exhibit temperature sensitivity. Nevertheless, the fact that they can be temperature sensitive with an elevated Q_{10} in particular conditions has led us to include them in this chapter.

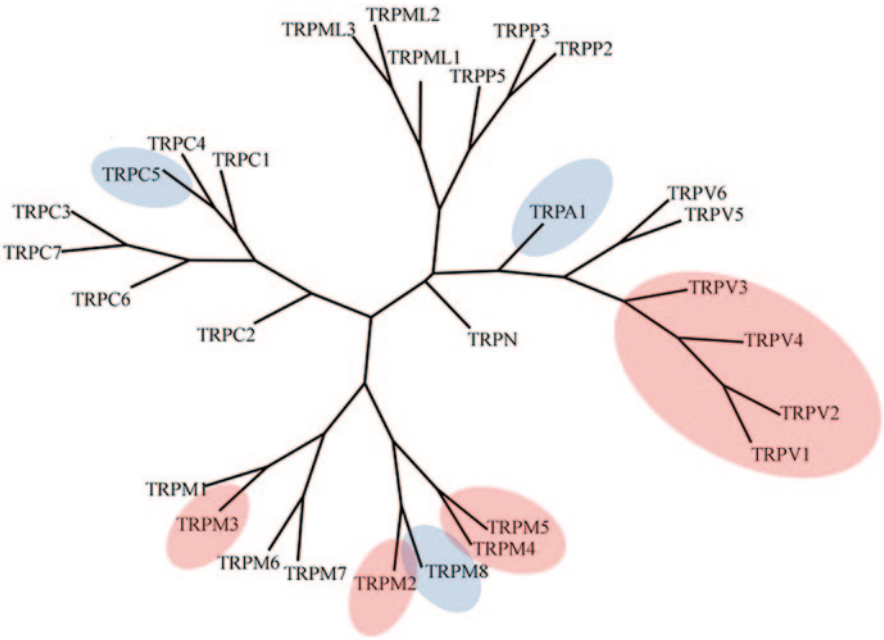

Fig. 1.2 Phylogenetic tree of TRP channels. ThermoTRP channels are indicated according to thermosensitivity: cold (*blue*) and heat (Zaritsky et al. 2001). Dendrogram was based on full length sequence comparisons of human TRP channel protein using the UniProt services website: TRPV1 (Q8NER1); TRPV2 (Q9Y5S1); TRPV3 (Q8NET8); TRPV4 (Q9HBA0); TRPV5 (Q9NQA5); TRPV6 (Q9H1D0); TRPM1 (Q7Z4N2); TRPM2 (O94759); TRPM3 (A2A3F7); TRPM4 (Q8TD43); TRPM5 (Q9NZQ8); TRPM6 (Q9BX84); TRPM7 (Q96QT4); TRPM8 (Q7Z2W7); TRPC1 (P48995); TRPC3 (Q13507); TRPC4 (Q9UBN4); TRPC5 (Q9UL62); TRPC6 (Q9Y210); TRPC7 (Q9HCX4); TRPML1 (Q9GZU1); TRPML2 (Q8IZK6); TRPML3 (Q8TDD5); TRPP2 (Q13563); TRPP3 (Q9P0L9); TRPP5 (Q9NZM6); TRPA1 (O75762). TRPC2 is a pseudogene in humans and TRPNs are not present in mammals; therefore we used mouse TRPC2 (Q9R244) and zebrafish TRPN (Q7T1G6)

TRPV1 This TRP channel was first characterized by McNaugthon and his group (Cesare and McNaughton 1996), and was subsequently cloned by the group led by David Julius (Caterina et al. 1997). It is a non-selective cation permeable channel activated by a wide range of stimuli including capsaicin and low pH (< 6), and by physical factors such as heat and membrane depolarization (Caterina et al. 1997; Tominaga et al. 1998; Voets et al. 2004). Activation threshold by heat is in the range of 41–43 °C, likely reaching a maximum over 50 °C. This channel can be directly activated by ethanol (Trevisani et al. 2002), several endogenous lipids (i.e., endocannabinoid, anandamide and N-arachidonoyl dopamine) (Ross 2003; De Petrocellis et al. 2004), metabolic products of lipoxygenase (Hwang et al. 2000), topical analgesics such as camphor (Xu et al. 2005), and pungent compounds present in black pepper (piperine) (McNamara et al. 2005) and garlic (allicin) (Macpherson et al. 2005; Salazar et al. 2008). Consequently, TRPV1 is viewed as a signaling integrator for many noxious stimuli.

TRPV2 Although this channel shares 50% of sequence homology with TRPV1, it is insensitive to capsaicin, low pH and responds to higher temperatures with an activation threshold of 52 °C (Caterina et al. 1999). TRPV2 is activated in vitro by three different types of physical stimuli: heat, osmotic stress and mechanical stretch (Caterina et al. 1999; Muraki et al. 2003). It is also activated by chemicals such as 2-aminoethoxydiphenyl borate (2-APB) (Hu et al. 2004) and by cannabidiol, a natural component of the marijuana plant (Qin et al. 2008). The physiological role of TRPV2 in thermal nociception remains unknown because TRPV2$^{-/-}$ mice showed no evident deficits in thermal sensation (Park et al. 2011).

TRPV3 This ion channel is implicated in the perception of warmth in the skin with an activation threshold of 34–38 °C (Peier et al. 2002b; Smith et al. 2002; Xu et al. 2002). TRPV3 is activated by 2-APB (Chung et al. 2004) and by various natural compounds such as carvacrol, thymol and eugenol, which are present in essential oils from herbs and spices such as *Thymus vulgaris*, *Origanum syriacum*, and *Caryophilli flos* (Moqrich et al. 2005; Xu et al. 2006). TRPV3$^{-/-}$ mice showed some deficits in sensing hot temperatures in the innocuous to noxious range (Moqrich et al. 2005).

TRPV4 TRPV4 is a non-selective cation channel that shares ~ 40% amino acid identity with TRPV1 (Liedtke et al. 2000; Strotmann et al. 2000; Delany et al. 2001). This thermo-TRP is activated by moderate temperatures (27–34 °C) (Watanabe et al. 2002b), by hypotonic solutions, suggesting that it serves as a sensor for osmolarity or mechanical stretch (Liedtke et al. 2000; Strotmann et al. 2000; Wissenbach et al. 2000; Delany et al. 2001; Alessandri-Haber et al. 2005), and by phorbol esters (Watanabe et al. 2002a). Knockout studies suggest that TRPV4 also plays a role in thermal hyperalgesia (Todaka et al. 2004).

TRPM2 TRPM2 channels are Ca^{2+} -selective TRP channels, modulated by ADP-ribose, intracellular Ca^{2+} and oxidative stress. In the presence of ADP-ribose and intracellular Ca^{2+}, they are heat-activated by warm temperatures. They are expressed mainly in the brain, pancreas and immune cells. As for most of the TRP channels, they also have several modulating pathways in addition to those just mentioned (Eisfeld and Luckhoff 2007).

TRPM3 TRPM3 channels are non-selective cation channels permeable to Ca^{2+}, which were classically described as osmo- and volume-regulated TRP channels, denoting properties in common with TRPV4 channels. They are mostly expressed in the kidney, neurons and eyes, and have several splice variants in the N and C-terminus plus the S1-S6 region (some of them including the pore region) (Grimm et al. 2003; Oberwinkler and Phillipp 2007). These channels are activated by noxious heat (> 40 °C) and are also strongly associated and modulated by steroids (pregnenolone sulfate), acting as membrane receptors for these lipids (Wagner et al. 2008).

TRPM4 and TRPM5 Both are non-selective Ca^{2+}-activated cation channels. They also share 50% of their sequences, being modulated by similar mechanisms. Sev-

eral splice variants have been identified for TRPM4 channels (a, b and c), being TRPM4b the most widespread. They are both heat-activated in a warm temperature range and in the presence of intracellular Ca^{2+}. Just like most TRP channels, their function is modulated by a wide range of extra and intracellular ligands. Whereas TRPM4s are widely expressed throughout the organism, TRPM5s appear to be confined mostly to taste receptors (Talavera et al. 2005; Guinamard et al. 2011).

TRPM8 TRPM8 was the first TRP channels found to sense cold temperatures (McKemy 2002; Peier 2002a). Activation is measurable at ~ 33 °C in sensory neurons, with currents increasing in magnitude at lower temperatures (McKemy et al. 2002; Peier 2002a; Brauchi 2004; Brauchi et al. 2004; Madrid et al. 2006; Bautista et al. 2007; Malkia et al. 2007). Furthermore, this channel is activated by voltage (Brauchi 2004; Voets et al. 2004) and chemical agonists that mimic cooling, such as menthol, eucalyptol or icilin (McKemy 2002; Peier 2002a; Chuang 2004; Bodding et al. 2007). TRPM8 is also activated by the regulatory lipid PIP_2 (Liu and Qin 2005; Rohacs et al. 2005). In vivo studies of $TRPM8^{-/-}$ mice showed deficits in reacting to cool temperatures, indicating that this channel is a major sensor of peripheral innocuous coolness (Bautista et al. 2007; Colburn et al. 2007; Dhaka et al. 2007).

TRPA1 TRPA1 is activated within the noxious cold range of temperatures (~≤17 °C), suggesting that it may be responsible for detecting painful levels of cold (Story et al. 2003). The active constituents of mustard oil (allyl isothionate) and garlic (allicin) robustly activate TRPA1 currents (Bandell et al. 2004; Jordt et al. 2004; Macpherson et al. 2005). TRPA1 is directly activated by Ca^{2+} binding to an EF-hand domain located within its intracellular N-terminal region. This suggested that apparent cold sensitivity may not result from direct temperature-sensitive gating, but instead from a cold induced increase in intracellular Ca^{2+}(Doerner et al. 2007; Zurborg et al. 2007). Furthermore, analyses of $TRPA1^{-/-}$ mice has yielded conflicting data, with one group reporting no deficits in acute cold sensing, while a second group observing reduced cold sensitivity in female but not in male mice, and a third group showing cold deficits after prolonged exposure to cold (Bautista et al. 2006; Kwan et al. 2006; Karashima et al. 2009). However, these apparent conflicting results are a consequence of species differences. Thus, cold activates rat and mouse TRPA1 but not human or rhesus monkey TRPA1 (Chen et al. 2013).

TRPC5 TRPC5 is cold-sensitive in the temperature range of 37–25 °C (Q_{10}~10). It is present in mouse and human sensory neurons of dorsal root ganglia and in the dorsal lamina of the spinal cord that receives sensory input from the skin (Zimmermann et al. 2011). It is modulated by receptors coupled to the phosphoinositoside signaling pathway. The use of ratiometric Ca^{2+} imaging of dissociated cultured DRG from WT and $TRPC5^{-/-}$ mice has demonstrated that TRPM8 and/or other menthol-sensitive channels appear to underpin a much larger component of noxious cold sensing after *TRPC5* deletion (Zimmermann et al. 2011).

1.3.3 Gating by Temperature and by Voltage in Thermosensitive TRP Channels

As seen above, temperature change in the appropriate range can activate eight heat-activated channels (TRPV1–4, TRPM2–5) and three cold-activated channels (TRPM8, TRPA1, TRPC5) (Clapham 2003; Jordt et al. 2003; Dhaka et al. 2006; Venkatachalam and Montell 2007; Zimmermann et al. 2011). What happens to these channels, however, when temperature changes? A possible answer can be found in the lipids tightly bound to the proteins or surrounding them, thus, these molecules could serve as temperature sensors converting thermal energy into protein conformational changes. However, TRPV1 and TRPM8 channels remain activated by temperature, following perturbations of membrane fluidity by either cholesterol changes (Liu et al. 2003) or by the reconstitution of TRPM8 and TRPV1 into planar lipid bilayer membranes and artificial liposomes, respectively (Zakharian et al. 2010; Cao et al. 2013a).

The key domains or residues for thermosensitivity have not yet been identified and the situation is at present quite unclear. Some studies reveal that the C-terminal of thermo TRPs may be a potential candidate because: (1) swapping the C-terminal domain of TRPV1 with TRPM8 exchanges their temperature sensitivity, channel gating kinetics and PIP_2 modulation (Brauchi et al. 2006, 2007); and (2) sequential deletions of the distal half C-terminal in TRPV1 were shown to shift the temperature threshold to lower temperatures (Vlachova 2003). On the other hand, the pore region has also been postulated as a potential temperature sensor, since it was found that point mutations in the vicinity of the pore region were able to significantly decrease TRPV1 and TRPV3 temperature sensitivity without affecting their activation by voltages (Grandl et al. 2008, 2010). Finally, the TRPV1 N-terminus and *Drosophila* TRPA1 have also been identified as possible thermosensitive structures. Specifically, temperature- dependence has been associated with regions flanking the N-terminal ARD (Cordero-Morales et al. 2011; Yao et al. 2011).

Several TRP channels have been reported to be voltage-sensitive, specifically TRPV1, TRPV3, TRPM8, and TRPM4 (Nilius et al. 2003, 2005b; Voets et al. 2004; Chung et al. 2005). Their voltage sensitivity is quite low in comparison to the classic voltage-gated channels, such as Shaker channels, where movement of S4 across the transmembrane electric field contributes to what would be equivalent to about 13 e_0 gating (Schoppa and Sigworth 1998). What temperature does is shift the voltage activation curve towards more negative voltages according to the heat or cold thermosensitivity of the channels with an unusually large Q_{10} for a protein. This general allosteric mechanism (similar to the role of intracellular Ca^{2+} for BK/Slo1 channels) is also modulated by many intra and extracellular ligands, making TRP channels special sensors and transducer molecules, where many signals can be integrated at the same time, thus behaving like polymodal receptors (Baez-Nieto et al. 2011).

TRPM8, for example, has an apparent gating charge of only 0.6–0.8 e_0. One possible explanation for this could be the scarcity of positive charges in the human TRPM8 S4 domain, having only one arginine residue on the S4 linker (R842) and one lysine on the S4-S5 linker (K856) (Voets 2007). This domain also contains a histidine residue that can be positively charged depending on its pK in the protein milieu. Voets (Voets 2007) designed different charge-neutralizing S4 and S4-S5 linkers for TRPM8, which reduce the channel's gating charge, indicating that this region would be part of the voltage sensor. Similar to TRPM8, TRPV1 presents an apparent gating charge of 0.5–0.7 e_0 (Brauchi 2004; Voets et al. 2004, 2007). This channel does not have charged residues in the predicted S4 segment; however it contains an arginine-glutamate pair in the S2 segment (R474 and E478 in rat TRPV1).

As can be seen, there seems to be no consensus regarding the localization of the temperature and voltage sensors. It is clear, however, that they both reside in different channel molecular structures, because it is possible to eliminate the temperature sensitivity of a channel without altering its voltage sensitivity and vice versa (Boukalova et al. 2010; Yang et al. 2010).

1.3.4 Allosteric-Polymodal Gating in ThermoTRP Channels

In the last decade, significant progress has been made in the biophysical and thermodynamic characterization of some thermoTRP channels, especially of TRPV1 and TRPM8 (Brauchi et al. 2004; Voets et al. 2004; Hui et al. 2005; Matta and Ahern 2007). Nevertheless, there is still controversy as to which kinetic model best explains the behavior of these channels. While Voets points out that the two-state model represents a good approximation to describe TRPM8 and TRPV1 temperature and voltage gating (Voets et al. 2004, 2007), analyses of conductance-voltage curves or of single channel data (Fernandez et al. 2011) indicate that this model is an oversimplification. Brauchi made the simplifying assumption that voltage and temperature sensors move in concert, giving rise to a cubic eight-state kinetic model (Horrigan and Aldrich 2002; Brauchi 2004; Latorre et al. 2007a).

The term *allosteric* is quite popular in enzyme kinetics. A typical way of using it in this sense is when a ligand binds to a regulatory site of an enzyme and alters the form of the protein, resulting in changes in enzyme effectiveness. The regulatory site in the protein can be altogether different from the catalytic site, where the chemistry takes place. Thus, to explain TRP channel data (mainly for TRPM8 and TRPV1), an allosteric activation mechanism that involves the following three separate two-state equilibria has been used: voltage sensor activation, temperature sensor activation, channel opening, as well as some interactions among these three processes (Fig. 1.3).

One of the key features that defines the behavior of TRPM8 and TRPV1 channels is that neither temperature nor voltage would be strictly necessary for channel

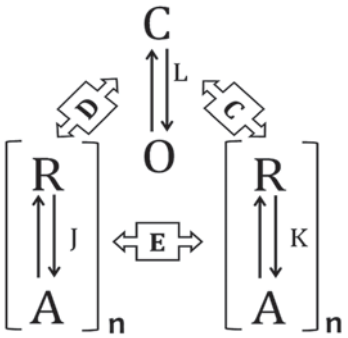

Fig. 1.3 Allosteric kinetic model of thermo-RP channel gating. The channel undergoes transitions between closed (*C*) and open (*O*) states with an equilibrium constant *L*, being the voltage and temperature sensors in resting condition. In addition, the sensors undergo transitions between resting (*R*) ((Q9BX84); TRPM7 (Q96QT4); TRPM8 (Q7Z2W7). Alignments were performed with the cluster method using the UniProt service (ClustalO).) and activated (*A*) states with equilibrium constants *J* and *K* for the voltage and temperature sensors, respectively. The voltage and temperature sensors are coupled to channel gating by allosteric coupling constants *C* and *D*, respectively. The allosteric factor, *E*, establishes the interaction between the voltage and temperature sensors, and *n* represents the number of sensors involved in channel activation. *R*, Resting state we must be careful not to confuse this with the ideal gas constant (*R)*

activation (Brauchi 2004; Latorre et al. 2007a; Matta and Ahern 2007). Thus, at high temperatures, the channel is activated by significant depolarization and when all voltage sensors are resting, the open probability of the channel can be increased by lowering temperature. Furthermore, the allosteric model demands that temperature and voltage sensors be contained in different structures. This has been corroborated by different findings, mainly in TRPM8 and TRPV1 channels, as follows:

1. Specific mutations introduced into the C-terminal of TRPM8 channels abolished temperature sensitivity, yet maintained voltage dependence (Brauchi et al. 2007).
2. Grandl and coworkers (Grandl et al. 2010) found three mutations in TRPV1, localized in the pore region (N628K, N652T and Y653T), which decrease their heat sensitivity as determined by a right shift in the temperature threshold. The double and triple mutants N652T-Y653T and N628K-N652T-Y653T have a stronger phenotype than the single-point mutants, showing greater decreases in their heat sensitivity without affecting their activation by agonists (capsaicin and 2-APB), voltage and acid pH.
3. Analyses of current activation time courses at different temperatures in TRPM8 channels show that they become mono-exponential after brief delays (Latorre et al. 2007a). Such delays are shortened when pre-pulses reach more depolarized values (Cole and Moore 1960), indicating that the channels transit between several voltage-dependent closed states. Similarly, large hyperpolarizing voltages would populate the closed states further removed from open states. These

changes in delays do not show temperature dependence, suggesting that temperature dependence of voltage-dependent transitions is weak.
4. Analyses of single channel data indicate the existence of a minimum of five closed states and two open states for TRPM8 (Fernandez et al. 2011) and at least three closed and three open states for TRPV1, whose duration is practically temperature-independent (Liu et al. 2003).

All these results strongly suggest that voltage and temperature sensors are different entities. Allosteric models have been successful in explaining the workings of other polymodal ion channels, such as BK potassium channels (Horrigan and Aldrich 2002; Orio and Latorre 2005) and can be useful to describe the complex regulation of thermo TRP channels, such as TRPV1 and TRPM8, and to understand their contribution to heat and cold sensitivity.

1.3.5 Pore and Selectivity of Thermosensitive TRP Channels

TRP channels are non-selective cation channels, permeable to both monovalent and divalent cations. However, the degree of specificity of permeation to either monovalent or divalent cations depends on the type of TRP channel. In some cases, it seems that the permeation properties of a channel may be associated with its complex polymodal gating, as it has recently been shown by Cao et al. (Cao et al. 2013b).

1.3.5.1 The TRPV Subfamily

While TRPV1–4 channels are mostly involved in nociception and thermal reception, TRPV5/6 channels are mostly involved in Ca^{2+} reabsorption in the kidney (Owsianik et al. 2006). Thus, TRPV5/6 channels are highly permeable to Ca^{2+}, whereas TRPV1–4 channels are mildly Ca^{2+} permeable. They also exhibit rectification and modulation by divalent cations and protons. In contrast to TRPV 5/6 channels that show strong inward rectification in the absence of divalent cations (Voets et al. 2003), TRPV1 exhibits outward rectification (Premkumar et al. 2002; Voets et al. 2003) and extracellular divalents can potentiate the TRPV1 current, promoting changes in the extracellular region (Yang et al. 2014).

Alignments of TRPV channels show that there are conserved residues (L, F, I; see Fig. 1.4a) in the putative pore region among all these subfamily members, and also that the mutations of aspartates have profound effects on the selectivity and blocking properties of TRPV4–6 channels (Nilius et al. 2001; Voets et al. 2002, 2003). Accordingly, it has been proposed that the aspartates in these channels would form a tetrameric ring with two binding sites for Ca^{2+} (Nilius et al. 2001; Voets and Nilius 2003). Thus, D646 has been identified to be relevant for Ca^{2+} selectivity and blocking by Ruthenium Red in TRPV1 (Garcia-Martinez et al. 2000). Structure-function studies in TRPV1 channels have revealed that the S5-S6 loop is related to the permeation pathway (Lopez et al. 1994). Recently, electron cryo-microscopy

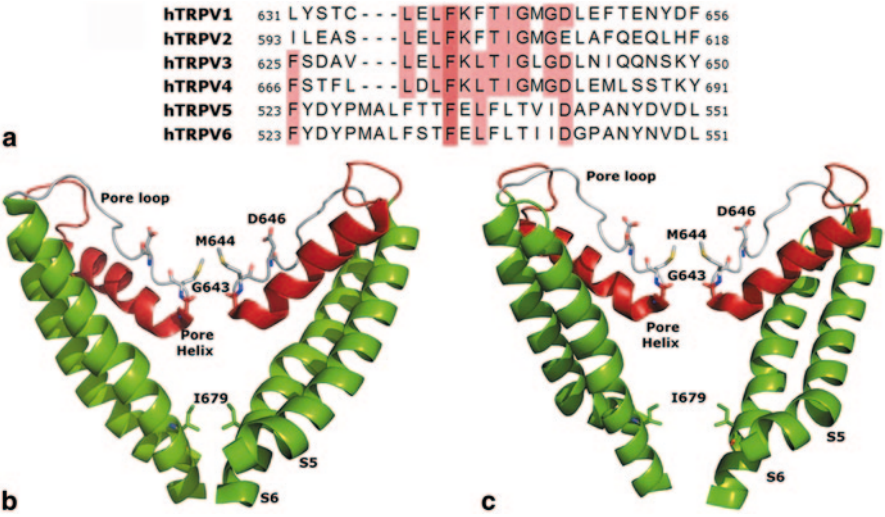

Fig. 1.4 The putative pore structure in TRPV channels subfamily. **a** Sequence alignment of the pore region of human TRPV channels subfamily. The conserved amino acids that in all or mostly members of subfamily are indicated in *red*. Sequences used in the alignments were extracted from UniProt database: TRPV1 (Q8NER1); TRPV2 (Q9Y5S1); TRPV3 (Q8NET8); TRPV4 (Q9HBA0); TRPV5 (Q9NQA5); TRPV6 (Q9H1D0). Alignments were performed by cluster method using UniProt service (ClustalO). **b** Molecular structure of the rat TRPV1 pore region in closed (PDB 3J5P) and **c** open states (PDB 3J5R), showing pore loop (Trevisani et al.) and pore helix domain (Zaritsky et al.), selectivity filter (GMDG), and lower gate (I679). Transmembrane domain 5 (S5) and 6 (S6) of adjacent subunits are represented in *green*

has been applied to the TRPV1 channel with an average resolution of 3.4 Å, which was revealed to have a wide outer pore followed by a short-narrow selectivity filter (G643-M644-G645-D646) and a lower gate (I679) (Liao et al. 2013) (Fig. 1.4b).

As stated before, temperature gating in TRPV1 (and TRPM8) is allosterically linked to gating by chemicals and by voltage (Brauchi et al. 2006; Latorre et al. 2007a). It has also been shown that thermal or chemical stimulations of some TRPV channels have profound effects on their pore properties and structure, especially the outer pore region (Bautista and Julius 2008). Recent studies have shown that single pore residues in an extension of 50 amino acids in the outer mouth of the pore in both TRPV1/3 channels exhibit temperature-dependent conformations (Kim et al. 2013). However, some authors have reported significant conformational changes of the pore turret in TRPV1 channels due to temperature (Yang et al. 2010), whereas others implicate the inner pore region as one of the critical domains determining TRPV1 channel gating, also modulating capsaicin and thermal sensitivity (Susankova et al. 2007; Myers et al. 2008). Differences in the pore region may account for the differences observed between species for TRPV1 heat activation (Papakosta et al. 2011).

Finally, modulation by protons in TRPV5 has been shown to alter pore dimensions by cysteine scanning mutagenesis. Extra or intracellular acidification has been seen to facilitate pore collapse in TRPV5 through pore helix rotations (Yeh

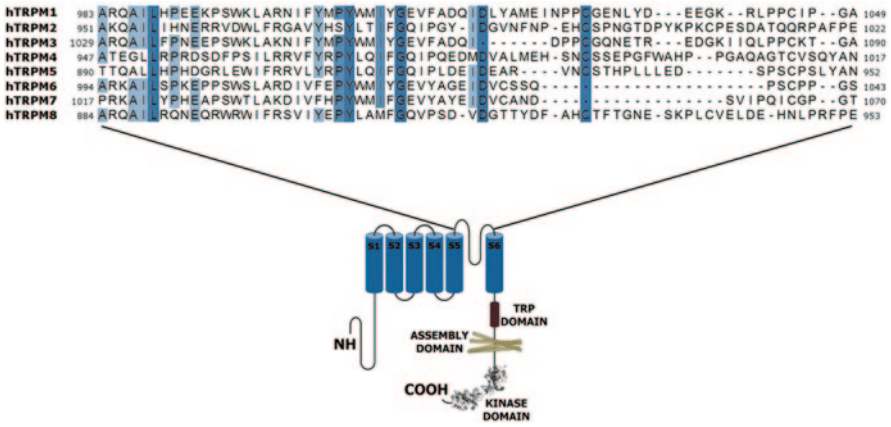

Fig. 1.5 Sequence alignment of the putative pore region of the human TRPM channel subfamily. Conserved amino acids in all or most members of the subfamily are indicated in *blue*. Sequences used in the alignments were extracted from the UniProt database: TRPM1 (Q7Z4N2); TRPM2 (O94759); TRPM3 (A2A3F7); TRPM4 (Q8TD43); TRPM5 (Q9NZQ8); TRPM6 (Q9BX84); TRPM7 (Q96QT4); TRPM8 (Q7Z2W7). Alignments were performed with the cluster method using the UniProt service (ClustalO)

et al. 2005). In TRPV1, which is reported to be quite permeable to protons ($P_{H+}/P_{Cs+} \sim 1130$) (Hellwig et al. 2004), the critical regions related to pH sensitivity seem to be related to two key domains located in the helix pore and the S4-S5 linker (Ryu et al. 2007). Wirkner et al. found that glycosylation of residues near the S5-S6 loop (N604) diminishes capsaicin and pH sensitivity of the channel (Wirkner et al. 2005) (see Fig. 1.2).

1.3.5.2 The TRPM Subfamily

Unlike TRPV channels, some members of this subfamily are absolutely impermeant to divalents such as Ca^{2+}, though they are gated by it (TRPM4/5) (Launay et al. 2002; Hofmann et al. 2003; Lopez et al. 2014). TRPM2/3/8 and TRPM6/7 channels are weakly and highly permeant to divalent ions, respectively (Monteilh-Zoller et al. 2003; Owsianik et al. 2006). The loop between S5 and S6 (where the putative pore is) has been observed to be quite conserved among all TRPM channels (amino acids Y908-F912, along with an aspartate at 920 (see Fig. 1.5), though there are some subtle differences that may account for their varying selectivity to Ca^{2+} permeation (i.e., changes from E (Glu) to Q (Gln)). Thus, the neutralization of D920 in TRPM8 yields non-functional channels, whereas mutation Q914E reduces Ca^{2+} permeability in these channels (Perraud et al. 2003; Nilius et al. 2005a; Topala et al. 2007). Similar to TRPV1, a glycosylation site (N934) was identified near the pore region (Dragoni et al. 2006), which might modulate TRPM8 sensitivity to cold (Per-

tusa et al. 2012). The site is flanked by two cysteines (C929 and C940), which are essential for channel function (Dragoni et al. 2006) (Fig. 1.5).

1.3.5.3 The TRPA Subfamily (TRPA1)

While TRPM8 is activated by moderate cold (Temp < approx. 25 °C), TRPA1 is activated by noxious cold (< 17 °C) (Story et al. 2003). These channels are quite permeable to Ca^{2+}, being the third among TRP channels (TRPV5/6 channels being the most permeable to Ca^{2+}). D918 and E923 seem to be critical for Ca^{2+} permeation (Karashima et al. 2010). As it has been reported for TRPV channels, there have been studies linking the pore and gating of these channels to temperature (Benedikt et al. 2009; Banke et al. 2010; Wang et al. 2013). Using organic cations of different sizes, it has been shown that activation of TRPA1 by cold involves reversible pore dilation, yielding pore diameters of about 11 Å (Chen et al. 2009; Banke et al. 2010; Alpizar et al., 2013).

1.3.5.4 The TRPC5 Channel

TRPC5 modulation by temperature was unknown until 2011, when it was established as a cold-activated channel in the peripheral nervous system (Zimmermann et al. 2011). Homotetrameric TRPC5 channels show an unusual biphasic rectification property that might be explained by voltage-dependent blocking by intracellular Mg^{2+} (Obukhov and Nowycky 2005). Unlike other TRP channels, the region between S5 and S6 in TRPC channels is not conserved. Negative residues inside and nearly outside the S5-S6 loop, like E559/E570, might be related to the selectivity properties of the TRPC5 channel, suggesting that the pore region in this subfamily is also related to the S5-S6 loop and helices, as in the rest of the TRP channel families (Gutman et al. 2003).

1.3.6 Gating Modifiers in the Context of Molecular Models of Thermo TRP Channels

TRPV channels are voltage-activated channels, whose voltage thresholds for channel opening can be allosterically modified by heat (temp. > 42 °C), capsaicin and protons in an allosteric polymodal way (Latorre et al. 2010; Baez-Nieto et al. 2011). It has been known that TRPV1 channels are activated by pH < 6 (Caterina et al. 1997). Lowering the *pH* was seen to diminish the threshold for activation by capsaicin and related compounds as well as heat. Proton binding residues in TRPV1 were characterized as E600 and 648, and they were shown to be relevant for TRPV1 channel modulation by other extracellular cations, as well as for the modulation of sensitivity to chemical agonists (Jordt et al. 2000; Ahern et al. 2005; Ryu et al. 2007)

Capsaicin and its natural and artificial derivatives (capsacinoids) activate the TRPV1 channel and they seem to do so from within the intracellular moieties of the channel. Sensitivity to capsaicin and its endogenous analog, anandamide, was shown to be closely related to transmembrane segment 3 (S3 or TM3). Based on site-directed mutagenesis of TRPV1, Jordt and Julius (Jordt and Julius 2002) proposed that tyrosine at position 511 (Y511) and its aromatic nature, located in or nearby the intracellular TM3 loop, is critical for TRPV1 capsaicin sensitivity. Residues close to Y511, like S512 or R491, have been observed to have an important influence on capsaicin activity as well. These results led the authors to propose that all such residues constitute a "pocket" for vanilloid-like agonist molecules.

The *N-terminal* cytosolic segment of TRPV channels contains ankyrin repeat domains (Stewart et al. 2010), whose sequences are well conserved. The ARDs have been shown to be important for channel gating modulation and sensitization through ATP binding and Ca^{2+}/calmodulin interaction (TRPV1–4, but not TRPV2/5–6) (Rosenbaum et al. 2004; Lishko et al. 2007; Phelps et al. 2010). In TRPV1/3 channels, Ca^{2+}-calmodulin binding to ARDs in addition to a C-terminal binding site promote desensitization, though it potentiates TRPV4 channels (Lishko et al. 2007; Phelps et al. 2010; Lau et al. 2012). Sensitization in TRPV3 takes place when Ca^{2+} is released from the inhibiting binding sites located in the ARD calmodulin interaction site (residues 108–130), as well as the release of Ca^{2+} from the pore to the extracellular side (Asp 641 inside the pore) (Xiao et al. 2008). Regarding TRPV4, it has been suggested that potentiation may arise from interactions between the N-terminus and C-terminus Ca^{2+} -calmodulin binding-sites (Strotmann et al. 2003, 2010). All these results indicate that despite the sequence conservation among thermosensitive TRPV1–4 channels, they are differentially modulated by the same ligands and sites, suggesting different 3D structures that might be explained by distinct fits of the ARDs inside the cytoplasmic side of the channel (Huynh et al. 2014).

The *C-terminus* (the proximal region to S6) contains a very well conserved sequence among all TRP channels of 25 residues, named TRP domain where the *TRP-Box* (IWKLQR) (Ramsey et al. 2006). The following coiled-coil region (E684 to R721), also comprising part of the rat TRP domain, is involved in TRPV1 tetramerization (Vlachova et al. 2003; Garcia-Sanz et al. 2004). Similar residues have been identified for mouse TRPV1 (752–772), receiving the name *tetrameric assembly domain* (Wirkner et al. 2005) (Zhang et al. 2011). Furthermore, in TRPV1 it has been established that this region might participate in calmodulin-binding (E767 to T801), phosphorylation and thermal sensitivity (Numazaki et al. 2003; Brauchi et al. 2006; Huang et al. 2006).

Ca^{2+}-calmodulin binding has been implicated in TRPV1 desensitization (Numazaki et al. 2003). However, the C-terminus calmodulin-binding site (named CT, from W787 to L796) has distinct effects on desensitization compared to the N-terminus ARD calmodulin-binding site (Lau et al. 2012). Interestingly, the CT calmodulin-binding site does not contain a classic calmodulin binding sequence motif. The crystallized 3D structure of the TRPV1-CT segment shows that it is quite similar to the canonical Ca^{2+}-calmodulin peptide complexes, having two lobes of Ca^{2+}-calmodulin wrapped around the TRPV1-CT segment (Lau et al. 2012). Unlike

TRPV1, the calmodulin interaction site in TRPV2 has been characterized as a classic calmodulin binding sequence (Holakovska et al. 2011) and Ca^{2+}-desensitization is through PIP_2 hydrolysis (Mercado et al. 2010). Furthermore, differential thermal sensitivity shorting this region in TRPV1 channels was reported by Vlachova et al. (Vlachova et al. 2003).

Bearing all of this in mind, Brauchi et al. (2006) switched the C-terminal tail between hot-sensing TRPV1 and cold-sensing TRPM8. Switching the channel's tail resulted in a chimeric channel, whose phenotype was determined by the C-terminal tail, not only for thermal sensing, but also for some gating properties such as modulation of channel function by PIP_2 binding. Recently, it has been shown that PIP_2 activates the channel by directly interacts with TRPV1, and also that the PIP_2 binding-site is located proximal to the TRPV1 channel C-terminus (Ufret-Vincenty et al. 2011). The precise interaction location of PIP_2 with the C-terminus has been reported to take place at residues K688-K718 and L777-S820, constituting two independent sites that overlap with the CT segment. In addition, a third binding site for PIP_2 has also been reported in the TRPV1 channel N-terminus (F189-V221) (Grycova et al. 2012).

TRPM channels are also voltage-activated, just like TRPV channels. In contrast with TRPV1–4 channels, the TRPM8 channel opening voltage threshold is activated by cold (temp. < 25 °C) as well as by menthol and derivatives. High-throughput screening of random mutagenesis in mouse TRPM8 has identified a tyrosine residue in S2 (Y745), which is critical for *menthol* sensitivity (Bandell et al. 2006). It has been suggested that there should be many other residues in the channel contributing to the menthol-binding site, as some TRPM8 antagonists that supposedly interact with the menthol-binding site are still active in Y745 TRPM8 mutated channels (Malkia et al. 2009). Mutations in S4 (R842H) can also change menthol sensitivity, suggesting that its site of action could be placed in the cleft between S2 and S4 (Voets et al. 2007). Subthreshold concentrations of menthol move TRPM8 activation cold temperature threshold to warmer temperatures. However, repeated menthol stimulation rapidly desensitizes TRPM8 in a Ca^{2+}-dependent manner (McKemy et al. 2002; Reid 2002).

TRPM8 is also activated by the so-called "super-cooling agent" *icilin* (as it is 200 times more potent than menthol) in the presence of intracellular Ca^{2+}(Chuang et al. 2004). The binding site for icilin seems to be located in residues along S3 (N799, D802 and G805). This finding implies that icilin and menthol-binding sites are different and that they would also probably differ in terms of their channel activation mechanisms (Chuang et al. 2004). In line with this view, it has been shown that icilin and cold, but not menthol sensitivity, is modulated by intracellular pH and viceversa (Andersson et al. 2004).

As discussed above, the intracellular *N-terminus* of TRPM channels has a large conserved region among channel subfamily members (~700 residues), and are characterized by having 4 subdomains labeled TRPM homology regions or *MHRs* involved in trafficking (TRPM2/4/8) (Clapham 2003; (Perraud et al. 2003; Launay et al. 2004; Phelps and Gaudet 2007).

In TRPM2/4/5 channels, activity critically depends on the level of intracellular Ca^{2+} and has been implicated in channel gating and desensitization (McHugh et al. 2003; Tong et al. 2006); Vennekens and Nilius 2007 (Nilius et al. 2004a). In TRPM2, intracellular Ca^{2+} activates the channel by binding to calmodulin, presumably located at this IQ sequence motif in the N-terminus of the channel (residues 406–416) (McHugh et al. 2003; Tong et al. 2006). Ca^{2+}-sensitivity is also determined by the binding of four Ca^{2+} ions, which are cooperatively bound to the inner mouth of the channel in the open state (Csanady and Torocsik 2009). In TRPM4, ATP binding to the N-terminus and to the S3-S4 intracellular loop appears to be needed for Ca^{2+} channel sensitivity. Calmodulin binding sites in the N-terminus, however, appear to be irrelevant for TRPM4 function (Nilius et al. 2004b)

Like TRPV channels, the *C-terminus* contains the *TRP domain* and box that is well conserved among all TRPM channels (McKemy et al. 2002). The C-terminus distal region contains a coiled-coil structure involved in multimerization, trafficking and activation gating of TRPM8 (residues 1064–1104), which is conserved among TRPM channels (Erler et al. 2006; Mei et al. 2006; Tsuruda et al. 2006; Phelps and Gaudet 2007).

Regulation of thermoTRPM channels by PIP2 includes activation or potentiation of TRPM4/5/8 (Guinamard et al. 2011; Prescott and Julius 2003; Vennekens and Nilius 2007). In TRPM8, some key positively charged residues present in the TRP box (K995, K998 and R1008) were found to be crucial in PIP_2 binding and modulation (Rohacs et al. 2005). Ca^{2+} -dependent desensitization of TRPM8 seems to occur through hydrolysis of PIP_2 (Liu and Qin 2005; Rohacs et al. 2005). Y1005 and L1009 residues have been implicated in the modulation of menthol sensitivity, but they do not seem to be involved in the initial event needed for menthol activation (Bandell et al. 2006). The results obtained with splice variants of the TRPM8 channel C-terminus (named α and β) suggest that these short isoforms seem to mimic heat, hence inhibiting channel activation. Therefore, all of these results are consistent with the hypothesis that the C-terminus in TRPM8 channels is essential for their cold sensing ability, channel activation and modulation by PIP_2 (Brauchi et al. 2006; Bidaux et al. 2012; Fernandez et al. 2012).

TRPA1 channels, as we discussed above, are quite undiscriminating when it comes to their gating modulation. In addition to the agents that commonly modulate most of the TRP channels described in this review (such as Ca^{2+}, phosphorylation/dephosphorylation and $PIP_{2)}$, TRPA1 channels are also modulated by a plethora of different compounds that have pharmacological and physiological significance. These natural or artificial chemicals can produce nucleophilic covalent modifications (reviewed in (Nilius et al. 2012)) (i.e., electrophilic compounds such as isothiocyanates, cinnamaldehyde, allicin, acrolein, O_2, CO_2, reactive oxygen species (ROS) and reactive nitrogen species (Watanabe et al. 2002a)). Other types of interactions like non-electrophilic compounds (menthol, icilin, caffeine) are unlikely to modify channel gating through covalent interaction, exhibiting bimodal action (bell-shaped dose response curves) (Nilius et al. 2012). Thus, activation by cold in TRPA1 is potentiated in the presence of many of the chemical agonists mentioned above (Fajardo et al. 2008).

The *N-terminus* is characterized by having a long stretch of 18 ARDs, which are important for protein-protein interaction and channel trafficking. Intracellular Ca^{2+} can bind to the N-terminus and change its structure (Nilius et al. 2012). However, the deletion of the putative EF-hands that might act as N-terminal Ca^{2+}-binding sites only have effects on TRPA1 membrane trafficking (just mild effects on the modulation of TRPA1 by intracellular Ca^{2+}) (Nilius et al. 2011). There are cysteine residues in the N-terminus that could be quite important for TRPA1 interaction through disulfide bridges, because they are located at the interface between TRPA1 subunits, hence being able to interfere with gating mechanisms. In addition, Sura et al. (2012) reported that site-directed mutagenesis in the *C-terminus*, which contains a stretch of several negative amino acids arranged in a manner similar to BK Ca^{2+}, impairs TRPA1 modulation by means of intracellular Ca^{2+} (residues E1077, D1080, D1081 and D1082) (Sura et al. 2012). Other residues like Lys 1048, Lys 1052, Lys 1092 and Arg 1099 have also been observed to modify a significant amount of TRPA1 chemical and voltage-dependent activation gating. These basic residues in the C-terminus might also explain the diversity of electrophilic compounds that can affect TRPA1 channel gating (Samad et al. 2011).

TRPC5 channels are present in mouse and human sensory neurons of dorsoal root ganglia, a substantial number of peripheral nerves, and in dorsal lamina of the spinal cord. These channels are non-selective cation channels permeable to Ca^{2+} and modulated by a large variety of signals, as it is usually reported for TRP channels (Zeng et al. 2004): intracellular Ca^{2+}, ATP, protons, PIP_2 and phosphorylation/dephosphorylation (Semtner et al. 2007; Dattilo et al. 2008). Some of these mechanisms are complex and seem to require different sites of action. In particular, it has been reported that intracellular Ca^{2+} usually potentiates agonist-activated TRPC5 channels (Blair et al. 2009). The *C-terminus* seems to contain Ca^{2+}-binding protein interaction sites, through which intracellular Ca^{2+} may exert its effects (calmodulin, Ca^{2+} binding protein 1, NCS-1) (Ordaz et al. 2005; Shimizu et al. 2006; Beech 2007).

In addition, these channels are activated by hypo-osmotic and pressure related changes in the membrane, revealing their role in mechanosensation (Gomis et al. 2008). Some of these changes are potentiated upon G-protein coupled receptor (GPCR) activation (Jemal et al. 2013). G proteins released from the GPCR increase channel responses to cold (Zimmermann et al. 2011) and might mediate PKA action in TRPC5 (Sung et al. 2011). Sung *et al* identified serines located in the C-terminus (S794 and S796) as a PKA phosphorylation site. TRPC5 channels are also strongly modulated by oxidizing agents that act through nitrosylation, like nitric oxide (NO). They can activate the channel by reacting with cysteine residues located at 553–558 in the S5-S6 linker, which are accessible from intracellular media (Yoshida et al. 2006).

1.3.7 Evolution of Thermosensitive TRP Channels

Since evolution involves a selection of the most adapted individuals to the environment in which they live, TRP channels are critical for such adaptation because of their nature as sensing and integrating molecules. Studies on TRP channels from an evolutionary perspective are very relevant to the field, as information about sites for particular chemical or physical stimuli can be obtained by comparing ortholog and paralog genes in different species that appeared on Earth at different times. They may also reveal critical interaction sites necessary for TRP channel function and interaction with other proteins (Sardar et al. 2012). The phylogenetic tree for the seven subfamilies of TRP channels (mostly including human TRP channels) is shown in Fig. 1.2 above.

TRP channels have only been described in protozoa (*Leishmania*), fungi and animalia thus far (Prole and Taylor 2011; Wolstenholme et al. 2011). Though TRP channels are not endogenously expressed in prokaryotes (Li et al. 2011), bacteria still have ways for osmo and mechano-transduction, which are not as elaborated as in eukaryotes, where channel sensing molecules functioning as precursors of TRP channels are involved (Jin et al. 2011). TRPN channels are only present in invertebrates and some fish, and absent in most vertebrates, which only have six subfamilies. TRPN channels are named as such because of the "No-mechano-potential-C" channels from *C. Elegans*. Although these channels (TRPN) were lost in vertebrates during evolution, the TRP channels almost doubled in numbers when implicated in Ca^{2+} and Mg^{2+} homeostasis as well as in thermosensation (Nilius and Owsianik 2011). In this regard, sponges, which are one of the most ancient metazoans with active responses to stimuli, though lacking nervous systems, contain a cilia-sensing system with TRP channels (Ludeman et al. 2014).

Animal adaptation to temperature changes occurs by sensing both body and environmental temperature. Toward this aim, thermal stimuli are monitored by thermal receptors expressing thermoTRP channels. Hence, changes in thermoTRP channels and thermosensitivity are crucial in understanding animal adaptation to different environments with different temperatures. Most of these genes emerged from common teleost fish ancestors through gene duplication (Saito and Shingai 2006). Regarding TRPVs, thermo-TRPVs (TRPV1–4) and nonthermo-TRPVs (TRPV5–6) diverged early in evolution (Fig. 1.2). ThermoTRPVs split first into the TRPV4 and TRPV1–3 branches, and later divided into the TRPV1–2 branch and TRPV3 channels (Saito et al. 2011). TRPV3–4 channels diverged earlier than the separation of the TRPV1–2 branch. TRPV1–2 channels are activated by noxious heat (temp. > 40 °C), whereas, TRPV3–4 are activated by warm temperatures (T > 35 °C). Because of that, they have been implicated in temperature regulation in homeothermic vertebrates. Consistent with this idea, TRPV3-null mice lack adequate control of their body and environmental temperatures (Moqrich et al. 2005). A TRPV3 ortholog of the mammalian TRPV3 channel found in western claw frogs has been seen to significantly diverge from mammalian TRPV3 channels in their C-terminus and N-terminus. In addition, they lack responses to several agents known to modulate mammalian TRPV3. All these results are consistent with previous findings that

suggested critical roles of the N-terminus and C-terminus in TRPV channels in heat and chemical modulation (Saito et al. 2011; Brauchi et al. 2004).

These results are quite interesting, since they show that the range of temperature sensitivity in thermoTRP channels can be changed and adapted during evolution, hence being flexible. Unlike mammals, which are homeothermic vertebrates with body temperatures around 35–37 °C, western clawed frogs are ectothermic vertebrates adapted to lower temperatures. Western clawed frogs live in an environment with temperatures within the range of 22–28 °C. Just like mammals, in which TRPV3 senses adequate temperatures in the body surface, TRPV3 in the western clawed frog is also crucial in perceiving skin temperature (Mandadi et al. 2009; Saito et al. 2011). In this sense, TRPV3 channels in western clawed frogs lack the ability to be modulated by heat and cold, and tend to mainly detect noxious cold, as it is of more advantage for the survival of the frog-environment relationship. The results are also consistent with the idea of modular "hot spots" in the functional structure of TRP channels, related to their different sensing abilities (Latorre et al. 2007a).

Opposite thermosensitivity among other ortholog thermoTRP channels besides TRPV3 has been reported in TRPA1 channels. While such thermosensitivity is activated by warm or hot temperatures in reptiles, frogs and insects, it is activated by cold in mice and it is not activated by temperature in zebrafish or humans (Viswanath et al. 2003; Kwan et al. 2006; Karashima et al. 2009; Gracheva et al. 2010; Saito et al. 2012); Chen et al. 2013). TRPA1 channels are quite ancient among animals and are interesting in that their thermal sensitivity was shifted from hot to cold during the course of evolution. Invertebrates like *Drosophila melanogaster* and *C. elegans* show that TRPA1 is sensitive to heat and nociceptive chemical stimulation (Wang et al. 2009; Kang et al. 2012). Except for zebrafish, where TRPA1 is insensitive to temperature, early vertebrates like amphibians, reptiles and lizards possess TRPA1 channels sensitive to heat with variable thresholds. In all cases, nociception by chemicals was maintained through time (Prober et al. 2008). Swapping the N-terminus of snake and mammal TRPA1 channels seemed to have changed their temperature dependence (Cordero-Morales et al. 2011). This suggests that the module related to TRPA1 channel thermosensitivity could be placed in the N-terminus, whilst nociception by chemicals would be due to binding to different regions of TRPA1 channels.

TRPA1 and TRPV1 are co-expressed in the same cells and might form heteromers as well (Salas et al. 2009; Staruschenko et al. 2010; Akopian 2011). TRPV1 came from the TRPV1/2 branch separation later in evolution after TRPA1 (Saito and Shingai 2006). Once TRPV1 channels appeared, they maintained their gating characteristics by heat as well as their temperature thresholds through the span of evolution. It has been hypothesized that the appearance of TRPV1 channels in evolution after TRPA1 channels as efficient sensors for noxious heat and nociception favored the transition of TRPA1 channels as receptors of noxious cold, while maintaining their nociception abilities. Hence, both channels are frequently expressed together in neurons related to nociception (Saito et al. 2012).

1.4 Concluding Remarks

In this chapter we have reviewed the structural, biophysical and evolutionary details of thermoTRP channels, relevant for sensory transduction. Regarding their biophysics and physiology, their allosteric polymodal nature confers them, among other proteins, an enormous advantage for sensing and transducing signals. The wide repertoire of chemical and physical agents that are able to gate these channels provides sophisticated fine-tuning for cell and organism living conditions. Their non-selective cation permeation properties and their mild voltage gating, which are strongly modulated by chemical and physical agents that can interact allosterically, make them unique machineries for cell homeostasis. Regarding their structure, their organization as tetramers with interrelated sensing modules has been revealed through structure-function studies. More recently, 3D models have shown that they have large cytoplasmic interacting regions, making them suitable for monitoring intracellular changes interacting with many ligands and proteins. All these features are reflected in evolution as they appear in molecules within more complex organisms. In line with the structural and biophysical details known to date, analyses of their properties in different organisms have revealed that they are one of the key factors involved in adapting to distinct thermal conditions. As such, these unique properties are conferred to cells and organisms expressing these molecules, indicating a clear advantage that makes them suitable for building complex interactions and networks. Network analysis of these channels in physiology and diseases has just begun (Chun et al. 2013) and have contributed in shedding light on our understanding as to how these channels play important roles in life within the context of systems biology.

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References

Ahern GP, Brooks IM, Miyares RL, Wang XB (2005) Extracellular cations sensitize and gate capsaicin receptor TRPV1 modulating pain signaling. J Neurosci 25:5109–5116

Akopian AN (2011) Regulation of nociceptive transmission at the periphery via TRPA1-TRPV1 interactions. Curr Pharm Biotechnol 12:89–94

Al-Fageeh MB, Smales CM (2006) Control and regulation of the cellular responses to cold shock: the responses in yeast and mammalian systems. Biochem J 397:247–259

Alessandri-Haber N, Joseph E, Dina OA, Liedtke W, Levine JD (2005) TRPV4 mediates pain-related behavior induced by mild hypertonic stimuli in the presence of inflammatory mediator. Pain 118:70–79

Alpizar YA, Gees M, Sanchez A, Apetrei A, Voets T, Nilius B, Talavera K (2013) Bimodal effects of cinnamaldehyde and camphor on mouse TRPA1. Pflugers Arch 465:853–864

Andersson DA, Chase HW, Bevan S (2004) TRPM8 activation by menthol, icilin, and cold is differentially modulated by intracellular pH. J Neurosci 24:5364–5369

Babes A (2009) Ion channels involved in cold detection in mammals: TRP and non-TRP mechanisms. Biophys Rev 1:193–200

Baez-Nieto D, Castillo JP, Dragicevic C, Alvarez O, Latorre R (2011) Thermo-TRP channels: biophysics of polymodal receptors. Adv Exp Med Biol 704:469–490

Bandell M, Story GM, Hwang SW, Viswanath V, Eid SR, Petrus MJ, Earley TJ, Patapoutian A (2004) Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. Neuron 41:849–857

Bandell M, Dubin AE, Petrus MJ, Orth A, Mathur J, Hwang SW, Patapoutian A (2006) High-throughput random mutagenesis screen reveals TRPM8 residues specifically required for activation by menthol. Nat Neurosci 9:493–500

Banke TG, Chaplan SR, Wickenden AD (2010) Dynamic changes in the TRPA1 selectivity filter lead to progressive but reversible pore dilation. Am J Physiol Cell Physiol 298:C1457–C1468

Bargal R, Avidan N, Ben-Asher E, Olender Z, Zeigler M, Frumkin A, Raas-Rothschild A, Glusman G, Lancet D, Bach G (2000) Identification of the gene causing mucolipidosis type IV. Nat Genet 26:118–123

Baroiller JF, D'Cotta H (2001) Environment and sex determination in farmed fish. Comp Biochem Physiol C Toxicol Pharmacol 130:399–409

Bassi MT, Manzoni M, Monti E, Pizzo MT, Ballabio A, Borsani G (2000) Cloning of the gene encoding a novel integral membrane protein, mucolipidin-and identification of the two major founder mutations causing mucolipidosis type IV. Am J Hum Genet 67:1110–1120

Bautista D, Julius D (2008) Fire in the hole: pore dilation of the capsaicin receptor TRPV1. Nat Neurosci 11:528–529

Bautista DM, Jordt SE, Nikai T, Tsuruda PR, Read AJ, Poblete J, Yamoah EN, Basbaum AI, Julius D (2006) TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. Cell 124:1269–1282

Bautista DM, Siemens J, Glazer JM, Tsuruda PR, Basbaum AI, Stucky CL, Jordt SE, Julius D (2007) The menthol receptor TRPM8 is the principal detector of environmental cold. Nature 448:204–208

Beech, DJ (2007) Canonical transient receptor potential 5. Handb Exp Pharmacol 179:109-123

Benedikt J, Samad A, Ettrich R, Teisinger J, Vlachova V (2009) Essential role for the putative S6 inner pore region in the activation gating of the human TRPA1 channel. Biochim Biophys Acta 1793:1279–1288

Bidaux G, Beck B, Zholos A, Gordienko D, Lemonnier L, Flourakis M, Roudbaraki M, Borowiec AS, Fernandez J, Delcourt P, Lepage G, Shuba Y, Skryma R, Prevarskaya N (2012) Regulation of activity of transient receptor potential melastatin 8 (TRPM8) channel by its short isoforms. J Biol Chem 287:2948–2962

Blair NT, Kaczmarek JS, Clapham DE (2009) Intracellular calcium strongly potentiates agonist-activated TRPC5 channels. J Gen Physiol 133:525–546

Bodding M, Wissenbach U, Flockerzi V (2007) Characterisation of TRPM8 as a pharmacophore receptor. Cell Calcium 42:618–628

Boukalova S, Marsakova L, Teisinger J, Vlachova V. 2010. Conserved residues within the putative S4-S5 region serve distinct functions among thermosensitive vanilloid transient receptor potential (TRPV) channels. J Biol Chem 285:41455–41462

Brauchi S, Orio P, Latorre R (2004) Clues to understanding cold sensation: thermodynamics and electrophysiological analysis of the cold receptor TRPM8. Proc Natl Acad Sci USA 101:15494–15499

Brauchi S, Orta G, Salazar M, Rosenmann E, Latorre R (2006) A hot-sensing cold receptor: C-terminal domain determines thermosensation in transient receptor potential channels. J Neurosci 26:4835–4840

Brauchi S, Orta G, Mascayano C, Salazar M, Raddatz N, Urbina H, Rosenmann E, Gonzalez-Nilo F, Latorre R (2007) Dissection of the components for PIP2 activation and thermosensation in TRP channels. Proc Natl Acad Sci U S A 104:10246–10251

Cao E, Cordero-Morales JF, Liu B, Qin F, Julius D (2013a) TRPV1 channels are intrinsically heat sensitive and negatively regulated by phosphoinositide lipids. Neuron 77:667–679
Cao E, Liao M, Cheng Y, Julius D (2013b) TRPV1 structures in distinct conformations reveal activation mechanisms. Nature 504:113–118
Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389:816–824
Caterina MJ, Rosen TA, Tominaga M, Brake AJ, Julius D (1999) A capsaicin-receptor homologue with a high threshold for noxious heat. Nature 398:436–441
Cesare P, McNaughton P (1996) A novel heat-activated current in nociceptive neurons and its sensitization by bradykinin. Proc Natl Acad Sci U S A 93:15435–15439
Chen J, Kim D, Bianchi BR, Cavanaugh EJ, Faltynek CR, Kym PR, Reilly RM (2009) Pore dilation occurs in TRPA1 but not in TRPM8 channels. Mol Pain 5:3
Chen J, Kang D, Xu J, Lake M, Hogan JO, Sun C, Walter K, Yao B, Kim D (2013) Species differences and molecular determinant of TRPA1 cold sensitivity. Nat Commun 4:2501
Chuang HH, Neuhausser WM, Julius D (2004) The super-cooling agent icilin reveals a mechanism of coincidence detection by a temperature-sensitive TRP channel. Neuron 43:859–869
Chun JN, Lim JM, Kang Y, Kim EH, Shin YC, Kim HG, Jang D, Kwon D, Shin SY, So I, Jeon JH (2013) A network perspective on unraveling the role of TRP channels in biology and disease. Pflugers Archiv: Eur J Physiol. doi:10.1007/s00424-013-1292-2
Chung MK, Lee H, Caterina MJ (2003) Warm temperatures activate TRPV4 in mouse 308 keratinocytes. J Biol Chem 278:32037–32046
Chung MK, Lee H, Mizuno A, Suzuki M, Caterina MJ (2004) 2-aminoethoxydiphenyl borate activates and sensitizes the heat-gated ion channel TRPV3. J Neurosci 24:5177–5182
Chung MK, Guler AD, Caterina MJ (2005) Biphasic currents evoked by chemical or thermal activation of the heat-gated ion channel, TRPV3. J Biol Chem 280:15928–15941
Clapham DE (2003) TRP channels as cellular sensors. Nature 426:517–524
Clapham DE, Runnels LW, Strubing C (2001) The TRP ion channel family. Nat Rev Neurosci 2:387–396
Colburn RW, Lubin ML, Stone DJ Jr, Wang Y, Lawrence D, D'Andrea MR, Brandt MR, Liu Y, Flores CM, Qin N (2007) Attenuated cold sensitivity in TRPM8 null mice. Neuron 54:379–386
Cole KS, Moore JW (1960) Potassium ion current in the squid giant axon: dynamic characteristic. Biophys J 1:1–14
Cordero-Morales JF, Gracheva EO, Julius D (2011) Cytoplasmic ankyrin repeats of transient receptor potential A1 (TRPA1) dictate sensitivity to thermal and chemical stimuli. Proc Natl Acad Sci U S A 108:E1184–1191
Cosens DJ, Manning A (1969) Abnormal electroretinogram from a *Drosophila* mutant. Nature 224:285–287
Csanady L, Torocsik B (2009) Four Ca2+ ions activate TRPM2 channels by binding in deep crevices near the pore but intracellularly of the gate. J Gen Physiol 133:189–203
Dattilo M, Penington NJ, Williams K (2008) Inhibition of TRPC5 channels by intracellular ATP. Mol Pharmacol 73:42–49
De Petrocellis L, Chu CJ, Moriello AS, Kellner JC, Walker JM, Di Marzo V (2004) Actions of two naturally occurring saturated N-acyldopamines on transient receptor potential vanilloid 1 (TRPV1) channels. Br J Pharmacol 143:251–256
del Camino D, Murphy S, Heiry M, Barrett LB, Earley TJ, Cook CA, Petrus MJ, Zhao M, D'Amours M, Deering N, Brenner GJ, Costigan M, Hayward NJ, Chong JA, Fanger CM, Woolf CJ, Patapoutian A, Moran MM (2010) TRPA1 contributes to cold hypersensitivity. J Neurosci 30:15165–15174
Delany NS, Hurle M, Facer P, Alnadaf T, Plumpton C, Kinghorn I, See CG, Costigan M, Anand P, Woolf CJ, Crowther D, Sanseau P, Tate SN (2001) Identification and characterization of a novel human vanilloid receptor-like protein, VRL-2. Physiol Genomics 4:165–174
Denis V, Cyert MS (2002) Internal Ca(2+) release in yeast is triggered by hypertonic shock and mediated by a TRP channel homologue. J Cell Biol 156:29–34
Dhaka A, Viswanath V, Patapoutian A (2006) TRP ion channels and temperature sensation. Annu Rev Neurosci 29:135–161

Dhaka A, Murray AN, Mathur J, Earley TJ, Petrus MJ, Patapoutian A (2007) TRPM8 is required for cold sensation in mice. Neuron 54:371–378
Digel I, Kayser P, Artmann GM (2008) Molecular processes in biological thermosensation. J Biophys 2008:60287
Doerner JF, Gisselmann G, Hatt H, Wetzel CH (2007) Transient receptor potential channel A1 is directly gated by calcium ions. J Biol Chem 282:13180–13189
Dragoni I, Guida E, McIntyre P (2006) The cold and menthol receptor TRPM8 contains a functionally important double cysteine motif. J Biol Chem 281:37353–37360
Eisfeld J, Luckhoff A (2007) TRPM2. Handb Exp Pharmacol 179:237–252
Erler I, Al-Ansary DM, Wissenbach U, Wagner TF, Flockerzi V, Niemeyer BA (2006) Trafficking and assembly of the cold-sensitive TRPM8 channel. J Biol Chem 281:38396–38404
Fajardo O, Meseguer V, Belmonte C, Viana F (2008) TRPA1 channels mediate cold temperature sensing in mammalian vagal sensory neurons: pharmacological and genetic evidence. J Neurosci 28:7863–7875
Fernandez JA, Skryma R, Bidaux G, Magleby KL, Scholfield CN, McGeown JG, Prevarskaya N, Zholos AV (2011) Voltage- and cold-dependent gating of single TRPM8 ion channels. J Gen Physiol 137:173–195
Fernandez JA, Skryma R, Bidaux G, Magleby KL, Scholfield CN, McGeown JG, Prevarskaya N, Zholos AV (2012) Short isoforms of the cold receptor TRPM8 inhibit channel gating by mimicking heat action rather than chemical inhibitors. J Biol Chem 287:2963–2970
Garcia-Martinez C, Morenilla-Palao C, Planells-Cases R, Merino JM, Ferrer-Montiel A (2000) Identification of an aspartic residue in the P-loop of the vanilloid receptor that modulates pore properties. J Biol Chem 275:32552–32558
Garcia-Sanz N, Fernandez-Carvajal A, Morenilla-Palao C, Planells-Cases R, Fajardo-Sanchez E, Fernandez-Ballester G, Ferrer-Montiel A (2004) Identification of a tetramerization domain in the C terminus of the vanilloid receptor. J Neurosci 24:5307–5314
Gaudet R (2008) A primer on ankyrin repeat function in TRP channels and beyond. Mol Biosyst 4:372–379
Goel M, Sinkins WG, Schilling WP (2002) Selective association of TRPC channel subunits in rat brain synaptosomes. J Biol Chem 277:48303–48310
Gomis A, Soriano S, Belmonte C, Viana F (2008) Hypoosmotic- and pressure-induced membrane stretch activate TRPC5 channels. J Physiol 586:5633–5649
Gong Z, Son W, Chung YD, Kim J, Shin DW, McClung CA, Lee Y, Lee HW, Chang DJ, Kaang BK, Cho H, Oh U, Hirsh J, Kernan MJ, Kim C (2004) Two interdependent TRPV channel subunits, inactive and Nanchung, mediate hearing in *Drosophila*. J Neurosci 24:9059–9066
Gracheva EO, Ingolia NT, Kelly YM, Cordero-Morales JF, Hollopeter G, Chesler AT, Sanchez EE, Perez JC, Weissman JS, Julius D (2010) Molecular basis of infrared detection by snakes. Nature 464:1006–1011
Grandl J, Hu H, Bandell M, Bursulaya B, Schmidt M, Petrus M, Patapoutian A (2008) Pore region of TRPV3 ion channel is specifically required for heat activation. Nat Neurosci 11:1007–1013
Grandl J, Kim SE, Uzzell V, Bursulaya B, Petrus M, Bandell M, Patapoutian A (2010) Temperature-induced opening of TRPV1 ion channel is stabilized by the pore domain. Nat Neurosci 13:708–714
Grimm C, Kraft R, Sauerbruch S, Schultz G, Harteneck C (2003) Molecular and functional characterization of the melastatin-related cation channel TRPM3. J Biol Chem 278:21493–21501
Grycova L, Holendova B, Bumba L, Bily J, Jirku M, Lansky Z, Teisinger J (2012) Integrative binding sites within intracellular termini of TRPV1 receptor. PloS One 7:e48437
Guinamard R, Salle L, Simard C (2011) The non-selective monovalent cationic channels TRPM4 and TRPM5. Adv Exp Med Biol 704:147–171
Gutman GA, Chandy KG, Adelman JP, Aiyar J, Bayliss DA, Clapham DE, Covarriubias M, Desir GV, Furuichi K, Ganetzky B, Garcia ML, Grissmer S, Jan LY, Karschin A, Kim D, Kuperschmidt S, Kurachi Y, Lazdunski M, Lesage F, Lester HA, McKinnon D, Nichols CG, O'Kelly I, Robbins J, Robertson GA, Rudy B, Sanguinetti M, Seino S, Stuehmer W, Tamkun MM, Vandenberg CA, Wei A, Wulff H, Wymore RS (2003) International Union of Pharmacology. XLI. Compendium of voltage-gated ion channels: potassium channels. Pharmacol Rev 55:583–586

Hardie RC, Minke B (1992) The trp gene is essential for a light-activated Ca2+ channel in *Drosophila* photoreceptors. Neuron 8:643–651
Hellwig N, Plant TD, Janson W, Schafer M, Schultz G, Schaefer M (2004) TRPV1 acts as proton channel to induce acidification in nociceptive neurons. J Biol Chem 279:34553–34561
Hille B (2001) Ion channels of excitable membranes. Sinauer Associates Inc., Sunderland
Hofmann T, Schaefer M, Schultz G, Gudermann T (2002) Subunit composition of mammalian transient receptor potential channels in living cells. Proc Natl Acad Sci U S A 99:7461–7466
Hofmann T, Chubanov V, Gudermann T, Montell C (2003) TRPM5 is a voltage-modulated and Ca(2+)-activated monovalent selective cation channel. Curr Biol 13:1153–1158
Holakovska B, Grycova L, Bily J, Teisinger J (2011) Characterization of calmodulin binding domains in TRPV2 and TRPV5 C-tails. Amino Acids 40:741–748
Hori T, Katafuchi T (1998) Cell biology and the functions of thermosensitive neurons in the brain. Prog Brain Res 115:9–23
Horrigan FT, Aldrich RW (2002) Coupling between voltage sensor activation, Ca2+ binding and channel opening in large conductance (BK) potassium channels. J Gen Physiol 120:267–305
Hu HZ, Gu Q, Wang C, Colton CK, Tang J, Kinoshita-Kawada M, Lee LY, Wood JD, Zhu MX (2004) 2-aminoethoxydiphenyl borate is a common activator of TRPV1, TRPV2, and TRPV3. J Biol Chem 279:35741–35748
Huang J, Zhang X, McNaughton PA (2006) Modulation of temperature-sensitive TRP channels. Semin Cell Dev Biol 17:638–645
Hui K, Guo Y, Feng ZP (2005) Biophysical properties of menthol-activated cold receptor TRPM8 channels. Biochem Biophys Res Commun 333:374–382
Hurme R, Rhen M (1998) Temperature sensing in bacterial gene regulation—what it all boils down to. Mol Microbiol 30:1–6
Huynh KW, Cohen MR, Chakrapani S, Holdaway HA, Stewart PL, Moiseenkova-Bell VY (2014) Structural insight into the assembly of TRPV channels. Structure 22:260–268
Hwang SW, Cho H, Kwak J, Lee SY, Kang CJ, Jung J, Cho S, Min KH, Suh YG, Kim D, Oh U (2000) Direct activation of capsaicin receptors by products of lipoxygenases: endogenous capsaicin-like substances. Proc Natl Acad Sci U S A 97:6155–6160
Jara-Oseguera A, Islas LD (2013) The role of allosteric coupling on thermal activation of thermoTRP channels. Biophys J 104:2160–2169
Jemal I, Soriano S, Conte AL, Morenilla C, Gomis A (2014) G protein-coupled receptor signalling potentiates the osmo-mechanical activation of TRPC5 channels. Pflugers Arch466(8):1635–1646. doi:10.1007/s00424-013-1392-z
Jin M, Berrout J, O'Neil RG (2011) Regulation of TRP channels by osmomechanical stress. In: Zhu MX (ed) TRP channels. Boca Raton. ISBN:9781439818602
Jordt SE, Julius D (2002) Molecular basis for species-specific sensitivity to "hot" chili peppers. Cell 108:421–430
Jordt SE, Tominaga M, Julius D (2000) Acid potentiation of the capsaicin receptor determined by a key extracellular site. Proc Natl Acad Sci U S A 97:8134–8139
Jordt SE, McKemy DD, Julius D (2003) Lessons from peppers and peppermint: the molecular logic of thermosensation. Curr Opin Neurobiol 13:487–492
Jordt SE, Bautista DM, Chuang HH, McKemy DD, Zygmunt PM, Hogestatt ED, Meng ID, Julius D (2004) Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. Nature 427:260–265
Kang D, Choe C, Kim D (2005) Thermosensitivity of the two-pore domain K+ channels TREK-2 and TRAAK. J Physiol 564:103–116
Kang K, Panzano VC, Chang EC, Ni L, Dainis AM, Jenkins AM, Regna K, Muskavitch MA, Garrity PA (2012) Modulation of TRPA1 thermal sensitivity enables sensory discrimination in *Drosophila*. Nature 481:76–80
Karashima Y, Talavera K, Everaerts W, Janssens A, Kwan KY, Vennekens R, Nilius B, Voets T (2009) TRPA1 acts as a cold sensor in vitro and in vivo. Proc Natl Acad Sci U S A 106:1273–1278
Karashima Y, Prenen J, Talavera K, Janssens A, Voets T, Nilius B (2010) Agonist-induced changes in Ca(2+) permeation through the nociceptor cation channel TRPA1. Biophys J 98:773–783

Kim J, Chung YD, Park DY, Choi S, Shin DW, Soh H, Lee HW, Son W, Yim J, Park CS, Kernan MJ, Kim C (2003) A TRPV family ion channel required for hearing in *Drosophila*. Nature 424:81–84

Kim SE, Patapoutian A, Grandl J (2013) Single residues in the outer pore of TRPV1 and TRPV3 have temperature-dependent conformations. PloS One 8:e59593

Kiselyov K, Chen J, Rbaibi Y, Oberdick D, Tjon-Kon-Sang S, Shcheynikov N, Muallem S, Soyombo A (2005) TRP-ML1 is a lysosomal monovalent cation channel that undergoes proteolytic cleavage. J Biol Chem 280:43218–43223

Kwan KY, Allchorne AJ, Vollrath MA, Christensen AP, Zhang DS, Woolf CJ, Corey DP (2006) TRPA1 contributes to cold, mechanical, and chemical nociception but is not essential for hair-cell transduction. Neuron 50:277–289

Latorre R, Brauchi S, Orta G, Zaelzer C, Vargas G (2007a) ThermoTRP channels as modular proteins with allosteric gating. Cell Calcium 42:427–438

Latorre R, Vargas G, Orta G, Brauchi S (2007b) Voltage and temperature gating of thermoTRP channels. In: Liedtke WB, Heller S (eds) TRP ion channel function in sensory transduction and cellular signaling cascades. Boca Raton. ISBN: 0849340899780849340482

Latorre R, Morera FJ, Zaelzer C (2010) Allosteric interactions and the modular nature of the voltage- and Ca2+ -activated (BK) channel. J Physiol 588:3141–3148

Lau SY, Procko E, Gaudet R (2012) Distinct properties of Ca2+ -calmodulin binding to N- and C-terminal regulatory regions of the TRPV1 channel. J Gen Physiol 140:541–555

Launay P, Fleig A, Perraud AL, Scharenberg AM, Penner R, Kinet JP (2002) TRPM4 is a Ca2+ -activated nonselective cation channel mediating cell membrane depolarization. Cell 109:397–407

Launay P, Cheng H, Srivatsan S, Penner R, Fleig A, Kinet JP (2004) TRPM4 regulates calcium oscillations after T cell activation. Science 306:1374–1377

Li M, Yu Y, Yang J (2011) Structural biology of TRP channels. Adv Exp Med Biol 704:1–23

Liao M, Cao E, Julius D, Cheng Y (2013) Structure of the TRPV1 ion channel determined by electron cryo-microscopy. Nature 504:107–112

Liedtke W, Choe Y, Marti-Renom MA, Bell AM, Denis CS, Sali A, Hudspeth AJ, Friedman JM, Heller S (2000) Vanilloid receptor-related osmotically activated channel (VR-OAC), a candidate vertebrate osmoreceptor. Cell 103:525–535

Lishko PV, Procko E, Jin X, Phelps CB, Gaudet R (2007) The ankyrin repeats of TRPV1 bind multiple ligands and modulate channel sensitivity. Neuron 54:905–918

Littleton JT, Ganetzky B (2000) Ion channels and synaptic organization: analysis of the *Drosophila* genome. Neuron 26:35–43

Liu B, Qin F (2005) Functional control of cold- and menthol-sensitive TRPM8 ion channels by phosphatidylinositol 4,5-bisphosphate. J Neurosci 25:1674–1681

Liu B, Hui K, Qin F (2003) Thermodynamics of heat activation of single capsaicin ion channels VR1. Biophys J 85:1–19

Lopez GA, Jan YN, Jan LY (1994) Evidence that the S6 segment of the shaker voltage-gated K+ channel comprises part of the pore. Nature 367:179–182

Lopez F, Delgado R, Lopez R, Bacigalupo J, Restrepo D (2014) Transduction for pheromones in the main olfactory epithelium is mediated by the Ca2+ -activated channel TRPM5. J Neurosci 34:3268–3278

Ludeman DA, Farrar N, Riesgo A, Paps J, Leys SP (2014) Evolutionary origins of sensation in metazoans: functional evidence for a new sensory organ in sponges. BMC Evol Biol 14:3

Macpherson LJ, Geierstanger BH, Viswanath V, Bandell M, Eid SR, Hwang S, Patapoutian A (2005) The pungency of garlic: activation of TRPA1 and TRPV1 in response to allicin. Curr Biol 15:929–934

Madrid R, Donovan-Rodriguez T, Meseguer V, Acosta MC, Belmonte C, Viana F (2006) Contribution of TRPM8 channels to cold transduction in primary sensory neurons and peripheral nerve terminals. J Neurosci 26:12512–12525

Madrid R, de la Pena E, Donovan-Rodriguez T, Belmonte C, Viana F (2009) Variable threshold of trigeminal cold-thermosensitive neurons is determined by a balance between TRPM8 and Kv1 potassium channels. J Neurosci 29:3120–3131

Maingret F, Lauritzen I, Patel AJ, Heurteaux C, Reyes R, Lesage F, Lazdunski M, Honore E (2000) TREK-1 is a heat-activated background K(+) channel. EMBO J 19:2483–2491
Malkia A, Madrid R, Meseguer V, de la Pena E, Valero M, Belmonte C, Viana F (2007) Bidirectional shifts of TRPM8 channel gating by temperature and chemical agents modulate the cold sensitivity of mammalian thermoreceptors. J Physiol 581:155–174
Malkia A, Pertusa M, Fernandez-Ballester G, Ferrer-Montiel A, Viana F (2009) Differential role of the menthol-binding residue Y745 in the antagonism of thermally gated TRPM8 channels. Mol Pain 5:62
Mandal M, Breaker RR (2004) Gene regulation by riboswitches. Nat Rev Mol Cell Biol 5:451–463
Mandadi S, Sokabe T, Shibasaki K, Katanosaka K, Mizuno A, et al (2009) TRPV3 in keratinocytes transmits temperature information to sensory neurons via ATP. Pflugers Arch 458:1093–1102
Manzoni M, Monti E, Bresciani R, Bozzato A, Barlati S, Bassi MT, Borsani G (2004) Overexpression of wild-type and mutant mucolipin proteins in mammalian cells: effects on the late endocytic compartment organization. FEBS Lett 567:219–224
Matta JA, Ahern GP (2007) Voltage is a partial activator of rat thermosensitive TRP channels. J Physiol 585:469–482
McHugh D, Flemming R, Xu SZ, Perraud AL, Beech DJ (2003) Critical intracellular Ca2+ dependence of transient receptor potential melastatin 2 (TRPM2) cation channel activation. J Biol Chem 278:11002–11006
McKemy DD, Neuhausser WM, Julius D (2002) Identification of a cold receptor reveals a general role for TRP channels in thermosensation. Nature 416:52–58
McNamara FN, Randall A, Gunthorpe MJ (2005) Effects of piperine, the pungent component of black pepper, at the human vanilloid receptor (TRPV1). Br J Pharmacol 144:781–790
Mei ZZ, Xia R, Beech DJ, Jiang LH (2006) Intracellular coiled-coil domain engaged in subunit interaction and assembly of melastatin-related transient receptor potential channel 2. J Biol Chem 281:38748–38756
Mercado J, Gordon-Shaag A, Zagotta WN, Gordon SE (2010) Ca2+ -dependent desensitization of TRPV2 channels is mediated by hydrolysis of phosphatidylinositol 4,5-bisphosphate. J Neurosci 30:13338–13347
Mercer JB, Simon E (1984) A comparison between total body thermosensitivity and local thermosensitivity in mammals and birds. Pflugers Arch 400:228–234
Monteilh-Zoller MK, Hermosura MC, Nadler MJ, Scharenberg AM, Penner R, Fleig A (2003) TRPM7 provides an ion channel mechanism for cellular entry of trace metal ions. J Gen Physiol 121:49–60
Montell C 2005. The TRP superfamily of cation channels. Sci STKE: Signal Transduct Knowl Environ 2005:re3
Montell C, Rubin GM (1989) Molecular characterization of the *Drosophila* trp locus: a putative integral membrane protein required for phototransduction. Neuron 2:1313–1323
Montell C, Birnbaumer L, Flockerzi V (2002a) The TRP channels, a remarkably functional family. Cell 108:595–598
Montell C, Birnbaumer L, Flockerzi V, Bindels RJ, Bruford EA, Caterina MJ, Clapham DE, Harteneck C, Heller S, Julius D, Kojima I, Mori Y, Penner R, Prawitt D, Scharenberg AM, Schultz G, Shimizu N, Zhu MX (2002b) A unified nomenclature for the superfamily of TRP cation channels. Mol Cell 9:229–231
Moqrich A, Hwang SW, Earley TJ, Petrus MJ, Murray AN, Spencer KS, Andahazy M, Story GM, Patapoutian A (2005) Impaired thermosensation in mice lacking TRPV3, a heat and camphor sensor in the skin. Science 307:1468–1472
Muraki K, Iwata Y, Katanosaka Y, Ito T, Ohya S, Shigekawa M, Imaizumi Y (2003) TRPV2 is a component of osmotically sensitive cation channels in murine aortic myocytes. Circ Res 93:829–838
Myers BR, Bohlen CJ, Julius D (2008) A yeast genetic screen reveals a critical role for the pore helix domain in TRP channel gating. Neuron 58:362–373

Nadler MJ, Hermosura MC, Inabe K, Perraud AL, Zhu Q, Stokes AJ, Kurosaki T, Kinet JP, Penner R, Scharenberg AM, Fleig A (2001) LTRPC7 is a Mg.ATP-regulated divalent cation channel required for cell viability. Nature 411:590–595

Nara T, Lee L, Imae Y (1991) Thermosensing ability of Trg and Tap chemoreceptors in *Escherichia coli*. J Bacteriol 173:1120–1124

Nauli SM, Alenghat FJ, Luo Y, Williams E, Vassilev P, Li X, Elia AE, Lu W, Brown EM, Quinn SJ, Ingber DE, Zhou J (2003) Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. Nat Genet 33:129–137

Nelson DL, Lehninger AL, Cox MM (2008) Lehninger principles of biochemistry. Macmillan. ISBN: 071677108X

Nilius B, Owsianik G (2011) The transient receptor potential family of ion channels. Genome Biol 12:218

Nilius B, Vennekens R, Prenen J, Hoenderop JG, Droogmans G, Bindels RJ (2001) The single pore residue Asp542 determines Ca2+ permeation and Mg2+ block of the epithelial Ca2+ channel. J Biol Chem 276:1020–1025

Nilius B, Prenen J, Droogmans G, Voets T, Vennekens R, Freichel M, Wissenbach U, Flockerzi V (2003) Voltage dependence of the Ca2+ -activated cation channel TRPM4. J Biol Chem 278:30813–30820

Nilius B, Prenen J, Janssens A, Voets T, Droogmans G (2004a) Decavanadate modulates gating of TRPM4 cation channels. J Physiol 560:753–765

Nilius B, Prenen J, Voets T, Droogmans G (2004b) Intracellular nucleotides and polyamines inhibit the Ca2+ -activated cation channel TRPM4b. Pflugers Arch 448:70–75

Nilius B, Prenen J, Janssens A, Owsianik G, Wang C, Zhu MX, Voets T (2005a) The selectivity filter of the cation channel TRPM4. J Biol Chem 280:22899–22906

Nilius B, Talavera K, Owsianik G, Prenen J, Droogmans G, Voets T (2005b) Gating of TRP channels: a voltage connection? J Physiol 567:35–44

Nilius B, Prenen J, Owsianik G (2011) Irritating channels: the case of TRPA1. J Physiol 589:1543–1549

Nilius B, Appendino G, Owsianik G (2012) The transient receptor potential channel TRPA1: from gene to pathophysiology. Pflugers Arch 464:425–458

Numazaki M, Tominaga T, Takeuchi K, Murayama N, Toyooka H, Tominaga M (2003) Structural determinant of TRPV1 desensitization interacts with calmodulin. Proc Natl Acad Sci U S A 100:8002–8006

Oberwinkler J, Phillipp SE (2007) TRPM3. Handb Exp Pharmacol 179: 253–267

Obukhov AG, Nowycky MC (2005) A cytosolic residue mediates Mg2+ block and regulates inward current amplitude of a transient receptor potential channel. J Neurosci 25:1234–1239

Ordaz B, Tang J, Xiao R, Salgado A, Sampieri A, Zhu MX, Vaca L (2005) Calmodulin and calcium interplay in the modulation of TRPC5 channel activity. Identification of a novel C-terminal domain for calcium/calmodulin-mediated facilitation. J Biol Chem 280:30788–30796

Orio P, Latorre R (2005) Differential effects of beta 1 and beta 2 subunits on BK channel activity. J Gen Physiol 125:395–411

Orio P, Madrid R, de la Pena E, Parra A, Meseguer V, Bayliss DA, Belmonte C, Viana F (2009) Characteristics and physiological role of hyperpolarization activated currents in mouse cold thermoreceptors. J Physiol 587:1961–1976

Owsianik G, Talavera K, Voets T, Nilius B (2006) Permeation and selectivity of TRP channels. Annu Rev Physiol 68:685–717

Palmer CP, Zhou XL, Lin J, Loukin SH, Kung C, Saimi Y (2001) A TRP homolog in *Saccharomyces cerevisiae* forms an intracellular Ca(2+)-permeable channel in the yeast vacuolar membrane. Proc Natl Acad Sci U S A 98:7801–7805

Papakosta M, Dalle C, Haythornthwaite A, Cao L, Stevens EB, Burgess G, Russell R, Cox PJ, Phillips SC, Grimm C (2011) The chimeric approach reveals that differences in the TRPV1 pore domain determine species-specific sensitivity to block of heat activation. J Biol Chem 286:39663–39672

Park U, Vastani N, Guan Y, Raja SN, Koltzenburg M, Caterina MJ (2011) TRP vanilloid 2 knock-out mice are susceptible to perinatal lethality but display normal thermal and mechanical nociception. J Neurosci 31:11425–11436

Peier AM, Moqrich A, Hergarden AC, Reeve AJ, Andersson DA, Story GM, Earley TJ, Dragoni I, McIntyre P, Bevan S, Patapoutian A (2002a) A TRP channel that senses cold stimuli and menthol. Cell 108:705–715

Peier AM, Reeve AJ, Andersson DA, Moqrich A, Earley TJ, Hergarden AC, Story GM, Colley S, Hogenesch JB, McIntyre P, Bevan S, Patapoutian A (2002b) A heat-sensitive TRP channel expressed in keratinocytes. Science 296:2046–2049

Perraud AL, Fleig A, Dunn CA, Bagley LA, Launay P, Schmitz C, Stokes AJ, Zhu Q, Bessman MJ, Penner R, Kinet JP, Scharenberg AM (2001) ADP-ribose gating of the calcium-permeable LTRPC2 channel revealed by Nudix motif homology. Nature 411:595–599

Perraud AL, Schmitz C, Scharenberg AM (2003) TRPM2 Ca2+ permeable cation channels: from gene to biological function. Cell Calcium 33:519–531

Pertusa M, Madrid R, Morenilla-Palao C, Belmonte C, Viana F (2012) N-glycosylation of TRPM8 ion channels modulates temperature sensitivity of cold thermoreceptor neurons. J Biol Chem 287:18218–18229

Petersen CC, Berridge MJ, Borgese MF, Bennett DL (1995) Putative capacitative calcium entry channels: expression of *Drosophila* trp and evidence for the existence of vertebrate homologues. Biochem J 311(Pt 1):41–44

Petsko GA, Ringe D (2004) Protein structure and function. New Science Press

Phelps CB, Gaudet R (2007) The role of the N terminus and transmembrane domain of TRPM8 in channel localization and tetramerization. J Biol Chem 282:36474–36480

Phelps CB, Wang RR, Choo SS, Gaudet R (2010) Differential regulation of TRPV1, TRPV3, and TRPV4 sensitivity through a conserved binding site on the ankyrin repeat domain. J Biol Chem 285:731–740

Phillips AM, Bull A, Kelly LE (1992) Identification of a *Drosophila* gene encoding a calmodulin-binding protein with homology to the trp phototransduction gene. Neuron 8:631–642

Pieau C, Dorizzi M, Richard-Mercier N (2001) Temperature-dependent sex determination and gonadal differentiation in reptiles. EXS 91: 117–141

Premkumar LS, Agarwal S, Steffen D (2002) Single-channel properties of native and cloned rat vanilloid receptors. J Physiol 545:107–117

Prescott ED, Julius D (2003) A modular PIP2 binding site as a determinant of capsaicin receptor sensitivity. Science 300:1284–1288

Prober DA, Zimmerman S, Myers BR, McDermott BM Jr, Kim SH, Caron S, Rihel J, Solnica-Krezel L, Julius D, Hudspeth AJ, Schier AF (2008) Zebrafish TRPA1 channels are required for chemosensation but not for thermosensation or mechanosensory hair cell function. J Neurosci 28:10102–10110

Prole DL, Taylor CW (2011) Identification of intracellular and plasma membrane calcium channel homologues in pathogenic parasites. PloS One 6:e26218

Qin N, Neeper MP, Liu Y, Hutchinson TL, Lubin ML, Flores CM (2008) TRPV2 is activated by cannabidiol and mediates CGRP release in cultured rat dorsal root ganglion neurons. J Neurosci 28:6231–6238

Ramsey IS, Delling M, Clapham DE (2006) An introduction to TRP channels. Annu Rev Physiol 68:619–647

Reid G (2005) ThermoTRP channels and cold sensing: what are they really up to? Pflugers Archiv 451:250–263

Rohacs T, Lopes CM, Michailidis I, Logothetis DE (2005) PI(4,5)P2 regulates the activation and desensitization of TRPM8 channels through the TRP domain. Nat Neurosci 8:626–634

Rosenbaum T, Gordon-Shaag A, Munari M, Gordon SE (2004) Ca2+/calmodulin modulates TRPV1 activation by capsaicin. J Gen Physiol 123:53–62

Ross RA (2003) Anandamide and vanilloid TRPV1 receptors. Br J Pharmacol 140:790–801

Runnels LW, Yue L, Clapham DE (2001) TRP-PLIK, a bifunctional protein with kinase and ion channel activities. Science 291:1043–1047

Ryu S, Liu B, Yao J, Fu Q, Qin F (2007) Uncoupling proton activation of vanilloid receptor TRPV1. J Neurosci 27:12797–12807

Saito S, Shingai R (2006) Evolution of thermoTRP ion channel homologs in vertebrates. Physiol Genomics 27:219–230

Saito S, Fukuta N, Shingai R, Tominaga M (2011) Evolution of vertebrate transient receptor potential vanilloid 3 channels: opposite temperature sensitivity between mammals and western clawed frogs. PLoS Genet 7:e1002041

Saito S, Nakatsuka K, Takahashi K, Fukuta N, Imagawa T, Ohta T, Tominaga M (2012) Analysis of transient receptor potential ankyrin 1 (TRPA1) in frogs and lizards illuminates both nociceptive heat and chemical sensitivities and coexpression with TRP vanilloid 1 (TRPV1) in ancestral vertebrates. J Biol Chem 287:30743–30754

Salas MM, Hargreaves KM, Akopian AN (2009) TRPA1-mediated responses in trigeminal sensory neurons: interaction between TRPA1 and TRPV1. Eur J Neurosci 29:1568–1578

Salazar H, Llorente I, Jara-Oseguera A, Garcia-Villegas R, Munari M, Gordon SE, Islas LD, Rosenbaum T (2008) A single N-terminal cysteine in TRPV1 determines activation by pungent compounds from onion and garlic. Nat Neurosci 11:255–261

Samad A, Sura L, Benedikt J, Ettrich R, Minofar B, Teisinger J, Vlachova V (2011) The C-terminal basic residues contribute to the chemical- and voltage-dependent activation of TRPA1. Biochem J 433:197–204

Sano Y, Inamura K, Miyake A, Mochizuki S, Yokoi H, Matsushime H, Furuichi K (2001) Immunocyte Ca2+ influx system mediated by LTRPC2. Science 293:1327–1330

Sardar P, Kumar A, Bhandari A, Goswami C (2012) Conservation of tubulin-binding sequences in TRPV1 throughout evolution. PloS One 7:e31448

Schaefer M (2005) Homo- and heteromeric assembly of TRP channel subunits. Pflugers Arch 451:35–42

Schlingmann KP, Weber S, Peters M, Niemann Nejsum L, Vitzthum H, Klingel K, Kratz M, Haddad E, Ristoff E, Dinour D, Syrrou M, Nielsen S, Sassen M, Waldegger S, Seyberth HW, Konrad M (2002) Hypomagnesemia with secondary hypocalcemia is caused by mutations in TRPM6, a new member of the TRPM gene family. Nat Genet 31:166–170

Schoppa NE, Sigworth FJ (1998) Activation of shaker potassium channels. III. An activation gating model for wild-type and V2 mutant channels. J Gen Physiol 111:313–342

Semtner M, Schaefer M, Pinkenburg O, Plant TD (2007) Potentiation of TRPC5 by protons. J Biol Chem 282:33868–33878

Shimizu S, Yoshida T, Wakamori M, Ishii M, Okada T, Takahashi M, Seto M, Sakurada K, Kiuchi Y, Mori Y (2006) Ca2+ -calmodulin-dependent myosin light chain kinase is essential for activation of TRPC5 channels expressed in HEK293 cells. J Physiol 570:219–235

Sidi S, Friedrich RW, Nicolson T (2003) NompC TRP channel required for vertebrate sensory hair cell mechanotransduction. Science 301:96–99

Smith GD, Gunthorpe MJ, Kelsell RE, Hayes PD, Reilly P, Facer P, Wright JE, Jerman JC, Walhin JP, Ooi L, Egerton J, Charles KJ, Smart D, Randall AD, Anand P, Davis JB (2002) TRPV3 is a temperature-sensitive vanilloid receptor-like protein. Nature 418:186–190

Staruschenko A, Jeske NA, Akopian AN (2010) Contribution of TRPV1-TRPA1 interaction to the single channel properties of the TRPA1 channel. J Biol Chem 285:15167–15177

Stayner C, Zhou J (2001) Polycystin channels and kidney disease. Trends Pharmacol Sci 22:543–546

Stewart AP, Egressy K, Lim A, Edwardson JM (2010) AFM imaging reveals the tetrameric structure of the TRPM8 channel. Biochem Biophys Res Commun 394:383–386

Story GM, Peier AM, Reeve AJ, Eid SR, Mosbacher J, Hricik TR, Earley TJ, Hergarden AC, Andersson DA, Hwang SW, McIntyre P, Jegla T, Bevan S, Patapoutian A (2003) ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. Cell 112:819–829

Strotmann R, Harteneck C, Nunnenmacher K, Schultz G, Plant TD (2000) OTRPC4, a nonselective cation channel that confers sensitivity to extracellular osmolarity. Nat Cell Biol 2:695–702

Strotmann R, Schultz G, Plant TD (2003) Ca2+ -dependent potentiation of the nonselective cation channel TRPV4 is mediated by a C-terminal calmodulin binding site. J Biol Chem 278:26541–26549

Strotmann R, Semtner M, Kepura F, Plant TD, Schoneberg T (2010) Interdomain interactions control Ca2+ -dependent potentiation in the cation channel TRPV4. PloS One 5:e10580

Strubing C, Krapivinsky G, Krapivinsky L, Clapham DE (2003) Formation of novel TRPC channels by complex subunit interactions in embryonic brain. J Biol Chem 278:39014–39019

Sun M, Goldin E, Stahl S, Falardeau JL, Kennedy JC, Acierno JS Jr, Bove C, Kaneski CR, Nagle J, Bromley MC, Colman M, Schiffmann R, Slaugenhaupt SA (2000) Mucolipidosis type IV is caused by mutations in a gene encoding a novel transient receptor potential channel. Hum Mol Genet 9:2471–2478

Sung TS, Jeon JP, Kim BJ, Hong C, Kim SY, Kim J, Jeon JH, Kim HJ, Suh CK, Kim SJ, So I (2011) Molecular determinants of PKA-dependent inhibition of TRPC5 channel. Am J Physiol Cell Physiol 301:C823–C832

Sura L, Zima V, Marsakova L, Hynkova A, Barvik I, Vlachova V (2012) C-terminal acidic cluster is involved in Ca2+ -induced regulation of human transient receptor potential ankyrin 1 channel. J Biol Chem 287:18067–18077

Susankova K, Ettrich R, Vyklicky L, Teisinger J, Vlachova V (2007) Contribution of the putative inner-pore region to the gating of the transient receptor potential vanilloid subtype 1 channel (TRPV1). J Neurosci 27:7578–7585

Talavera K, Yasumatsu K, Voets T, Droogmans G, Shigemura N, Ninomiya Y, Margolskee RF, Nilius B (2005) Heat activation of TRPM5 underlies thermal sensitivity of sweet taste. Nature 438:1022–1025

Todaka H, Taniguchi J, Satoh J, Mizuno A, Suzuki M (2004) Warm temperature-sensitive transient receptor potential vanilloid 4 (TRPV4) plays an essential role in thermal hyperalgesia. J Biol Chem 279:35133–35138

Togashi K, Hara Y, Tominaga T, Higashi T, Konishi Y, Mori Y, Tominaga M (2006) TRPM2 activation by cyclic ADP-ribose at body temperature is involved in insulin secretion. EMBO J 25:1804–1815

Tominaga M, Caterina MJ, Malmberg AB, Rosen TA, Gilbert H, Skinner K, Raumann BE, Basbaum AI, Julius D (1998) The cloned capsaicin receptor integrates multiple pain-producing stimuli. Neuron 21:531–543

Tong Q, Zhang W, Conrad K, Mostoller K, Cheung JY, Peterson BZ, Miller BA (2006) Regulation of the transient receptor potential channel TRPM2 by the Ca2+ sensor calmodulin. J Biol Chem 281:9076–9085

Topala CN, Groenestege WT, Thebault S, van den Berg D, Nilius B, Hoenderop JG, Bindels RJ (2007) Molecular determinants of permeation through the cation channel TRPM6. Cell Calcium 41:513–523

Trevisani M, Smart D, Gunthorpe MJ, Tognetto M, Barbieri M, Campi B, Amadesi S, Gray J, Jerman JC, Brough SJ, Owen D, Smith GD, Randall AD, Harrison S, Bianchi A, Davis JB, Geppetti P (2002) Ethanol elicits and potentiates nociceptor responses via the vanilloid receptor-1. Nat Neurosci 5:546–551

Tse-Dinh YC, Qi H, Menzel R (1997) DNA supercoiling and bacterial adaptation: thermotolerance and thermoresistance. Trends Microbiol 5:323–326

Tsuruda PR, Julius D, Minor DL Jr (2006) Coiled coils direct assembly of a cold-activated TRP channel. Neuron 51:201–212

Ufret-Vincenty CA, Klein RM, Hua L, Angueyra J, Gordon SE (2011) Localization of the PIP2 sensor of TRPV1 ion channels. J Biol Chem 286:9688–9698

Venkatachalam K, Montell C (2007) TRP channels. Annu Rev Biochem 76:387–417

Venkatachalam K, Hofmann T, Montell C (2006) Lysosomal localization of TRPML3 depends on TRPML2 and the mucolipidosis-associated protein TRPML1. J Biol Chem 281:17517–17527

Vennekens R, Nilius B (2007) Insights into TRPM4 function, regulation and physiological role. Handb Exp pharmacol. pp 269-285

Vigh L, Maresca B, Harwood JL (1998) Does the membrane's physical state control the expression of heat shock and other genes? Trends Biochem Sci 23:369–374
Viswanath V, Story GM, Peier AM, Petrus MJ, Lee VM, Hwang SW, Patapoutian A, Jegla T (2003) Opposite thermosensor in fruitfly and mouse. Nature 423:822–823
Vlachova V, Teisinger J, Susankova K, Lyfenko A, Ettrich R, Vyklicky L (2003) Functional role of C-terminal cytoplasmic tail of rat vanilloid receptor 1. J Neurosci 23:1340–1350
Voets T, Nilius B (2003) The pore of TRP channels: trivial or neglected? Cell Calcium 33:299–302
Voets T, Prenen J, Vriens J, Watanabe H, Janssens A, Wissenbach U, Bodding M, Droogmans G, Nilius B (2002) Molecular determinants of permeation through the cation channel TRPV4. J Biol Chem 277:33704–33710
Voets T, Janssens A, Prenen J, Droogmans G, Nilius B (2003) Mg2+ -dependent gating and strong inward rectification of the cation channel TRPV6. J Gen Physiol 121:245–260
Voets T, Droogmans G, Wissenbach U, Janssens A, Flockerzi V, Nilius B (2004) The principle of temperature-dependent gating in cold- and heat-sensitive TRP channels. Nature 430:748–754
Voets T, Owsianik G, Janssens A, Talavera K, Nilius B (2007) TRPM8 voltage sensor mutants reveal a mechanism for integrating thermal and chemical stimuli. Nat Chem Biol 3:174–182
Vriens J, Owsianik G, Hofmann T, Philipp SE, Stab J, Chen X, Benoit M, Xue F, Janssens A, Kerselaers S, Oberwinkler J, Vennekens R, Gudermann T, Nilius B, Voets T (2011) TRPM3 is a nociceptor channel involved in the detection of noxious heat. Neuron 70:482–494
Wagner TF, Loch S, Lambert S, Straub I, Mannebach S, Mathar I, Dufer M, Lis A, Flockerzi V, Philipp SE, Oberwinkler J (2008) Transient receptor potential M3 channels are ionotropic steroid receptors in pancreatic beta cells. Nat Cell Biol 10:1421–1430
Walder RY, Landau D, Meyer P, Shalev H, Tsolia M, Borochowitz Z, Boettger MB, Beck GE, Englehardt RK, Carmi R, Sheffield VC (2002) Mutation of TRPM6 causes familial hypomagnesemia with secondary hypocalcemia. Nat Genet 31:171–174
Walker RG, Willingham AT, Zuker CS (2000) A *Drosophila* mechanosensory transduction channel. Science 287:2229–2234
Wang G, Qiu YT, Lu T, Kwon HW, Pitts RJ, Van Loon JJ, Takken W, Zwiebel LJ (2009) Anopheles gambiae TRPA1 is a heat-activated channel expressed in thermosensitive sensilla of female antennae. Eur J Neurosci 30:967–974
Wang H, Schupp M, Zurborg S, Heppenstall PA (2013) Residues in the pore region of *Drosophila* transient receptor potential A1 dictate sensitivity to thermal stimuli. J Physiol 591:185–201
Watanabe H, Davis JB, Smart D, Jerman JC, Smith GD, Hayes P, Vriens J, Cairns W, Wissenbach U, Prenen J, Flockerzi V, Droogmans G, Benham CD, Nilius B (2002a) Activation of TRPV4 channels (hVRL-2/mTRP12) by phorbol derivatives. J Biol Chem 277:13569–13577
Watanabe H, Vriens J, Suh SH, Benham CD, Droogmans G, Nilius B (2002b) Heat-evoked activation of TRPV4 channels in a HEK293 cell expression system and in native mouse aorta endothelial cells. J Biol Chem 277:47044–47051
Wes PD, Chevesich J, Jeromin A, Rosenberg C, Stetten G, Montell C (1995) TRPC1, a human homolog of a Drosophila store-operated channel. Proc Natl Acad Sci U S A 92:9652–9656
Wirkner K, Hognestad H, Jahnel R, Hucho F, Illes P (2005) Characterization of rat transient receptor potential vanilloid 1 receptors lacking the N-glycosylation site N604. Neuroreport 16:997–1001
Wissenbach U, Bodding M, Freichel M, Flockerzi V (2000) Trp12, a novel Trp related protein from kidney. FEBS Lett 485:127–134
Wolstenholme AJ, Williamson SM, Reaves BJ (2011) TRP channels in parasites. Adv Exp Med Biol 704:359–371
Xiao R, Tang J, Wang C, Colton CK, Tian J, Zhu MX (2008) Calcium plays a central role in the sensitization of TRPV3 channel to repetitive stimulations. J Biol Chem 283:6162–6174
Xu H, Ramsey IS, Kotecha SA, Moran MM, Chong JA, Lawson D, Ge P, Lilly J, Silos-Santiago I, Xie Y, DiStefano PS, Curtis R, Clapham DE (2002) TRPV3 is a calcium-permeable temperature-sensitive cation channel. Nature 418:181–186
Xu H, Blair NT, Clapham DE (2005) Camphor activates and strongly desensitizes the transient receptor potential vanilloid subtype 1 channel in a vanilloid-independent mechanism. J Neurosci 25:8924–8937

Xu H, Delling M, Jun JC, Clapham DE (2006) Oregano, thyme and clove-derived flavors and skin sensitizers activate specific TRP channels. Nat Neurosci 9:628–635

Yang F, Cui Y, Wang K, Zheng J (2010) Thermosensitive TRP channel pore turret is part of the temperature activation pathway. Proc Natl Acad Sci U S A 107:7083–7088

Yang F, Ma L, Cao X, Wang K, Zheng J (2014) Divalent cations activate TRPV1 through promoting conformational change of the extracellular region. J Gen Physiol 143:91–103

Yao J, Liu B, Qin F (2011) Modular thermal sensors in temperature-gated transient receptor potential (TRP) channels. Proc Natl Acad Sci U S A 108:11109–11114

Yeh BI, Kim YK, Jabbar W, Huang CL (2005) Conformational changes of pore helix coupled to gating of TRPV5 by protons. EMBO J 24:3224–3234

Yoshida T, Inoue R, Morii T, Takahashi N, Yamamoto S, Hara Y, Tominaga M, Shimizu S, Sato Y, Mori Y (2006) Nitric oxide activates TRP channels by cysteine S-nitrosylation. Nat Chem Biol 2:596–607

Zakharian E, Cao C, Rohacs T (2010) Gating of transient receptor potential melastatin 8 (TRPM8) channels activated by cold and chemical agonists in planar lipid bilayers. J Neurosci 30:12526–12534

Zaritsky JJ, Redell JB, Tempel BL, Schwarz TL (2001) The consequences of disrupting cardiac inwardly rectifying K(+) current (I(K1)) as revealed by the targeted deletion of the murine Kir2.1 and Kir2.2 genes. J Physiol 533:697–710

Zeng F, Xu SZ, Jackson PK, McHugh D, Kumar B, Fountain SJ, Beech DJ (2004) Human TRPC5 channel activated by a multiplicity of signals in a single cell. J Physiol 559:739–750

Zhang F, Liu S, Yang F, Zheng J, Wang K (2011) Identification of a tetrameric assembly domain in the C terminus of heat-activated TRPV1 channels. J Biol Chem 286:15308–15316

Zhu X, Chu PB, Peyton M, Birnbaumer L (1995) Molecular cloning of a widely expressed human homologue for the *Drosophila* trp gene. FEBS Lett 373:193–198

Zimmermann K, Lennerz JK, Hein A, Link AS, Kaczmarek JS, Delling M, Uysal S, Pfeifer JD, Riccio A, Clapham DE (2011) Transient receptor potential cation channel, subfamily C, member 5 (TRPC5) is a cold-transducer in the peripheral nervous system. Proc Natl Acad Sci U S A 108:18114–18119

Zurborg S, Yurgionas B, Jira JA, Caspani O, Heppenstall PA (2007) Direct activation of the ion channel TRPA1 by Ca2+. Nat Neurosci 10:277–279

Chapter 2
Pharmacology of TRP Channels

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Abstract TRP channels are a family of ion channels involved in a plethora of physiological sensory processes. Since their discovery they have attracted the attention of academic and non-academic laboratories with the aim of developing modulators that could be used as pharmacological tools for unveiling their physiological and pathological activities, and as therapeutic compounds for intervening in TRP dysfunction. Intriguingly, TRP pharmacology shows dispersed progress, with vast pharmacology developed for some members of the so-called thermoTRP channel subfamily (TRPV1, TRPV3, TRPM8 and TRPA1), and very little, for all other TRP channels. Pharmacologically, the most investigated TRP channel is undoubtedly TRPV1 for which a large number of agonists and antagonists with *in vitro* and *in vivo* activities have been characterized. Recent interest has grown for TRPV3, TRPM8 and TRPA1 because of their implication in several human pathologies and disorders. Similarly, the TRPM3 channel is emerging as important targets for pain transduction. With the development of novel screening methods, the focus is slowly changing to other TRP members for whom we do not have appropriate agonists or antagonists. These include the TRPC family, which has limited our understanding of their role in pathological processes and whether pharmacological intervention in these channels will have a therapeutic benefit. A bright future is anticipated for TRP pharmacology, with the discovery of selective and potent modulators for this important family of sensory channels.

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2.1 Introduction

TRP channels are a superfamily of ion channels that includes seven subfamilies, namely TRPC, TRPV, TRPP, TRPM, TRPA, TRPML, and TRPN. These channels perform a wide diversity of physiological functions and are present in many tissues, and almost all cell types. Most TRP channels are non-selective cation channels with low voltage dependence. TRP channels use a wide variety of activation and regulatory mechanisms and carry out functions as diverse as thermosensation, phototransduction, pheromone reception, magnesium homeostasis, and vascular tone regulation (Montell 1999) (see Chap. 4 by Bacigalupo et al. in this Book). Thus, these channels are considered molecular gateways in sensory and regulatory systems.

Structurally, TRP channels are tetrameric assemblies of basic subunits organized around a central aqueous pore. Akin to voltage-gated K^+ channels, each subunit is composed of a transmembrane region containing 6 transmembrane segments. The recent structural model derived from cryo-electron microscopic images has clearly shown this molecular analogy (Liao et al. 2013). All TRP channels display this core transmembrane region, and differ in the cytosolic N- and C-termini domains, which are involved in channel gating and mediating intracellular signaling. Indeed, most of TRP channels, if not all, are part of protein complexes known as signalplexes (Devesa et al. 2011; Fernandez-Carvajal et al. 2011; Ferrer-Montiel et al. 2012).

Some TRP channels have been involved in the pathophysiology of human diseases. This pathological contribution could be the result of channel mutations, giving rise to channelopathies (Devesa et al. 2011; Fernandez-Carvajal et al. 2011; Ferrer-Montiel et al. 2012), or the change in channel function due to alteration of the protein function and/or expression (Devesa et al. 2011; Fernandez-Carvajal et al. 2011; Ferrer-Montiel et al. 2012). The pivotal involvement in the etiology of pathological conditions has signaled members of this large channel family as druggable targets for therapeutic intervention, which has driven discovery programs in academic and non-academic institutions. This concerted effort has notably expanded the pharmacology of TRP channels, although, unfortunately, for a limited number of TRP members. For instance, large families of modulators have been obtained for TRPV1, TRPV3, TRPM8 and TRPA1, while the pharmacology of other TRP channels is still in its infancy. A plausible reason for the pharmacological progress in these channels is the availability of natural ligands present in food spices. Nonetheless, the development of combinatorial chemistry and the large diversity of vegetal and marine extracts, along with the development of high throughput electrophysiological assays for ion channels will expand the pharmacology of TRP channels to the entire family. Here, we briefly expose the pharmacological data for the most studied TRP channels, namely TRPV1, TRPM8 and TRPA1, and include the data accrued for TRPV2, TRPV4, TRPM3 and the TRPC5, most of them with an

important role in sensory transduction. We aim to illustrate the differential pharmacological progress in this exciting field and evidence a drift towards enhancing the pharmacology of other members, if not all, of this pivotal channel family.

2.2 TRPV1

TRP Vanillod 1, TRPV1, a non-selective $Ca2^{+}$ channel is a TRP channel activated by noxious temperatures (43 °C) acidic pH and vanilloid compounds, whose channel activity is highly potentiated by proalgesic mediators in response to inflammation, tissue injury and ischemia (Huang et al. 2006; Ueda et al. 2008). In addition, TRPV1 expression is markedly up-regulated under acute inflammatory conditions (Camprubi-Robles et al. 2009; Morenilla-Palao et al. 2004; Van Buren et al. 2005), and in human chronic pain states (Broad et al. 2008; Szallasi and Blumberg 2007). Consistent with a role in pain signaling, TRPV1 is highly expressed in C-type, peptidergic nociceptors in the peripheral nervous system. Thus, TRPV1 is considered a gateway for pain transduction, and a pivotal target for drug intervention in pain syndromes. In addition, due to a widespread tissue distribution of this TRP channel, it may be involved in the etiology of other human pathologies or disorders (Avelino et al. 2002; Inoue et al. 2002).

TRPV1 sensitization by inflammatory conditions is produced through two distinct, but complementary mechanisms, namely: (i) covalent modification of the channel by protein kinase A (PKA) and/or protein kinase C (PKC) phosphorylation (Bhave et al. 2003; Tominaga et al. 2001; Varga et al. 2006; Vellani et al. 2001); and, (ii) rapid recruitment of a vesicular population of TRPV1 channels to the neuronal surface through a $Ca2^{+}$ -dependent, SNARE-mediated exocytosis mechanism in response to pro-algesic agents (Camprubi-Robles et al. 2009; Zhang et al. 2005).

Pharmacologically, TRPV1 is primarily activated by a diverse collection of chemical ligands known as vanilloids (Caterina et al. 2000; Khairatkar-Joshi and Szallasi 2009) (Fig. 2.1). The most known agonist of TRPV1 is capsaicin, the pungent compound of chili peppers. Resiniferatoxin (RTX), a vanilloid from *Euphorbia resinifera*, is also a potent agonist of the receptor. Furthermore, TRPV1 may also be activated by non-vanilloid compounds, such as allicin, piperine, camphor, olvanil, 2-aminoethoxydiphenylborate (2-APB), and tarantula venom peptide toxins (Bohlen et al. 2010) (Table 2.1). In addition, there is a family of endogenous compounds, referred to as endovanilloids, that also act as agonists of TRPV1 (Van Der Stelt and Di 2004). These compounds may be divided into conjugates of biogenic amines [e.g., N-arachidonoylathanolamine (AEA, anandamide), N-arachidonoyldopamine (NADA), N-oleoylethanolamine (OLEA), N-arachidonolylserine, and various N-acyltaurines and N-acylsalsolinols (Appendino et al. 2008), and oxygenated eicosatetraenoic acids like the lipoxygenase products 5-, 12-, and 15-hydroperoxyeicosatetraenoic acids (5S-, 12S-, 15S-HPETE), their reduced hydroxyl analogs, prostaglandins, and leukotriene B4 (Ahern 2003; Huang et al. 2006; Wang et al. 2005) (Fig. 2.1).

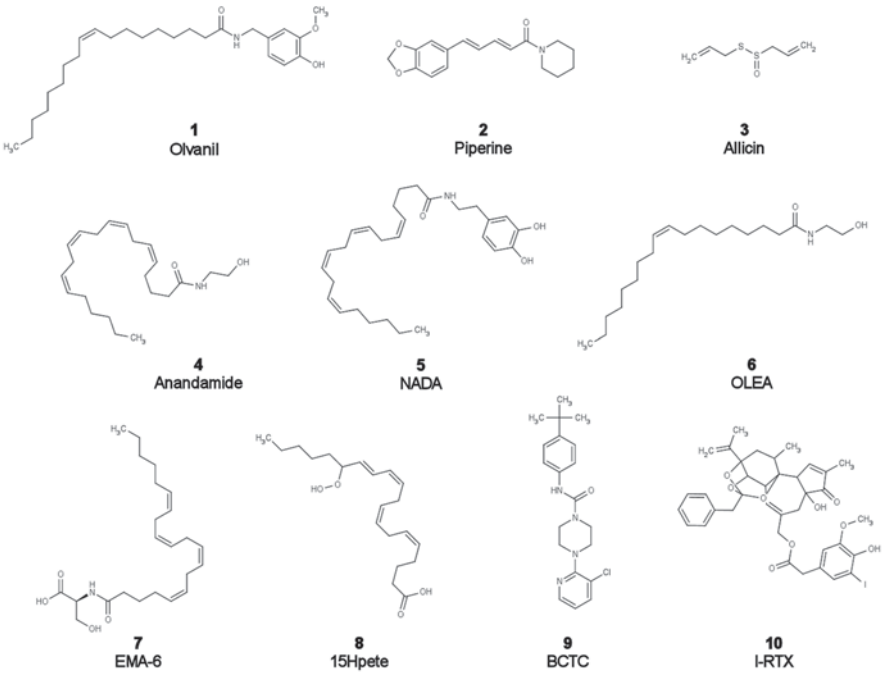

Fig. 2.1 Selected examples of activators (1–8) and inhibitors (9–10) of TRPV1. **1** Olvanil (CID 5311093). **2** Piperine (CID 638024). **3** Allicin (CID 65036). **4** Anandamide (CID 5281969). **5** NADA: N-arachidonoyl dopamine. (CID 5282105). **6** OLEA: N-oleoyl ethanolamine (CID 5283454). **7** EMA-6: N-arachidonoyl serine (CID 10596625). **8** 15-Hpete: 15-hydroperoxy eicosatetraenoic acid (CID 6437084). **9** BCTC (CID 9929425). **10** I-RTX: 5-iodoresiniferatoxin (CID 16219535)

As expected, the activation of TRPV1 in nociceptors with vanilloids causes a burning pain sensation and irritation. Paradoxically, capsaicin has been in use for many years as anti-nociceptive compound in peripheral neuropathies (e.g., post-herpetic neuralgia, neuropathy, mastectomy, amputation and skin cancer). Capsaicin is used as an analgesic, because in addition to activate the channel, it also induces its desensitization. Furthermore, the repetitive application of the vanilloid produces a rundown of channel activity known as tachyphylaxia that results in a strong anti-nociceptive effect (Knotkova et al. 2008). This analgesia may be accompanied by reversible and/or irreversible loss of the capsaicin sensitive C-fibers (Hiura 2000).

Although TRPV1 agonists may have some therapeutic application, their low in vivo activity, along with their poor bioavailability and secondary effects has limited their development as anti-nociceptives, and promoted the research into the design of potent antagonists that display higher therapeutic index. The efforts in developing TRPV1 antagonists have been concentrated in obtaining both competitive and non-competitive (including uncompetitive) inhibitors (Planells-Cases et al. 2003; Szallasi and Appendino 2004). Uncompetitive antagonists acting as open channel

blockers are activity-dependent blockers that preferentially bind to over-activated receptors, with minimal interaction with the physiologically working channels. Accordingly, they are supposed to display lower side-effects than conventional antagonists.

Among the competitive TRPV1 antagonists (Fig. 2.1), capsazepine was the first identified, although with poor in vivo activity (Bevan et al. 1992; Walker et al. 2003). A vanilloid with better therapeutic potential is 5-iodo-RTX, a potent TRPV1antagonist (IC_{50} = 3.9 nM) (McDonnell et al. 2002; Wahl et al. 2001). This compound produced notable analgesic activity in vivo and it is currently under clinical studies.

The family of competitive antagonists grew tremendously thanks to the contribution of pharmaceutical companies that established strong drug discovery programs for TRPV1 channels. As a result, ultra-high affinity synthetic antagonists were discovered for analgesic drug development. However, most of the clinical trials for these compounds had to be cancelled in Phase I because the indiscriminate blockade of TRPV1 channels with these compounds resulted in significant hyperthermia in humans, suggesting that this receptor also plays a pivotal role in core body temperature (Gavva et al. 2008).

The first non-competitive TRPV1 antagonist was the trinuclear polyamine complex, ruthenium red that was followed by arginine-rich peptides, and peptidomimetic compounds such as peptoids DD00069 and DD01050 (Garcia-Martinez et al. 2002, 2006). All these compounds resulted in unacceptable in vivo side effects and toxicity that prevented their clinical development. Recently, an uncompetitive antagonist, based in a triazine scaffold (triazine 8aA) that block TRPV1 channel by an activity-dependent mechanism was reported (Vidal-Mosquera et al. 2011). Triazine 8aA showed a strong voltage-dependent TRPV1 blockade by inhibiting at negative membrane potential, a hallmark of open-channel blockers. This compound holds promise for therapeutic development, although in vivo activity in pain models has not been yet reported.

Allosteric modulators of TRPV1 activity are another class of non-competitive antagonists. These compounds interfere with the allosteric mechanism that gates the channel. Structure-function analysis of TRPV1 channels demonstrated that the intracellular TRP domain, a highly conserved region adjacent to the receptor internal gate (Venkatachalam and Montell 2007), is essential for subunit tetramerization and allosteric activation (Garcia-Sanz et al. 2004, 2007). Thus, this protein interface could be used as an allosteric site to modulate channel function. Indeed, compound TRP-p5, a palmitoylated 13-mer peptide patterned after the N-terminus region of the TRP domain, displays in vitro and in vivo inhibitory activity (Valente et al. 2011). This finding is proof-of-concept that allosteric modulators such as TRPducins represent another family of non-competitive antagonists that could be developed therapeutically as anti-nociceptives.

A complementary approach to reduce the inflammatory sensitization of TRPV1 has been to interfere with the recruitment of the channel to the neuronal surface. This strategy has proven that blockers of neuronal exocytosis such as compound DD04107 display analgesic activity (Ponsati et al. 2012). In vitro experiments with DD04107 showed that it blocked the inflammatory over expression of TRPV1

channels to the plasma membrane (Camprubi-Robles et al. 2009). In vivo, this compound displays long-lasting anti-nociceptive activity against inflammatory and neuropathic pain, without apparent side effects, demonstrating that acting on the TRPV1 signalplex may be a valuable pharmacological strategy (Ponsati et al. 2012). This compound is being developed clinically.

2.3 TRPV2

At variance with TRPV1 channels, the pharmacology of its close homologue TRPV2 is still in its infancy (Peralvarez-Marin et al. 2013). This non-selective $Ca2^{+}$ channel is also present in the peripheral nervous system and co-localizes with TRPV1 in a subset of nociceptors (Liapi and Wood 2005). The physiological role of this TRP channel is yet elusive. Initially was considered a thermoTRP channel that activated at 52 °C, and also responded to hypotonicity (Caterina et al. 1999; Muraki et al. 2003). However, these are still highly debated functions (Park et al. 2011; Peralvarez-Marin et al. 2013), thus requiring further investigation, including the discovery of agonists and antagonists that could be used as pharmacological tools.

The identification of specific TRPV2 modulators is, surprisingly, inexistent, probably due to the species-specific pharmacology coupled with problems in developing stable recombinant cell lines due to cytotoxic effects of TRPV2 expression (Penna et al. 2006). Several chemical compounds have been shown to modulate TRPV2, however, virtually all of them are non-specific (Table 2.1 and Fig. 2.2). Indeed, TRPV2 is activated by general TRP channel agonists, such as 2-aminoethoxy-diphenyl borate (2-APB), probenecid, lysophospholipids, and cannabinoids (Juvin et al. 2007; Monet et al. 2009; Qin et al. 2008). However, the response to these ligands is low and variable and quite species-dependent (Neeper et al. 2007).

To date, only general blockers such as ruthenium red and trivalent cations ($La3^{+}$ and $Gn3^{+}$) (Table 2.1), have been described as blockers of TRPV2 (Leffler et al. 2007). In addition, the potassium channel blockers tetraethylamonium (TEA), 4-aminopyridine (4-AP), and 1-(2-(trifluoromethyl)phenyl)imidazole are also able to block TRPV2 currents (Vriens et al. 2009). Other reported inhibitors are SKF96365, amiloride, and Tranilast, an antiallergic drug (Juvin et al. 2007; Mihara et al. 2010) (Fig. 2.2).

2.4 TRPV3

TRPV3 is a non-selective $Ca2^{+}$ channel that plays a pivotal role in various physiological processes in the skin and hair follicles. This channel displays a moderate sequence homology to TRPV1. TRPV3 is mainly located in keratinocytes and epithelial cells (Nilius and Owsianik 2011; Valdes-Rodriguez et al. 2013), and marginally in sensory neurons (Nilius et al. 2014). This TRP channel is a polymodal receptor

Table 2.1 Representative modulators of depicted TRP channels

Ion channel	Activators	Representative blockers
TRPV1	Capsaicin, resiniferatoxin, olvanil, piperine, eugenol, camphor, 2-APB, allicin, anandamide, NADA, OLEA, N-arachidonolylserine 5S-, 12S-, 15S-HPETE, prostagalandine, leukotriene B4	Capsazepine, ruthenium red, DD01050, 5-iodo-RTX, Triazine 8aA, TRP-p5
TRPV2	2-APB, probenecid, lysophospholipids, cannabinoids	Ruthenium red, La^{3+}, Gn^{3+}, TEA, 4-aminopyridine, 1-(2-(trifluoromethyl)phenyl) imidazole, SKF96365, amiloride, Tranilast.
TRPV3	2-APB, 17(R)-resolvin D1, PIP_2, diphenylboronic anhydride, farnesyl pyrophosphate camphor, carvacrol, eugenol, menthol, thymol, borneol, cresol, carveol, gerianool, propofol, linalool, incensole, citral	Ruthenium red, icilin, isopentenyl pyrophosphate chromane-, fused pyrimidine-, fused pyrimidinones-, chromanone- and fused imidazole-derivatives
TRPV4	Endocannabinoids, arachidonic acid metabolites, nitric oxide, diaculglycerol, bisandrographolide A, 4αPDD phorbol derivatives, GSK1016790A, RN-1747	RN-1734
TRPC5	Thioredoxin, lysophosphatidylcholine, lanthanides, genistein, diadzein	SKF-96365, BTP-2, flufenamic acid, chlorpromazine, W-13, calmidazolium, W-7, 2-APB, ML-7, ML-9
TRPM3	Pregnenolone sulphate, dihydro-D-erythro-sphingosine, N,N-dimethyl-D-erythro-sphingosine, dihydropyridine nifedipine	2-APB, Gd^{3+}, rosiglitazone, troglitazone, mefenamic acid, cholesterol, naringenin, hesperetin
TRPM8	Menthol, icilin, geraniol, D3263	AMTB, JNJ41876666, BCTC, Thio-BCTC, clotrimazole, econazole, SKF-96365
TRPA1	Allyl isocyanate, cinnamaldehyde, allicin, nifedipine, chlorpromazine, auranofin, clotrimazole, clioquinol, apomorphine, glibenclamide, BCTC	HC-030031, GRC-17536, A-967079, piperazineurea, N-1-Alkyl-2-oxo-2-aryl amide, 1,8-cineole, chlorpromazine, toxin ProTx-I

activated by non-painful temperatures (Peier et al. 2002a; Smith et al. 2002; Xu et al. 2002), and chemical stimuli (Xu et al. 2006a), including natural irritants and synthetic ligands (Xu et al. 2006a), and endogenous compounds, some of them involved in the downstream inflammatory cascade (Doerner et al. 2011; Sherkheli et al. 2009). Stimulation of TRPV3 releases inflammatory mediators from keratinocytes including ATP, prostaglandin E2 and IL-1, which supports its contribution to pain transduction and inflammatory signaling. Indeed, in certain human disease states there are changes in the expression of TRPV3, such as an increase in painful breast tissue (Matta et al. 2008), or a decrease in keratinocytes in diabetic neuropathy (Facer et al. 2007).

Some evidence points to phosphatidyl inositol-4,5-bisphosphate and 17(R)-resolvin D1 as putative in vivo modulators of TRPV3 (Bang et al. 2012; Doerner et al. 2011) (Table 2.1). A role of 17(R)-resolvin D1 as potential analgesic mediated by TRPV3 has been described, although a direct evidence is still missing (Bang et al.

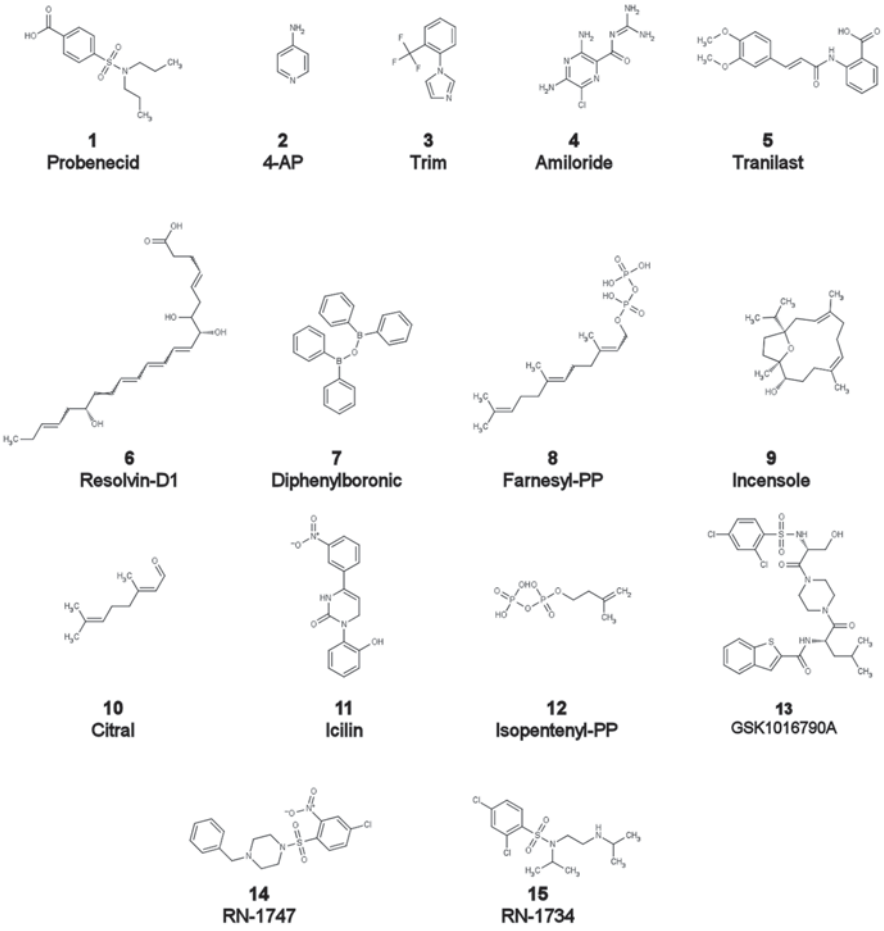

Fig. 2.2 Selected examples of TRPV2-4 effectors**.** TRPV2 activators (1) and inhibitors (2–5). TRPV3 activators (6–10) and inhibitors (11–12). TRPV4 activators (13–14) and inhibitors (15). **1** probenecid (CID 4911). **2** 4-AP: 4-aminopyridine (CID 1727). **3** Trim: 1-(2-(trifluoromethyl) phenyl) imidazole (CID 1359). **4** Amiloride (CID 16231). **5** Tranilast (CID 5282230). **6** Resolvin-D1: 17(R)-resolvin D1 (CID 71434077). **7** Diphenylboronic anhydride (CID 596810). **8** Farnesyl pyrophosphate (CID 44134714). **9** Incensole (CID 44583885). **10** Citral (CID 638011). **11** Icilin (CID 161930). **12** Isopentenyl pyrophosphate (CID 1195). **13** GSK1016790A (CID 23630424). **14** RN-1747 (CID 5068295). **15** RN-1734 (CID 3601086)

2012). Similarly, TRPV3 has been related with the production of nitric oxide via a nitrite independent pathway (Miyamoto et al. 2011). Furthermore, farnesyl pyrophosphate and isopentenyl pyrophosphate, intermediates of the melanovate pathway, are activator and inhibitor respectively, suggesting a fine-tuning of TRPV3

function (Bang et al. 2010; 2011). Alfa-hydroxy acids are proton donors commonly used in cosmetics to produce skin exfoliation mediated by TRPV3 activation (Cao et al. 2012).

The pharmacology of the TRPV family is far from simple, and TRPV3 is not an exception (Table 2.1 and Fig. 2.2). 2-APB also activates TRPV3 (Chung et al. 2004; Hu et al. 2004, 2009). Prolonged exposure of TRPV3 to 2-APB induced sensitization (Sherkheli et al. 2009). Structurally related 2-APB compounds such as diphenylboronic anhydride also act as potent TRPV3 agonists (Chung et al. 2005).

Natural aromatic monoterpenes, such as camphor, carvacrol, eugenol, menthol, thymol, as well as borneol, cresol, and others are an additional class of TRPV3 ligands (Moqrich et al. 2005; Vriens et al. 2009; Xu et al. 2006a). Camphor is a weak agonist for TRPV3 that activates currents only at concentrations of 10 mM. Carvacrol is responsible for arterial vasodilation by activating TRPV3 channels in the endothelium (Earley et al. 2010), which may account for some of their attributed cardioprotective effects. In addition to camphor and carvacrol, thymol and eugenol have also been shown to enhance the temperature response of TRPV3 (Macpherson et al. 2006; Xu et al. 2006a).

Non-aromatic monoterpenes such as carveol and derivatives (monocyclic), or gerianool, propofol and linalool (acyclic) display strong TRPV3 agonism (Vogt-Eisele et al. 2007). Incensole and incensole acetate are diterpenic cembrenoids found in incense (Boswellia papyrifera) potently activate TRPV3. The traditional use of these natural products is related to anti-inflammatory effects through the activation of TRPV3 in the skin. Interestingly, incensole acetate produces anxiolytic and antidepressive effects in mice (Moussaieff and Mechoulam 2009; Paul and Jauch 2012). Citral, a bioactive component of lemongrass is also an agonist of TRPV3 (Stotz et al. 2008), adding to the list of compounds acting on this channel (Fig. 2.2).

Cannabinoids such as cannabidiol or delta-9-tetrahydrocannabinol modulate nonspecifically TRPV3. Other derivatives such as cannabigerovarin or cannabigerolic desensitize TRPV3 (De Petrocellis et al. 2012). Active research is necessary in this field because, interestingly, the activation of TRPV3 by these compounds may contribute to their described in vivo activity (Anand 2003; Galeotti et al. 2001; Santos and Rao 2001; Umezu et al. 2001; Xu et al. 2005a).

TRPV3 antagonists include the non-specific ruthenium red, that blocks all TRPV family member at negative potentials (Vennekens et al. 2008). The compound icilin, which is a strong agonist of TRPM8 channel, is an inhibitor of TRPV3 at low doses (Sherkheli et al. 2012). Novel inhibitors are under study and have promising analgesic effects, which further suggests the involvement of TRPV3 in pain transduction (Reilly and Kym 2011). Several pharmaceutical industries have reported strong and selective TRPV3 antagonists including series of chromane-, fused pyrimidine-, fused pyrimidinones-, chromanone- and fused imidazole-derivatives (Ferrer-Montiel et al. 2012). Some of these antagonists are currently under clinical studies to treat human pain conditions.

2.5 TRPV4

Transient Receptor Potential Vanilloid 4 (TRPV4) a non-selective $Ca2^{+}$ channel is a homologue of the OSM-9 osmosensory channel first described in C. elegans. TRPV4 is activated by warm temperatures (27–35 °C) (Guler et al. 2002; Liedtke et al. 2000), and is sensitive to cell swelling and shear stress (Gao et al. 2003; Kohler et al. 2006; Loukin et al. 2010; Strotmann et al. 2000). Functions include temperature monitoring in skin keratinocytes, osmolarity sensing in the kidney (Pochynyuk et al. 2013), and shear stress detection in blood vessels, which indicates that TRPV4 functions as a putative mechanosensor (Nilius et al. 2003a, b), and is involved in nociception (Alessandri-Haber et al. 2005, 2006). It has been reported that TRPV4 may be activated by hypotonic solutions, and by mechanical forces in membrane patches (Loukin et al. 2009). TRPV4 may contribute to development of mechanical hyperalgesia after inflammation and injury (Alessandri-Haber et al. 2006). This channel is expressed in several tissues, including primary sensory neurons (Alvarez et al. 2006; Birder et al. 2007; Guler et al. 2002; Pochynyuk et al. 2013; Strotmann et al. 2000; Tabuchi et al. 2005; Watanabe et al. 2002b; Yang et al. 2006).

TRPV4 is activated by endogenous chemical ligands, such as endocannabinoids, arachidonic acid metabolites and nitric oxide (Birder et al. 2007) (Table 2.1 and Fig. 2.2). Phorbol esters that do not activate PKC, mediate TRPV4 heat responses (Watanabe et al. 2002a). TRPV4 sensitivity to osmotic and mechanical stimuli may depend on phospholipase A2 activation and the generation of arachidonic acid metabolites (Fernandes et al. 2008; Liedtke et al. 2000; Strotmann et al. 2000; Vriens et al. 2004). Furthermore, TRPV4 is activated by hypotonicity, diacylglycerol, and PKC-activating phorbol esters (Watanabe et al. 2002a, b, 2003).

Natural plant extracts (Klausen et al. 2009), bisandrographolide A (Smith et al. 2006) and synthetic compounds, such as a phorbol derivative (Birder et al. 2007), or GSK1016790A (Thorneloe et al. 2008) also activate TRPV4 channels. In addition, small molecules such as compound RN-1747 was also found to be a TRPV4 agonist (Vincent et al. 2009) (Table 2.1 and Fig. 2.2).

TRPV4 antagonism is being considered for inflammatory and neuropathic pain treatment (Vincent and Duncton 2011). However, selective TRPV4 antagonists have not been described appropriately. Ventilator-induced lung injury has emerged as a potential indicator for TRPV4 antagonists (Jin et al. 2011) (Table 2.1). The small molecule RN-1734 2,4-Dichloro-N-isopropyl-N-(2-isopropylaminoethyl)benzenesulfonamide was observed to inhibit ligand- and hypotonicity-activated TRPV4 (Vincent et al. 2009). In addition, the compound showed selective properties for TRPV4 over other TRPs such as TRPV1, TRPV3 and TRPM8, being a valuable pharmacological tool for TRPV4 studies (Vincent et al. 2009).

2.6 TRPC5

Several mammalian and Drosophila TRP canonical, TRPC proteins (TRPC1-7) have been identified (Plant and Schaefer 2003; Wes et al. 1995; Hardie and Minke 1995). All mammalian TRPCs seem to be enhanced with G-protein-coupled receptors and tyrosine kinases receptors (Montell 1999). The channels may be divided in three subgroups according to sequence homology: C1-C4-C5, C3-C6-C7, and C2 (Zufall et al. 2005). Particularly, TRPC5 is a functional plasma membrane ion channel (Beech 2007) activated by hypo-osmotic stimuli, which is dependent on phosphoinositides (Gomis et al. 2008). The inhibition of TRPC5 has been shown to suppress inflammatory pain induced by the component of the bee venom mellitin (Ding et al. 2011). Several studies support the conclusion that TRPC5 plays a role in growth cone extension and axonal guidance (Davare et al. 2009). A variety of other functions have been assigned to TRPC5, indicating a central role of this channel in physiology (Jiang et al. 2011; Nath et al. 2009; Premkumar and Abooj 2013; Wu et al. 2010; Wuensch et al. 2010; Xu et al. 2008), although the channel is not essential for life (Riccio et al. 2009).

TRPC5 modulation is not well known. A common stimulus for TRPC5 is the activation of G protein-coupled receptor. Many different receptors may be involved, including receptors for adenosine 5'-triphosphate, bradykinin, acetylcholine, histamine, prostaglandin E2, thrombin, uridine 5'-triphosphate, sphingosine-1-phosphate, glutamate and cholecystokinin (Meis et al. 2007; Riccio et al. 2009; Xu et al. 2006b; Zeng et al. 2004). TRPC5 is stimulated by activation of growth factor receptors (Bezzerides et al. 2004). TRPC5 is also a target for thioredoxin, an endogenous redox protein with established intracellular functions. Reduced thioredoxin activates TRPC5 expressed in secretory fibroblast-like synoviocytes when secreted extracellularly in patients with rheumatoid arthritis (Xu et al. 2008). Lysophosphatidylcholine has been identified as a TRPC5 activator (Flemming et al. 2006). Current data suggest a complex arrangement between TRPC5 activity and various lipid factors, supporting the hypothesis that a physiological function of TRPC5 channels is to act as lipid signal transducers.

An unusual feature of TRPC5 is its stimulation by external lanthanides (Jung et al. 2003; Schaefer et al. 2000; Xu et al. 2005b; Zeng et al. 2004). It was reported that ionic lead ($Pb2^{+}$) mimics the effect of lanthanides, leading to the hypothesis that TRPC5 may confer survival advantage by acting as a sensor of heavy metal ions (Sukumar and Beech 2010). Stimulation of TRPC5 by isoflavones like genistein or diadzein has also been reported (Wong et al. 2010) (Table 2.1, Fig. 2.3).

Although no specific or potent exogenous chemical inhibitors of TRPC5 are known, various chemicals have effects on TRPC5 function (Table 2.1). In many of these cases, it is not clear if the agent acts directly on the channel. TRPC5 has been reported to be inhibited by SKF-96365 (Okada et al. 1998), 3,5-bis(tri-fluoromethyl)pyrazole derivative BTP-2 (He et al. 2005; Kiyonaka et al. 2009), flufenamic acid (Lee et al. 2003b), W-13 or chlorpromazine (Shimizu et al. 2006), W-7 or calmidazolium (Kim et al. 2006), Pyr2, 2-APB (Xu et al. 2005b), and the myosin light chain kinase inhibitors ML-7 or ML-9 (Shimizu et al. 2006) (Fig. 2.3).

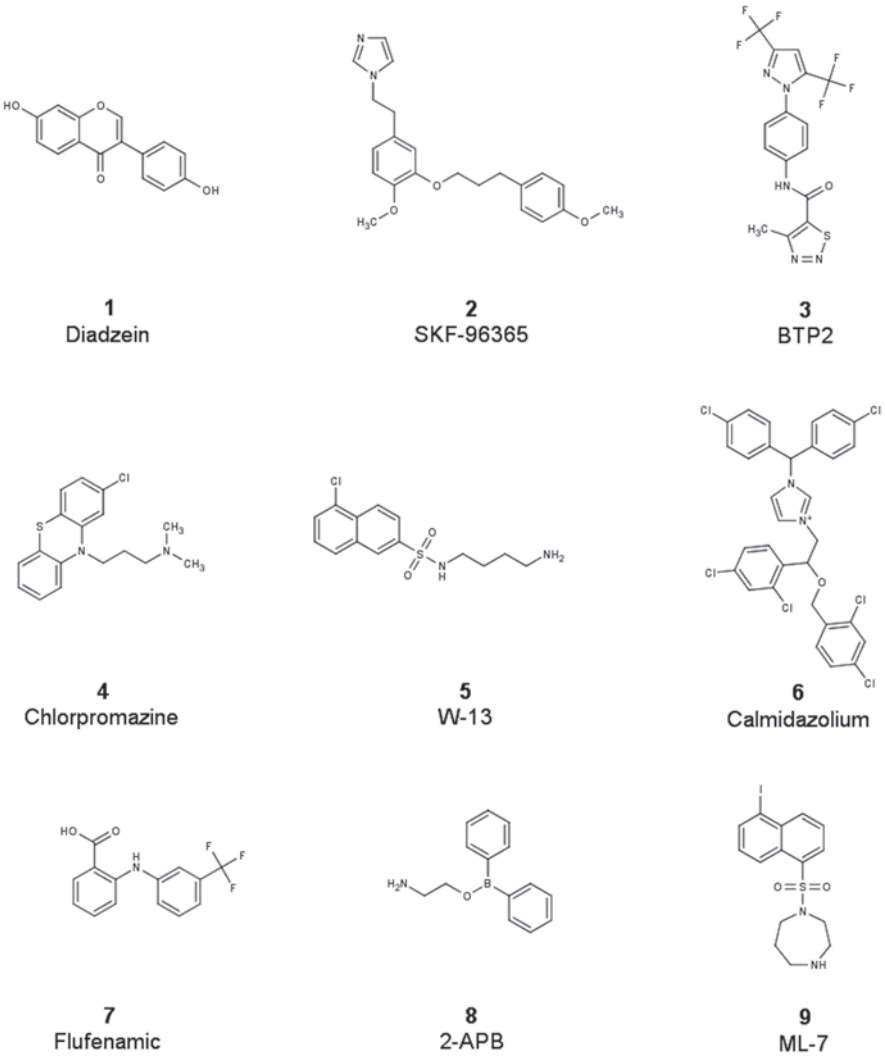

Fig. 2.3 Selected examples of activators (1) and inhibitors (2–9) of TRPC5. ***1*** Diadzein (CID 5281708). ***2*** SKF-96365 (CID 11957693). ***3*** BTP2 (CID 2455). ***4*** Flufenamic acid (CID 3371). ***5*** Chlorpromazine (CID 2726). ***6*** W-13 (CID 4299). ***7*** Calmidazolium (CID 644274). ***8*** 2-APB (CID 1598). ***9*** ML-7 (CID 4216)

2.7 TRPM3

TRPM3, TRP melastatin 3, non-selective $Ca2^{+}$ channel is one of the least investigated proteins of the TRP family of ion channels. In humans, it is highly expressed in the kidney (Grimm et al. 2003; Lee et al. 2003a), brain (Lee et al. 2003a; Oberwinkler 2007), sensory neurons, human pituitary (Fonfria et al. 2006), vascular smooth

muscle (Naylor et al. 2010) and pancreatic beta cells (Thiel et al. 2013; Wagner et al. 2008). However, its physiological role is still under investigation. Activation of TRPM3 has been linked to insulin secretion in pancreatic beta-cells (Wagner et al. 2008), to vascular smooth muscle cell contraction (Naylor et al. 2010), and to potentiating glutamatergic transmission in cerebellar Purkinje neurons of developing rats (Zamudio-Bulcock et al. 2011). A role in pain transduction has also been reported for TRPM3 (Vriens et al. 2011).

Recently, pharmacological investigations have been initiated in order to identify substances that influence TRPM3 channel activity. TRPM3 is rapidly and reversibly activated by extracellular pregnenolone sulphate, a neuroactive steroid. Application of pregnenolone sulphate led to a rapid calcium influx and enhanced insulin secretion from pancreatic islets (Wagner et al. 2008). Pregnenolone sulfate also activates TRPM3 channels in HEK293 cells, vascular smooth muscle cells, and synovial fibroblasts (Ciurtin et al. 2010; Klose et al. 2011; Majeed et al. 2012; Naylor et al. 2010), confirming the functional relevance of TRPM3 in contractile function. However, the concentration of pregnenolone sulfate required to stimulate TRPM3 channels is in the micromolar range, suggesting that pregnenolone sulfate is not a physiological agonist of TRPM3 and may have only pharmacological relevance. Moreover, the fact that TRPM3 deficient mice did not show alterations in resting blood glucose levels (Vriens et al. 2011) suggests that TRPM3 plays a marginal role in controlling β-cell functions.

Two structural analogs of sphingosine, dihydro-D-erythro-sphingosine and N, N-dimethyl-D-erythro-sphingosine, are able to activate TRPM3 (Grimm et al. 2005) (Table 2.1 and Fig. 2.4). Surprisingly, TRPM3 channels are also activated by the dihydropyridine nifedipine, an inhibitor of voltage-gated $Ca2^{+}$ channels, while the structurally related compounds nimodipine, nicardipine, and nitrendipine were inactive (Wagner et al. 2008).

As for many other members of the TRP ion channel family, 2-APB and $Gd3^{+}$ have been reported to inhibit $Ca2^{+}$ influx through TRPM3 channels (Grimm et al. 2003; Harteneck and Schultz 2007; Xu et al. 2005b). Other TRPM3 channel blockers described thus far include the antidiabetic PPARγ-agonists rosiglitazone and troglitazone (Majeed et al. 2012). Nonsteroidal anti-inflammatory drugs (NSAIDs) of the fenamate group, like mefenamic acid are able to selective block TRPM3-mediated $Ca2^{+}$ entry as well as insulin release (Klose et al. 2011). Cholesterol, the precursor metabolite of pregnenolone and progesterone, also prevents TRPM3 channel activation. (Naylor et al. 2010).

Recently, the screening of a compound library revealed that citrus fruit flavanones, such as naringenin and hesperetin, and fabacea secondary metabolites selectively inhibit TRPM3 channel activation with potencies ranged from upper nanomolar to lower micromolar concentrations (Straub et al. 2013) (Table 2.1).

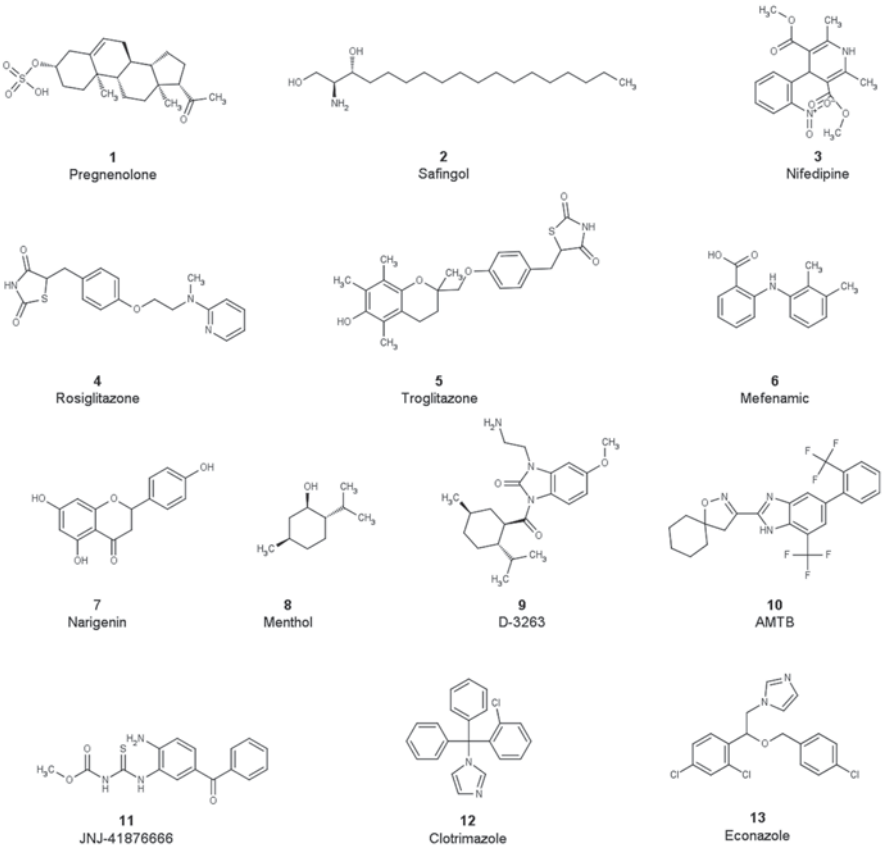

Fig. 2.4 Selected examples of TRPM3 and TRPM8 effectors. TRPM3 activators (1–3) and inhibitors (4–7). TRPM8 activators (8–9) and inhibitors (10–13). **1** Pregnenolone sulphate (CID 105074). **2** Safingol: Dihydro-D-erythro-sphingosine (CID 91486). **3** Nifedipine (CID 4485). **4** Rosiglitazone (CID 77999). **5** Troglitazone (CID 5591). **6** Mefenamic acid (CID 4044). **7** Naringenin (CID 932). **8** Menthol (CID 16666). **9** D-3263 (CID 44137358). **10** AMTB (CID 3036972). **11** JNJ-41876666: Johnson&Johnson patent. **12** Clotrimazole (CID 2812). **13** Econazole (CID 3198)

2.8 TRPM8

TRPM8 is a $Ca2^{+}$ permeable channel that was first identified in prostate cancer cells (Tsavaler et al. 2001; Bidaux et al. 2007), but also is present along the male urogenital tract (De Blas et al. 2009), artery myocytes (Johnson et al. 2009), and lung epithemium cells (Sabnis et al. 2008), although its role in many of these tissues still remains unclear. These channels are expressed in primary sensory neurons in skin and mucosae, with a physiological role in detecting low temperature signals (10–33 °C) (Babes et al. 2011), and in sensing cooling chemicals like menthol and icilin (McKemy et al. 2002; Peier et al. 2002) (Bharate and Bharate 2012; Chuang

et al. 2004). Under pathological conditions, there are increasing experimental evidences that confirm the anomalous over-expression of TRPM8 channels in sensory neurons after nerve injury or inflammation, as well as their involvement in cold allodynia and hyperalgesia (Kapoor 2012; Xing et al. 2007) (Abe et al. 2006; Ramachandran et al. 2013). The activation of TRPM8 also attenuates pain in certain acute and inflammatory pain states, mediating for instance the analgesic effects of menthol (Liu et al. 2013). Therefore, both TRPM8 agonists and antagonists could be valuable analgesic agents (Liu and Qin 2011; Maelkiae et al. 2011). TRPM8 channels are also expressed in corneal afferent neurons implicated in the regulation of ocular surface wetness, and in this respect TRPM8 modulators could have application in dry eye syndrome and excessive lacrimation dysfunction (Fernández-Peña and Viana 2013; Parra et al. 2010).

On the other hand, TRPM8 is abnormally over-expressed in androgen-sensitive prostate cancer (Gkika and Prevarskaya 2011; Tsavaler et al. 2001), breast cancer (Dhennin-Duthille et al. 2011; Ouadid-Ahidouch et al. 2012), skin melanoma cells (Yamamura et al. 2008), human pancreatic adenocarcinoma (Yee et al. 2010), oral scamous cell carcinoma (Okamoto et al. 2012), and osteosarcoma tissues and cell lines (Wang et al. 2014). Again, both agonists and antagonists of TRPM8 have proved to be valid as pharmacological tools for reducing growth and progression of neoplasias with intense expression of TRPM8 channels. Therefore, TRPM8 may also be considered an attractive target for therapeutic intervention in the search for new antitumor agents (Knowlton and McKemy 2011; Lehen'kyi and Prevarskaya 2011).

The crucial role of TRPM8 in the human pathologies is behind the intensive drug-discovery programs developed in recent years around this channel (with more than 25 patents filed since 2009). Among the antagonists, different families having benzotiophene, benzimidazole and arylglycine moieties as the central scaffold have been reported (Calvo et al. 2012; Matthews et al. 2012; Parks et al. 2011; Zhu et al. 2013). Some of these compounds showed excellent *in vitro* and *in vivo* profiles, including activity in animal models of inflammatory and neuropathic pain, thus emerging as strong candidates for future development (Table 2.1). The benzothiophene derivative JNJ41876666 has been used, along with other antagonist, AMTB, and RNAi to determine that the inhibition of either the expression or function of TRPM8 channels reduces the proliferation rate of prostate tumor cells, while no effect was observed in non-tumor cells (Valero et al. 2012).

The commercial antagonist N-(3-Aminopropyl)-2-[(3-methylphenyl)methoxy]-N-(2-thienylmethyl) benzamide hydrochloride AMTB has also served to suggest the potential of TRPM8 channel blocker as a new therapeutic opportunity for treating overactive bladder and painful bladder syndrome (Lashinger et al. 2008). Some tetrahydroquinoline and aza-analogues are TRPM8 antagonists, and a selected compound from this series reduced icilin-induced wet-dog shakes (WDS) in a dose dependent manner (Tamayo et al. 2012). Other chemotypes able to inhibit TRPM8 channels include the piperazine urea derivative BCTC, used to demonstrate that the menthol- and cold-induced allergic responses of mast cells are mediated by TRPM8 (Cho et al. 2010). A series of spiro-chromene-piperidines endowed with high po-

tency and favorable ADME properties are effective in rodent models of neuropathic pain (Chaudhari et al. 2013). SKF-96365 and the antifungic drugs clotrimazole and econazole are also effective blockers of TRPM8 (Madrid et al. 2006; Mälkiä et al. 2009 Meseguer et al. 2014; Table 2.1 and Fig. 2.4).

It has also been described that the Gαq protein, formed after activation of GPCRs, blocks TRPM8 activity by the direct formation of a complex with the channel (Zhang et al. 2012). This could indirectly arbitrate the inhibition of TRPM8 by the inflammatory mediators bradykinin and histamine in sensory nerves, and could open new strategies for modulating these channels, interfering within protein-protein interactions.

TRPM8 agonists are also therapeutically important for attenuating pain, and may induce apoptosis in TRPM8 expressing cancer cells (Maelkiae et al. 2011) (Table 2.1). The most remarkable result in this field refers to the ability of 3-(2-aminoethyl)-1(R)-[(2(S)-isopropyl-5(R)-methyl cyclohexanecarbonyl)]− 5-methoxy-1,3-dihydro-benzoimidazol-2-1 hydrochloride D3263 to inhibit the growth of TRPM8 expressing tumors. This new orally bioavailable chemical entity has already completed Phase I clinical trials in healthy individuals. The company also reported clinical studies on patients with advanced solid tumors, for which the preliminary results indicate disease stabilization after treatment. Some preclinical data also demonstrated the potential of D3263 to treat benign prostatic hyperplasia by itself or in combination with the synthetic 5α-reductase inhibitor finasteride.

2.9 TRPA1

Transient receptor potential ankyrin 1 (TRPA1) is a non-selective $Ca2^{+}$ channel characterized by a high number of ankyrin repeats (14) at the N-terminal domain (Andrade et al. 2012). This channel is activated by multiple stimuli, including temperature, acids, and numerous chemicals, like different noxious environmental and industrial pollutants, oxidant agents and bacterial endotoxins (Bandell et al. 2004; Bautista et al. 2005; Jordt et al. 2004; Levine and Alessandri-Haber 2007; Peterlin et al. 2007; Meseguer et al. 2014). Experimental evidence suggests that TRPA1 is activated by low temperatures, near the threshold of harmful cold for humans (Abrahamsen et al. 2008; del Camino et al. 2010; Karashima et al. 2009), although the categorization of TRPA1 as a cold thermosensor has been controversial.

TRPA1 receptors are vastly expressed in different unmyelinated sensory neurons (Story et al. 2003). They are also expressed in different organs, including the cardiovascular, gastrointestinal, and urinary systems (Andrade et al. 2012). TRPA1 co-localizes with TRPV1 channels at least in a subpopulation of C-type nociceptors (Fajardo et al. 2008). Akin to TRPV1, a number of studies have also established a crucial role of TRPA1 channels in neuronal and non-neuronal neuropathic pain (Barriere et al. 2012; Chen et al. 2011; Wei et al. 2010).

TRPA1 is also involved in respiratory reflexes due to inhaled pollutants (Bessac et al. 2009). These results suggest that the activation of this channel might con-

tribute to asthma and chronic obstructive pulmonary disease (Andre et al. 2008; Materazzi et al. 2010). TRPA1 is also activated by electrophilic products generated during oxidative processes, suggesting that these channels may act as sensors for the tissue damage during inflammatory processes (Taylor-Clark et al. 2008). Furthermore, TRPA1 channels have been identified as targets of allyl isocyanate, cinnamaldehyde, nifedipine, chlorpromazine, auranofin, as well as of clotrimazole, clioquinol, apomorphine, glibenclamide (Andrade et al. 2012; Babes et al. 2013), which provides some pharmacological basis for painful side effects of some of these drugs (Table 2.1 and Fig. 2.5).

The TRPA1 channel is considered a promising target for the development of new, clinically relevant drugs in different therapeutic areas (Baraldi et al. 2010). In this respect, the past few years have seen the emergence of novel TRPA1 antagonists, with more than 30 patents filed by different academic and non-academic institutions. Compounds claimed in these patents belong to different chemical

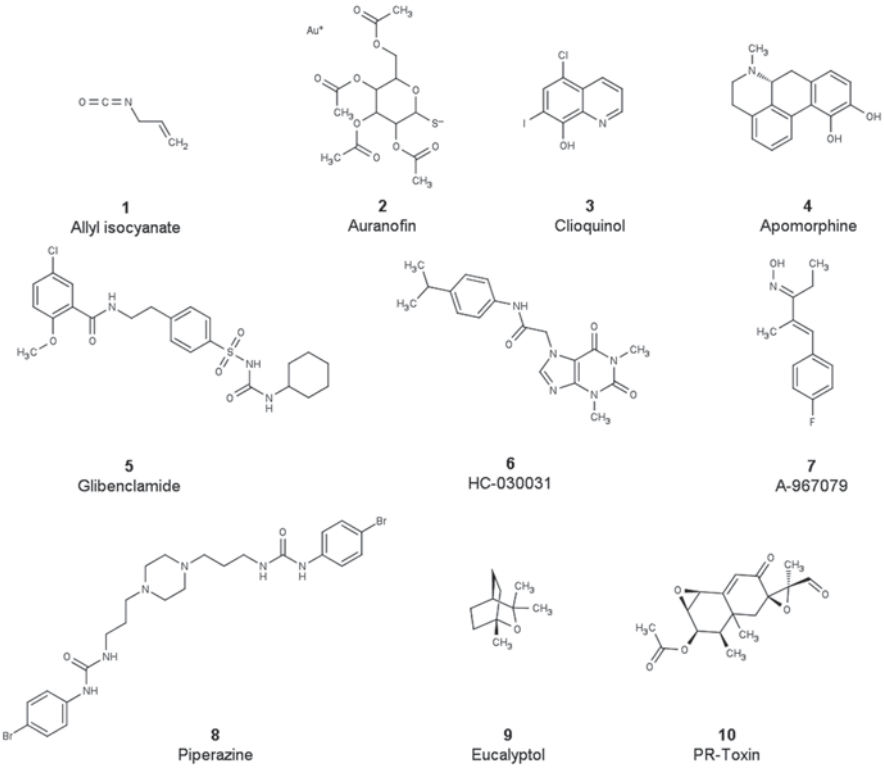

Fig. 2.5 Selected examples of activators (1–5) and inhibitors (6–10) of TRPA1. **1** Allyl isocyanate (CID15123). **2** Auranofin triethylphosphane (CID 24199313). **3** Clioquinol (CID 2788). **4** Apomorphine (CID 6005). **5** Glibenclamide (CID 3488). **6** HC-030031 (CID 1150897). **7** A-967079 (CID 42641861). **8** Piperazine urea (CID 96934). **9** Eucalyptol: 1,8-cineole (CID 2758). **10** PR-Toxin (CID 56844124)

families, but a number of them are related to fused pyrimidindione derivatives displaying efficacy in various models of pain (Table 2.1). Among the pyrimidindione antagonists, we can mention compound HC-030031 and its isobutyl analogue Chembridge-5861528, which are being profusely used as pharmacological tools for studying the implication of TRPA1 channels in pathophysiological pain, among other disorders (Koivisto et al. 2012; Meotti et al. 2013; Samer et al. 2008; Shigetomi et al. 2012). A small library of related pyrrolo[3,2-d]pyrimidinone derivatives with micromolar antagonist potencies was described (Baraldi et al. 2012). In addition, compound GRC-17536 has successfully completed Phase I clinical trials and, since 2012, is under Phase II studies in patients with painful diabetic neuropathy. Good efficacy was also observed when this selective compound was administered by the inhalation route, and a Phase IIa study is ongoing in people with refractory chronic cough.

The oxime derivative A-967079 is a TRPA1 antagonist that inhibits $Ca2^{+}$ influx through this channel at nanomolar concentrations, displayed moderate/good oral biovailability, and was active in models of inflammatory and neuropatic pain (Chen et al. 2011). Related analogues, showing either modest TRPA1 agonist or antagonist properties, have been described (DeFalco et al. 2010). Other chemotypes displaying potent TRPA1 antagonist properties include N-arylsufonyl-proline derivatives, piperazineurea, and N-1-Alkyl-2-oxo-2-aryl amide (Vallin et al. 2012). In addition, a series of 3-ylidenephtahlides and some leucettamol marine products were described to display a dual action, since they are able to activate the TRPA1 channel and to block the related cold sensor TRPM8 (Chianese et al. 2012; Ortar et al. 2013). On the contrary, the 1,8-cineole, an essential oil from eucalyptus, evoked inward currents through human TRPM8, but inhibited TRPA1 activation (Takaishi et al. 2012). BCTC, a good blocker of TRPM8 at micromolar concentrations is also a strong activator of TRPA1 at similar concentrations (Madrid et al. 2006) (Table 2.1 and Fig. 2.5). All these structures may be considered versatile templates towards novel TRP channel modulators.

Very recently, toxin ProTx-I was identified as a high-affinity TRPA1 antagonist. This Cys-rich peptide, isolated from the venom of the Peruvian green-velvet tarantula, also behave as an antagonist of voltage-gated sodium (NaV1.2) channels (Gui et al. 2014). However, mutations by Ala-scan indicated subtle differences in the structural requirements for binding to both ion channels. These findings open the possibility of using this peptide as the starting point for the development of new TRPA1 modulators. The use of ProTx-I, its mutants and the above indicated antagonists as pharmacological tools could certainly contribute to deepen our knowledge of TRPA1 function under physiological and pathological conditions, and to shed light on its gating mechanism.

2.10 Concluding Remarks

TRP channels pharmacology has been evolving a different pace for the members of this channel family, resulting in a plethora of modulators for few TRP proteins and a lack of ligands for the vast majority. However, technical advances in automated electrophysiology, along with an increase in the chemical diversity provided by synthetic and natural libraries most likely will change this conspicuous pharmacological unbalance. Furthermore, the discovery that allosteric modulators may be derived from the protein sequence will probably accelerate the discovery of TRP modulators. Moreover, the finding that TRP channel signalplexes are central to their function open new venues for drug intervention by targeting protein complexes involved in channel expression or signaling. Taken together, all these strategies will expand TRP pharmacology, even to previously considered non-druggable TRP channels.

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References

Abe J, Hosokawa H, Sawada Y, Matsumura K, Kobayashi S (2006) Ca^{2+} -dependent PKC activation mediates menthol-induced desensitization of transient receptor potential M8. Neurosci Lett 397:140–144

Abrahamsen B, Zhao J, Asante CO, Cendan CM, Marsh S, Martinez-Barbera JP, Nassar MA, Dickenson AH, Wood JN (2008) The cell and molecular basis of mechanical, cold, and inflammatory pain. Science 321:702–705

Ahern GP (2003) Activation of TRPV1 by the satiety factor oleoylethanolamide. J Biol Chem 278:30429–30434

Alessandri-Haber N, Joseph E, Dina OA, Liedtke W, Levine JD (2005) TRPV4 mediates pain-related behavior induced by mild hypertonic stimuli in the presence of inflammatory mediator. Pain 118:70–79

Alessandri-Haber N, Dina OA, Joseph EK, Reichling D, Levine JD (2006) A transient receptor potential vanilloid 4-dependent mechanism of hyperalgesia is engaged by concerted action of inflammatory mediators. J Neurosci 26:3864–3874

Alvarez DF, King JA, Weber D, Addison E, Liedtke W, Townsley MI (2006) Transient receptor potential vanilloid 4-mediated disruption of the alveolar septal barrier: a novel mechanism of acute lung injury. Circ Res 99:988–995

Anand P (2003) Capsaicin and menthol in the treatment of itch and pain: recently cloned receptors provide the key. Gut 52:1233–1235

Andrade EL, Meotti FC, Calixto JB (2012) TRPA1 antagonists as potential analgesic drugs. Pharmacol Ther 133:189–204

Andre E, Campi B, Materazzi S, Trevisani M, Amadesi S, Massi D, Creminon C, Vaksman N, Nassini R, Civelli M, Baraldi PG, Poole DP, Bunnett NW, Geppetti P, Patacchini R (2008) Cigarette smoke-induced neurogenic inflammation is mediated by alpha, beta-unsaturated aldehydes and the TRPA1 receptor in rodents. J Clin Invest 118:2574–2582

Appendino G, Minassi A, Pagani A, Ech-Chahad A (2008) The role of natural products in the ligand deorphanization of TRP channels. Curr Pharm Des 14:2–17

Avelino A, Cruz C, Nagy I, Cruz F (2002) Vanilloid receptor 1 expression in the rat urinary tract 83. Neuroscience 109:787–798

Babes A, Ciobanu AC, Neacsu C, Babes R-M (2011) TRPM8, a sensor for mild cooling in mammalian sensory nerve endings. Curr Pharm Biotechnol 12:78–88

Babes A, Fischer MJM, Filipovic M, Engel MA, Flonta M-L, Reeh PW (2013) The anti-diabetic drug glibenclamide is an agonist of the transient receptor potential Ankyrin 1 (TRPA1) ion channel. Eur J Pharmacol 704:15–22

Bandell M, Story GM, Hwang SW, Viswanath V, Eid SR, Petrus MJ, Earley TJ, Patapoutian A (2004) Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. Neuron 41:849–857

Bang S, Yoo S, Yang TJ, Cho H, Hwang SW (2010) Farnesyl pyrophosphate is a novel pain-producing molecule via specific activation of TRPV3. J Biol Chem 285:19362–19371

Bang S, Yoo S, Yang TJ, Cho H, Hwang SW (2011) Isopentenyl pyrophosphate is a novel antinociceptive substance that inhibits TRPV3 and TRPA1 ion channels. Pain 152:1156–1164

Bang S, Yoo S, Yang TJ, Cho H, Hwang SW (2012) 17(R)-resolvin D1 specifically inhibits transient receptor potential ion channel vanilloid 3 leading to peripheral antinociception. Br J Pharmacol 165:683–692

Baraldi PG, Preti D, Materazzi S, Geppetti P (2010) Transient receptor potential ankyrin 1 (TRPA1) channel as emerging target for novel analgesics and anti-inflammatory agents. J Med Chem 53:5085–5107

Baraldi PG, Romagnoli R, Saponaro G, Tabrizi MA, Baraldi S, Pedretti P, Fusi C, Nassini R, Materazzi S, Geppetti P, Preti D (2012) 7-Substituted-pyrrolo 3,2-d pyrimidine-2,4-dione derivatives as antagonists of the transient receptor potential ankyrin 1 (TRPA1) channel: a promising approach for treating pain and inflammation. Bioorg Med Chem 20:1690–1698

Barriere DA, Rieusset J, Chanteranne D, Busserolles J, Chauvin M-A, Chapuis L, Salles J, Dubray C, Morio B (2012) Paclitaxel therapy potentiates cold hyperalgesia in streptozotocin-induced diabetic rats through enhanced mitochondrial reactive oxygen species production and TRPA1 sensitization. Pain 153:553–561

Bautista DM, Movahed P, Hinman A, Axelsson HE, Sterner O, Hogestatt ED, Julius D, Jordt SE, Zygmunt PM (2005) Pungent products from garlic activate the sensory ion channel TRPA1. Proc Natl Acad Sci U S A 102:12248–12252

Beech DJ (2007) Canonical transient receptor potential 5. Handb Exp Pharmacol 109–123

Bessac BF, Sivula M, von Hehn CA, Caceres AI, Escalera J, Jordt S-E (2009) Transient receptor potential ankyrin 1 antagonists block the noxious effects of toxic industrial isocyanates and tear gases. FASEB J 23:1102–1114

Bevan S, Hothi S, Hughes G, James IF, Rang HP, Shah K, Walpole CS, Yeats JC (1992) Capsazepine: a competitive antagonist of the sensory neurone excitant capsaicin. Br J Pharmacol 107: 544–552

Bezzerides VJ, Ramsey IS, Kotecha S, Greka A, Clapham DE (2004) Rapid vesicular translocation and insertion of TRP channels. Nat Cell Biol 6:709–720

Bharate SS, Bharate SB (2012) Modulation of thermoreceptor TRPM8 by cooling compounds. Acs Chem Neurosci 3:248–267

Bhave G, Hu HJ, Glauner KS, Zhu W, Wang H, Brasier DJ, Oxford GS, Gereau RW (2003) Protein kinase C phosphorylation sensitizes but does not activate the capsaicin receptor transient receptor potential vanilloid 1 (TRPV1). Proc Natl Acad Sci U S A 100:12480–12485

Bidaux G, Flourakis M, Thebault S, Zholos A, Beck B, Gkika D, Roudbaraki M, Bonnal J-L, Mauroy B, Shuba Y, Skryma R, Prevarskaya N (2007) Prostate cell differentiation status determines transient receptor potential melastatin member 8 channel subcellular localization and function. J Clin Invest 117:1647–1657

Birder L, Kullmann FA, Lee H, Barrick S, de GW, Kanai A, Caterina M (2007) Activation of urothelial transient receptor potential vanilloid 4 by 4alpha-phorbol 12,13-didecanoate contributes to altered bladder reflexes in the rat. J Pharmacol Exp Ther 323:227–235

Bohlen CJ, Priel A, Zhou S, King D, Siemens J, Julius D (2010) A bivalent tarantula toxin activates the capsaicin receptor, TRPV1, by targeting the outer pore domain. Cell 141:834–845

Broad LM, Keding SJ, Blanco MJ (2008) Recent progress in the development of selective TRPV1 antagonists for pain. Curr Top Med Chem 8:1431–1441

Calvo RR, Meegalla SK, Parks DJ, Parsons WH, Ballentine SK, Lubin ML, Schneider C, Colburn RW, Flores CM, Player MR (2012) Discovery of vinylcycloalkyl-substituted benzimidazole TRPM8 antagonists effective in the treatment of cold allodynia. Bioorg Med Chem Lett 22:1903–1907

Camprubi-Robles M, Planells-Cases R, Ferrer-Montiel A (2009) Differential contribution of SNARE-dependent exocytosis to inflammatory potentiation of TRPV1 in nociceptors. FASEB J 23:3722–3733

Cao X, Yang F, Zheng J, Wang K (2012) Intracellular proton-mediated activation of TRPV3 channels accounts for the exfoliation effect of alpha-hydroxyl acids on keratinocytes. J Biol Chem 287:25905–25916

Caterina MJ, Rosen TA, Tominaga M, Brake AJ, Julius D (1999) A capsaicin-receptor homologue with a high threshold for noxious heat. Nature 398:436–441

Caterina MJ, Leffler A, Malmberg AB, Martin WJ, Trafton J, Petersen-Zeitz KR, Koltzenburg M, Basbaum AI, Julius D (2000) Impaired nociception and pain sensation in mice lacking the capsaicin receptor. Science 288:306–313

Chaudhari SS, Kadam AB, Khairatkar-Joshi N, Mukhopadhyay I, Karnik PV, Raghuram A, Rao SS, Vaiyapuri TS, Wale DP, Bhosale VM, Gudi GS, Sangana RR, Thomas A (2013) Synthesis and pharmacological evaluation of novel N-aryl-3, 4-dihydro-1'H-spiro chromene-2,4'-piperidine – 1'-carboxamides as TRPM8 antagonists. Bioorg Med Chem 21:6542–6553

Chen J, Joshi SK, DiDomenico S, Perner RJ, Mikusa JP, Gauvin DM, Segreti JA, Han P, Zhang X-F, Niforatos W, Bianchi BR, Baker SJ, Zhong C, Simler GH, McDonald HA, Schmidt RG, McGaraughty SP, Chu KL, Faltynek CR, Kort ME, Reilly RM, Kym PR (2011) Selective blockade of TRPA1 channel attenuates pathological pain without altering noxious cold sensation or body temperature regulation. Pain 152:1165–1172

Chianese G, Fattorusso E, Putra MY, Calcinai B, Bavestrello G, Moriello AS, De Petrocellis L, Marzo V D, Taglialatela-Scafati O (2012) Leucettamols, bifunctionalized marine sphingoids, act as modulators of TRPA1 and TRPM8 channels. Mar Drugs 10:2435–2447

Cho Y, Jang Y, Yang YD, Lee C-H, Lee Y, Oh U (2010) TRPM8 mediates cold and menthol allergies associated with mast cell activation. Cell Calcium 48:202–208

Chuang HH, Neuhausser WM, Julius D (2004) The super-cooling agent icilin reveals a mechanism of coincidence detection by a temperature-sensitive TRP channel. Neuron 43:859–869

Chung MK, Lee H, Mizuno A, Suzuki M, Caterina MJ (2004) 2-aminoethoxydiphenyl borate activates and sensitizes the heat-gated ion channel TRPV3. J Neurosci 24:5177–5182

Chung MK, Guler AD, Caterina MJ (2005) Biphasic currents evoked by chemical or thermal activation of the heat-gated ion channel, TRPV3. J Biol Chem 280:15928–15941

Ciurtin C, Majeed Y, Naylor J, Sukumar P, English AA, Emery P, Beech DJ (2010) TRPM3 channel stimulated by pregnenolone sulphate in synovial fibroblasts and negatively coupled to hyaluronan. BMC Musculoskelet Disord 11:111

Davare MA, Fortin DA, Saneyoshi T, Nygaard S, Kaech S, Banker G, Soderling TR, Wayman GA (2009) Transient receptor potential canonical 5 channels activate Ca^{2+}/calmodulin kinase Igamma to promote axon formation in hippocampal neurons. J Neurosci 29:9794–9808

De Blas GA, Darszon A, Ocampo AY, Serrano CJ, Castellano LE, Hernandez-Gonzalez EO, Chirinos M, Larrea F, Beltran C, Trevino CL (2009) TRPM8, a versatile channel in human sperm. PLos One 4(6):e6095

DeFalco J, Steiger D, Gustafson A, Emerling DE, Kelly MG, Duncton MAJ (2010) Oxime derivatives related to AP18: agonists and antagonists of the TRPA1 receptor. Bioorg Med Chem Lett 20:276–279

del Camino D, Murphy S, Heiry M, Barrett LB, Earley TJ, Cook CA, Petrus MJ, Zhao M, D'Amours M, Deering N, Brenner GJ, Costigan M, Hayward NJ, Chong JA, Fanger CM, Woolf CJ, Patapoutian A, Moran MM (2010) TRPA1 contributes to cold hypersensitivity. J Neurosci 30:15165–15174

De Petrocellis L, Orlando P, Moriello AS, Aviello G, Stott C, Izzo AA, Di Marzo V (2012) Cannabinoid actions at TRPV channels: effects on TRPV3 and TRPV4 and their potential relevance to gastrointestinal inflammation. Acta Physiol (Oxf) 204:255–266

Devesa I, Planells-Cases R, Fernandez-Ballester G, Gonzalez-Ros JM, Ferrer-Montiel A, Fernandez-Carvajal A (2011) Role of the transient receptor potential vanilloid 1 in inflammation and sepsis. J Inflamm Res 4:67–81

Dhennin-Duthille I, Gautier M, Faouzi M, Guilbert A, Brevet M, Vaudry D, Ahidouch A, Sevestre H, Ouadid-Ahidouch H (2011) High expression of transient receptor potential channels in human breast cancer epithelial cells and tissues: correlation with pathological parameters. Cell Physiol Biochem 28:813–822

Ding J, Xiao Y, Lu D, Du YR, Cui XY, Chen J (2011) Effects of SKF-96365, a TRPC inhibitor, on melittin-induced inward current and intracellular Ca^{2+} rise in primary sensory cells. Neurosci Bull 27:135–142

Doerner JF, Hatt H, Ramsey IS (2011) Voltage- and temperature-dependent activation of TRPV3 channels is potentiated by receptor-mediated PI(4,5)P2 hydrolysis. J Gen Physiol 137:271–288

Earley S, Gonzales AL, Garcia ZI (2010) A dietary agonist of transient receptor potential cation channel V3 elicits endothelium-dependent vasodilation. Mol Pharmacol 77:612–620

Facer P, Casula MA, Smith GD, Benham CD, Chessell IP, Bountra C, Sinisi M, Birch R, Anand P (2007) Differential expression of the capsaicin receptor TRPV1 and related novel receptors TRPV3, TRPV4 and TRPM8 in normal human tissues and changes in traumatic and diabetic neuropathy. BMC Neurol 7:11

Fajardo O, Meseguer V, Belmonte C, Viana F (2008) TRPA1 channels mediate cold temperature sensing in mammalian vagal sensory neurons: pharmacological and genetic evidence. J Neurosci 28:7863–7875

Fernandes J, Lorenzo IM, Andrade YN, Garcia-Elias A, Serra SA, Fernandez-Fernandez JM, Valverde MA (2008) IP3 sensitizes TRPV4 channel to the mechano- and osmotransducing messenger 5'-6'-epoxyeicosatrienoic acid. J Gen Physiol 131:i2

Fernandez-Carvajal A, Fernandez-Ballester G, Devesa I, Gonzalez-Ros JM, Ferrer-Montiel A (2011) New strategies to develop novel pain therapies: addressing thermoreceptors from different points of view. Pharmaceuticals 5:16–48

Fernández-Peña C, Viana F (2013) Targeting TRPM8 for pain relief. Open Pain J 6:154–164

Ferrer-Montiel A, Fernandez-Carvajal A, Planells-Cases R, Fernandez-Ballester G, Gonzalez-Ros JM, Messeguer A, Gonzalez-Muniz R (2012) Advances in modulating thermosensory TRP channels. Expert Opin Ther Pat 22:999–1017

Flemming PK, Dedman AM, Xu SZ, Li J, Zeng F, Naylor J, Benham CD, Bateson AN, Muraki K, Beech DJ (2006) Sensing of lysophospholipids by TRPC5 calcium channel. J Biol Chem 281:4977–4982

Fonfria E, Murdock PR, Cusdin FS, Benham CD, Kelsell RE, McNulty S (2006) Tissue distribution profiles of the human TRPM cation channel family. J Recept Signal Transduct Res 26:159–178

Galeotti N, Ghelardini C, Mannelli L, Mazzanti G, Baghiroli L, Bartolini A (2001) Local anaesthetic activity of (+)- and (−)-menthol. Planta Med 67:174–176

Gao X, Wu L, O'Neil RG (2003) Temperature-modulated diversity of TRPV4 channel gating: activation by physical stresses and phorbol ester derivatives through protein kinase C-dependent and -independent pathways. J Biol Chem 278:27129–27137

Garcia-Martinez C, Humet M, Planells-Cases R, Gomis A, Caprini M, Viana F, De La PE, Sanchez-Baeza F, Carbonell T, De FC, Perez-Paya E, Belmonte C, Messeguer A, Ferrer-Montiel A (2002) Attenuation of thermal nociception and hyperalgesia by VR1 blockers. Proc Natl Acad Sci U S A 99:2374–2379

Garcia-Martinez C, Fernandez-Carvajal A, Valenzuela B, Gomis A, Van Den Nest W, Ferroni S, Carreno C, Belmonte C, Ferrer-Montiel A (2006) Design and characterization of a noncompetitive antagonist of the transient receptor potential vanilloid subunit 1 channel with in vivo analgesic and anti-inflammatory activity. J Pain 7:735–746

Garcia-Sanz N, Fernandez-Carvajal A, Morenilla-Palao C, Planells-Cases R, Fajardo-Sanchez E, Fernandez-Ballester G, Ferrer-Montiel A (2004) Identification of a tetramerization domain in the C terminus of the vanilloid receptor. J Neurosci 24:5307–5314

Garcia-Sanz N, Valente P, Gomis A, Fernandez-Carvajal A, Fernandez-Ballester G, Viana F, Belmonte C, Ferrer-Montiel A (2007) A role of the transient receptor potential domain of vanilloid receptor I in channel gating. J Neurosci 27:11641–11650

Gavva NR, Treanor JJ, Garami A, Fang L, Surapaneni S, Akrami A, Alvarez F, Bak A, Darling M, Gore A, Jang GR, Kesslak JP, Ni L, Norman MH, Palluconi G, Rose MJ, Salfi M, Tan E, Romanovsky AA, Banfield C, Davar G (2008) Pharmacological blockade of the vanilloid receptor TRPV1 elicits marked hyperthermia in humans. Pain 136:202–210

Gkika D, Prevarskaya N (2011) TRP channels in prostate cancer: the good, the bad and the ugly? Asian J Androl 13:673–676

Gomis A, Soriano S, Belmonte C, Viana F (2008) Hypoosmotic- and pressure-induced membrane stretch activate TRPC5 channels. J Physiol 586:5633–5649

Grimm C, Kraft R, Sauerbruch S, Schultz G, Harteneck C (2003) Molecular and functional characterization of the melastatin-related cation channel TRPM3. J Biol Chem 278:21493–21501

Grimm C, Kraft R, Schultz G, Harteneck C (2005) Activation of the melastatin-related cation channel TRPM3 by D-erythro-sphingosine [corrected]. Mol Pharmacol 67:798–805

Gui J, Liu B, Cao G, Lipchik AM, Perez M, Dekan Z, Mobli M, Daly NL, Alewood PF, Parker LL, King GF, Zhou Y, Jordt S-E, Nitabach MN (2014) A tarantula-venom peptide antagonizes the TRPA1 nociceptor ion channel by binding to the S1–S4 gating domain. Curr Biol 24:473–483

Guler AD, Lee H, Iida T, Shimizu I, Tominaga M, Caterina M (2002) Heat-evoked activation of the ion channel, TRPV4. J Neurosci 22:6408–6414

Hardie RC, Minke B (1995) Phosphoinositide-mediated phototransduction in *Drosophila* photoreceptors: the role of Ca^{2+} and trp. Cell Calcium 18:256–274

Harteneck C, Schultz G (2007) TRPV4 and TRPM3 as volume-regulated cation channels. In: Liedtke Wb HS (ed) Trp ion channel function in sensory transduction and cellular signaling cascades. CRC Press

He LP, Hewavitharana T, Soboloff J, Spassova MA, Gill DL (2005) A functional link between store-operated and TRPC channels revealed by the 3,5-bis(trifluoromethyl)pyrazole derivative, BTP2. J Biol Chem 280:10997–11006

Hiura A (2000) Neuroanatomical effects of capsaicin on the primary afferent neurons. Arch Histol Cytol 63: 199–215

Hu HZ, Gu Q, Wang C, Colton CK, Tang J, Kinoshita-Kawada M, Lee LY, Wood JD, Zhu MX (2004) 2-aminoethoxydiphenyl borate is a common activator of TRPV1, TRPV2, and TRPV3. J Biol Chem 279:35741–35748

Hu G, Oboukhova EA, Kumar S, Sturek M, Obukhov AG (2009) Canonical transient receptor potential channels expression is elevated in a porcine model of metabolic syndrome. Mol Endocrinol 23:689–699

Huang J, Zhang X, McNaughton PA (2006) Inflammatory pain: the cellular basis of heat hyperalgesia. Curr. Neuropharmacol 4:197–206

Inoue K, Koizumi S, Fuziwara S, Denda S, Inoue K, Denda M (2002) Functional vanilloid receptors in cultured normal human epidermal keratinocytes. Biochem Biophys Res Commun 291:124–129

Jiang LH, Gamper N, Beech DJ (2011) Properties and therapeutic potential of transient receptor potential channels with putative roles in adversity: focus on TRPC5, TRPM2 and TRPA1. Curr Drug Targets 12:724–736

Jin M, Wu Z, Chen L, Jaimes J, Collins D, Walters ET, O'Neil RG (2011) Determinants of TRPV4 activity following selective activation by small molecule agonist GSK1016790A. PLoS One 6:e16713

Johnson CD, Melanaphy D, Purse A, Stokesberry SA, Dickson P, Zholos AV (2009) Transient receptor potential melastatin 8 channel involvement in the regulation of vascular tone. Am J Physiol Heart Circ Physiol 296:H1868–H1877

Jordt SE, Bautista DM, Chuang HH, McKemy DD, Zygmunt PM, Hogestatt ED, Meng ID, Julius D (2004) Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. Nature 427:260–265

Jung S, Muhle A, Schaefer M, Strotmann R, Schultz G, Plant TD (2003) Lanthanides potentiate TRPC5 currents by an action at extracellular sites close to the pore mouth. J Biol Chem 278:3562–3571

Juvin V, Penna A, Chemin J, Lin YL, Rassendren FA (2007) Pharmacological characterization and molecular determinants of the activation of transient receptor potential V2 channel orthologs by 2-aminoethoxydiphenyl borate. Mol Pharmacol 72:1258–1268

Kapoor MDS (2012) TRPM8 antagonists and their emerging role in the modulation of pain and allodynia. Biochem Biophys Res Commun 420:937–937

Karashima Y, Talavera K, Everaerts W, Janssens A, Kwan KY, Vennekens R, Nilius B, Voets T (2009) TRPA1 acts as a cold sensor in vitro and in vivo. Proc Natl Acad Sci U S A 106:1273–1278

Khairatkar-Joshi N, Szallasi A (2009) TRPV1 antagonists: the challenges for therapeutic targeting. Trends Mol Med 15:14–22

Kim MT, Kim BJ, Lee JH, Kwon SC, Yeon DS, Yang DK, So I, Kim KW (2006) Involvement of calmodulin and myosin light chain kinase in activation of mTRPC5 expressed in HEK cells. Am J Physiol Cell Physiol 290:C1031–C1040

Kiyonaka S, Kato K, Nishida M, Mio K, Numaga T, Sawaguchi Y, Yoshida T, Wakamori M, Mori E, Numata T, Ishii M, Takemoto H, Ojida A, Watanabe K, Uemura A, Kurose H, Morii T, Kobayashi T, Sato Y, Sato C, Hamachi I, Mori Y (2009) Selective and direct inhibition of TRPC3 channels underlies biological activities of a pyrazole compound. Proc Natl Acad Sci U S A 106:5400–5405

Klausen TK, Pagani A, Minassi A, Ech-Chahad A, Prenen J, Owsianik G, Hoffmann EK, Pedersen SF, Appendino G, Nilius B (2009) Modulation of the transient receptor potential vanilloid channel TRPV4 by 4alpha-phorbol esters: a structure-activity study. J Med Chem 52:2933–2939

Klose C, Straub I, Riehle M, Ranta F, Krautwurst D, Ullrich S, Meyerhof W, Harteneck C (2011) Fenamates as TRP channel blockers: mefenamic acid selectively blocks TRPM3. Br J Pharmacol 162:1757–1769

Knotkova H, Pappagallo M, Szallasi A (2008) Capsaicin (TRPV1 Agonist) therapy for pain relief: farewell or revival? Clin.J. Pain 24:142–154

Knowlton WM, McKemy DD (2011) TRPM8: from cold to cancer, peppermint to pain. Curr Pharm Biotechnol 12:68–77

Kohler R, Heyken WT, Heinau P, Schubert R, Si H, Kacik M, Busch C, Grgic I, Maier T, Hoyer J (2006) Evidence for a functional role of endothelial transient receptor potential V4 in shear stress-induced vasodilatation. Arterioscler Thromb Vasc Biol 26:1495–1502

Koivisto A, Hukkanen M, Saarnilehto M, Chapman H, Kuokkanen K, Wei H, Viisanen H, Akerman KE, Lindstedt K, Pertovaara A (2012) Inhibiting TRPA1 ion channel reduces loss of cutaneous nerve fiber function in diabetic animals: sustained activation of the TRPA1 channel contributes to the pathogenesis of peripheral diabetic neuropathy. Pharmacol Res 65:149–158

Lashinger ESR, Steiginga MS, Hieble JP, Leon LA, Gardner SD, Nagilla R, Davenport EA, Hoffman BE, Laping NJ, Su X (2008) AMTB, a TRPM8 channel blocker: evidence in rats for activity in overactive bladder and painful bladder syndrome. Am J Physiol Ren Physiol 295:F803–F810

Lee N, Chen J, Sun L, Wu S, Gray KR, Rich A, Huang M, Lin JH, Feder JN, Janovitz EB, Levesque PC, Blanar MA (2003a) Expression and characterization of human transient receptor potential melastatin 3 (hTRPM3). J Biol Chem 278:20890–20897

Lee YM, Kim BJ, Kim HJ, Yang DK, Zhu MH, Lee KP, So I, Kim KW (2003b) TRPC5 as a candidate for the nonselective cation channel activated by muscarinic stimulation in murine stomach. Am J Physiol Gastrointest Liver Physiol 284:G604–G616

Leffler A, Linte RM, Nau C, Reeh P, Babes A (2007) A high-threshold heat-activated channel in cultured rat dorsal root ganglion neurons resembles TRPV2 and is blocked by gadolinium. Eur J Neurosci 26:12–22

Lehen'kyi V, Prevarskaya N (2011) Oncogenic TRP Channels. In: Islam MS (ed) Transient receptor potential channels. Springer, Heidelberg, pp 929–945
Levine JD, Alessandri-Haber N (2007) TRP channels: targets for the relief of pain. Biochimica Biophysica Acta Mole Basis of Dis 1772: 989–1003
Liao M, Cao E, Julius D, Cheng Y (2013) Structure of the TRPV1 ion channel determined by electron cryo-microscopy. Nature 504:107–112
Liapi A, Wood JN (2005) Extensive co-localization and heteromultimer formation of the vanilloid receptor-like protein TRPV2 and the capsaicin receptor TRPV1 in the adult rat cerebral cortex. Eur J Neurosci 22:825–834
Liedtke W, Choe Y, Marti-Renom MA, Bell AM, Denis CS, Sali A, Hudspeth AJ, Friedman JM, Heller S (2000) Vanilloid receptor-related osmotically activated channel (VR-OAC), a candidate vertebrate osmoreceptor. Cell 103:525–535
Liu Y, Qin N (2011) TRPM8 in health and disease: cold sensing and beyond. In: Islam MS (ed) Transient receptor potential channels, pp 185–208
Liu B, Fan L, Balakrishna S, Sui A, Morris JB, Jordt S-E (2013) TRPM8 is the principal mediator of menthol-induced analgesia of acute and inflammatory pain. Pain 154:2169–2177
Loukin SH, Su Z, Kung C (2009) Hypotonic shocks activate rat TRPV4 in yeast in the absence of polyunsaturated fatty acids. FEBS Lett 583:754–758
Loukin S, Zhou X, Su Z, Saimi Y, Kung C (2010) Wild-type and brachyolmia-causing mutant TRPV4 channels respond directly to stretch force. J Biol Chem 285:27176–27181
Macpherson LJ, Hwang SW, Miyamoto T, Dubin AE, Patapoutian A, Story GM (2006) More than cool: promiscuous relationships of menthol and other sensory compounds. Mol. Cell Neurosci 32:335–343
Madrid R, Donovan-Rodriguez T, Meseguer V, Belmonte C, Viana F (2006) Contribution of TRPM8 channels to cold transduction in primary sensory neurons and peripheral nerve terminals. J Neurosci 26(48):12512–12525
Maelkiae A, Morenilla-Palao C, Viana F (2011) The emerging pharmacology of TRPM8 channels: hidden therapeutic potential underneath a cold surface. Curr Pharm Biotechnol 12:54–67
Majeed Y, Tumova S, Green BL, Seymour VA, Woods DM, Agarwal AK, Naylor J, Jiang S, Picton HM, Porter KE, O'Regan DJ, Muraki K, Fishwick CW, Beech DJ (2012) Pregnenolone sulphate-independent inhibition of TRPM3 channels by progesterone. Cell Calcium 51:1–11
Malkia A, Pertusa M, Fernández-Ballester G, Ferrer-Montiel A, Viana F (2009) Differential role of the menthol-binding residue Y745 in the antagonism of thermally gated TRPM8 channels. Molecular Pain 5:62
Materazzi S, Nassini R, Andre E, Gatti R, Trevisani M, Patacchini R, Geppetti P (2010) The contribute of TRPA1 channel in inflammatory respiratory diseases. Neuropeptides 44:522–522
Matta JA, Cornett PM, Miyares RL, Abe K, Sahibzada N, Ahern GP (2008) General anesthetics activate a nociceptive ion channel to enhance pain and inflammation. Proc Natl Acad Sci U S A 105: 8784–8789
Matthews JM, Qin N, Colburn RW, Dax SL, Hawkins M, McNally JJ, Reany L, Youngman MA, Baker J, Hutchinson T, Liu Y, Lubin ML, Neeper M, Brandt MR, Stone DJ, Flores CM (2012) The design and synthesis of novel, phosphonate-containing transient receptor potential melastatin 8 (TRPM8) antagonists. Bioorg Med Chem Lett 22:2922–2926
McDonnell ME, Zhang SP, Dubin AE, Dax SL (2002) Synthesis and in vitro evaluation of a novel iodinated resiniferatoxin derivative that is an agonist at the human vanilloid VR1 receptor. Bioorg Med Chem Lett 12:1189–1192
McKemy DD, Neuhausser WM, Julius D (2002) Identification of a cold receptor reveals a general role for TRP channels in thermosensation. Nature 416(6876):52–58
Meis S, Munsch T, Sosulina L, Pape HC (2007) Postsynaptic mechanisms underlying responsiveness of amygdaloid neurons to cholecystokinin are mediated by a transient receptor potential-like current. Mol. Cell Neurosci 35:356–367
Meotti FC, Forner S, Lima-Garcia JF, Viana AF, Calixto JB (2013) Antagonism of the transient receptor potential ankyrin 1 (TRPA1) attenuates hyperalgesia and urinary bladder overactivity in cyclophosphamide-induced haemorrhagic cystitis. Chem Biol Interact 203:440–447

Meseguer V, Alpizar YA, Luis E, Tajada S, Denlinger B, Fajardo O, Manenschijn JA, Fernández-Peña C, Talavera A, Kichko T, Navia B, Sánchez A, Señarís R, Reeh P5, Pérez-García MT, López-López JR, Voets T, Belmonte C, Talavera K, Viana F (2014) TRPA1 channels mediate acute neurogenic inflammation and pain produced by bacterial endotoxins. Nat Commun. 5:3125

Mihara H, Boudaka A, Shibasaki K, Yamanaka A, Sugiyama T, Tominaga M (2010) Involvement of TRPV2 activation in intestinal movement through nitric oxide production in mice. J Neurosci 30:16536–16544

Miyamoto T, Petrus MJ, Dubin AE, Patapoutian A (2011) TRPV3 regulates nitric oxide synthase-independent nitric oxide synthesis in the skin. Nat Commun 2:369

Monet M, Gkika D, Lehen'kyi V, Pourtier A, Vanden Abeele F, Bidaux G, Juvin V, Rassendren F, Humez S, Prevarsakaya N (2009) Lysophospholipids stimulate prostate cancer cell migration via TRPV2 channel activation. Biochim Biophys Acta 1793:528–539

Montell C (1999) Visual transduction in *Drosophila*. Annu Rev Cell Dev Biol 15:231–268

Moqrich A, Hwang SW, Earley TJ, Petrus MJ, Murray AN, Spencer KS, Andahazy M, Story GM, Patapoutian A (2005) Impaired thermosensation in mice lacking TRPV3, a heat and camphor sensor in the skin. Science 307:1468–1472

Morenilla-Palao C, Planells-Cases R, Garcia-Sanz N, Ferrer-Montiel A (2004) Regulated exocytosis contributes to protein kinase C potentiation of vanilloid receptor activity. J Biol Chem 279:25665–25672

Moussaieff A, Mechoulam R (2009) Boswellia resin: from religious ceremonies to medical uses; a review of in-vitro, in-vivo and clinical trials. J Pharm Pharmacol 61:1281–1293

Muraki K, Iwata Y, Katanosaka Y, Ito T, Ohya S, Shigekawa M, Imaizumi Y (2003) TRPV2 is a component of osmotically sensitive cation channels in murine aortic myocytes 39. Circ Res 93:829–838

Nath AK, Krauthammer M, Li P, Davidov E, Butler LC, Copel J, Katajamaa M, Oresic M, Buhimschi I, Buhimschi C, Snyder M, Madri JA (2009) Proteomic-based detection of a protein cluster dysregulated during cardiovascular development identifies biomarkers of congenital heart defects. PLoS One 4:e4221

Naylor J, Li J, Milligan CJ, Zeng F, Sukumar P, Hou B, Sedo A, Yuldasheva N, Majeed Y, Beri D, Jiang S, Seymour VA, McKeown L, Kumar B, Harteneck C, O'Regan D, Wheatcroft SB, Kearney MT, Jones C, Porter KE, Beech DJ (2010) Pregnenolone sulphate- and cholesterol-regulated TRPM3 channels coupled to vascular smooth muscle secretion and contraction. Circ Res 106:1507–1515

Neeper MP, Liu Y, Hutchinson TL, Wang Y, Flores CM, Qin N (2007) Activation properties of heterologously expressed mammalian TRPV2: evidence for species dependence. J Biol Chem 282:15894–15902

Nilius B, Owsianik G (2011) The transient receptor potential family of ion channels. Genome Biol 12:218

Nilius B, Droogmans G, Wondergem R (2003a) Transient receptor potential channels in endothelium: solving the calcium entry puzzle? Endothelium 10:5–15

Nilius B, Watanabe H, Vriens J (2003b) The TRPV4 channel: structure-function relationship and promiscuous gating behaviour. Pflugers Arch 446:298–303

Nilius B, Biro T, Owsianik G (2014) TRPV3: time to decipher a poorly understood family member! J Physiol 592:295–304

Oberwinkler J (2007) TRPM3, a biophysical enigma? Biochem Soc Trans 35:89–90

Okada T, Shimizu S, Wakamori M, Maeda A, Kurosaki T, Takada N, Imoto K, Mori Y (1998) Molecular cloning and functional characterization of a novel receptor-activated TRP Ca^{2+} channel from mouse brain. J Biol Chem 273:10279–10287

Okamoto Y, Ohkubo T, Ikebe T, Yamazaki J (2012) Blockade of TRPM8 activity reduces the invasion potential of oral squamous carcinoma cell lines. Int J Oncol 40:1431–1440

Ortar G, Moriello AS, Morera E, Nalli M, Marzo V D, De Petrocellis L (2013) 3-Ylidenephthalides as a new class of transient receptor potential channel TRPA1 and TRPM8 modulators. Bioorg Med Chem Lett 23:5614–5618

Ouadid-Ahidouch H, Dhennin-Duthille I, Gautier M, Sevestre H, Ahidouch A (2012) TRP calcium channel and breast cancer: expression, role and correlation with clinical parameters. Bull Cancer 99:655–664

Park U, Vastani N, Guan Y, Raja SN, Koltzenburg M, Caterina MJ (2011) TRP vanilloid 2 knockout mice are susceptible to perinatal lethality but display normal thermal and mechanical nociception. J Neurosci 31:11425–11436

Parks DJ, Parsons WH, Colburn RW, Meegalla SK, Ballentine SK, Illig CR, Qin N, Liu Y, Hutchinson TL, Lubin ML, Stone DJ Jr, Baker JF, Schneider CR, Ma J, Damiano BP, Flores CM, Player MR (2011) Design and optimization of benzimidazole-containing transient receptor potential melastatin 8 (TRPM8) antagonists. J Med Chem 54:233–247

Parra A, Madrid R, Echevarria D, del Olmo S, Morenilla-Palao C, Carmen Acosta M, Gallar J, Dhaka A, Viana F, Belmonte C (2010) Ocular surface wetness is regulated by TRPM8-dependent cold thermoreceptors of the cornea. Nat Med 16:1396–1399

Paul M, Jauch J (2012) Efficient preparation of incensole and incensole acetate, and quantification of these bioactive diterpenes in Boswellia papyrifera by a RP-DAD-HPLC method. Nat Prod Commun 7:283–288

Peier AM, Moqrich A, Hergarden AC, Reeve AJ, Andersson DA, Story GM, Earley TJ, Dragoni I, McIntyre P, Bevan S, Patapoutian A (2002a) A TRP channel that senses cold stimuli and menthol. Cell 108(5):705–715

Peier AM, Reeve AJ, Andersson DA, Moqrich A, Earley TJ, Hergarden AC, Story GM, Colley S, Hogenesch JB, McIntyre P, Bevan S, Patapoutian A (2002b) A heat-sensitive TRP channel expressed in keratinocytes. Science 296:2046–2049

Penna A, Juvin V, Chemin J, Compan V, Monet M, Rassendren FA (2006) PI3-kinase promotes TRPV2 activity independently of channel translocation to the plasma membrane. Cell Calcium 39:495–507

Peralvarez-Marin A, Donate-Macian P, Gaudet R (2013) What do we know about the transient receptor potential vanilloid 2 (TRPV2) ion channel? FEBS J 280:5471–5487

Peterlin Z, Chesler A, Firestein S (2007) A painful Trp can be a bonding experience. Neuron 53:635–638

Planells-Cases R, Perez-Paya E, Messeguer A, Carreno C, Ferrer-Montiel A (2003) Small molecules targeting the NMDA receptor complex as drugs for neuropathic pain. Mini Rev Med Chem 3:749–756

Plant TD, Schaefer M (2003) TRPC4 and TRPC5: receptor-operated Ca^{2+} -permeable nonselective cation channels. Cell Calcium 33:441–450

Pochynyuk O, Zaika O, O'Neil RG, Mamenko M (2013) Novel insights into TRPV4 function in the kidney. Pflugers Arch 465:177–186

Ponsati B, Carreno C, Curto-Reyes V, Valenzuela B, Duart MJ, Van den Nest W, Cauli O, Beltran B, Fernandez J, Borsini F, Caprioli A, Serio S D, Veretchy M, Baamonde A, Menendez L, Barros F, de la Pena P, Borges R, Felipo V, Planells-Cases R, Ferrer-Montiel A (2012) An inhibitor of neuronal exocytosis (DD04107) displays long-lasting in vivo activity against chronic inflammatory and neuropathic pain. J Pharmacol Exp Ther 341:634–645

Premkumar LS, Abooj M (2013) TRP channels and analgesia. Life Sci 92:415–424

Qin N, Neeper MP, Liu Y, Hutchinson TL, Lubin ML, Flores CM (2008) TRPV2 is activated by cannabidiol and mediates CGRP release in cultured rat dorsal root ganglion neurons. J Neurosci 28:6231–6238

Ramachandran R, Hyun E, Zhao L, Lapointe TK, Chapman K, Hirota CL, Ghosh S, McKemy DD, Vergnolle N, Beck PL, Altier C, Hollenberg MD (2013) TRPM8 activation attenuates inflammatory responses in mouse models of colitis. Proc Natl Acad Sci U S A 110:7476–7481

Reilly RM, Kym PR (2011) Analgesic potential of TRPV3 antagonists. Curr Top Med Chem 11:2210–2215

Riccio A, Li Y, Moon J, Kim KS, Smith KS, Rudolph U, Gapon S, Yao GL, Tsvetkov E, Rodig SJ, Van't Veer A, Meloni EG, Carlezon WA Jr, Bolshakov VY, Clapham DE (2009) Essential role for TRPC5 in amygdala function and fear-related behavior. Cell 137:761–772

Sabnis AS, Shadid M, Yost GS, Reilly CA (2008) Human lung epithelial cells express a functional cold-sensing TRPM8 variant. Am J Respir Cell Mol Biol 39:466–474

Samer RE, Eric DC, Eric LM, Hongyu AL, Kar-Chan C, Shelley D, Darrell AH, Stefanie AK, Mark OU (2008) HC-030031, a TRPA1 selective antagonist, attenuates inflammatory- and neuropathy-induced mechanical hypersensitivity. Mol Pain 4:48

Santos FA, Rao VS (2001) 1,8-cineol, a food flavoring agent, prevents ethanol-induced gastric injury in rats. Dig Dis Sci 46:331–337

Schaefer M, Plant TD, Obukhov AG, Hofmann T, Gudermann T, Schultz G (2000) Receptor-mediated regulation of the nonselective cation channels TRPC4 and TRPC5. J Biol Chem 275:17517–17526

Sherkheli MA, Benecke H, Doerner JF, Kletke O, Vogt-Eisele AK, Gisselmann G, Hatt H (2009) Monoterpenoids induce agonist-specific desensitization of transient receptor potential vanilloid-3 (TRPV3) ion channels. J Pharm Pharm Sci 12:116–128

Sherkheli MA, Gisselmann G, Hatt H (2012) Supercooling agent icilin blocks a warmth-sensing ion channel TRPV3. Sci World J 2012:982725

Shigetomi E, Tong X, Kwan KY, Corey DP, Khakh BS (2012) TRPA1 channels regulate astrocyte resting calcium and inhibitory synapse efficacy through GAT-3. Nat Neurosci 15:70–80

Shimizu S, Yoshida T, Wakamori M, Ishii M, Okada T, Takahashi M, Seto M, Sakurada K, Kiuchi Y, Mori Y (2006) Ca^{2+} -calmodulin-dependent myosin light chain kinase is essential for activation of TRPC5 channels expressed in HEK293 cells. J Physiol 570:219–235

Smith GD, Gunthorpe MJ, Kelsell RE, Hayes PD, Reilly P, Facer P, Wright JE, Jerman JC, Walhin JP, Ooi L, Egerton J, Charles KJ, Smart D, Randall AD, Anand P, Davis JB (2002) TRPV3 is a temperature-sensitive vanilloid receptor-like protein. Nature 418:186–190

Smith PL, Maloney KN, Pothen RG, Clardy J, Clapham DE (2006) Bisandrographolide from Andrographis paniculata activates TRPV4 channels. J Biol Chem 281:29897–29904

Story GM, Peier AM, Reeve AJ, Eid SR, Mosbacher J, Hricik TR, Earley TJ, Hergarden AC, Andersson DA, Hwang SW, McIntyre P, Jegla T, Bevan S, Patapoutian A (2003) ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. Cell 112:819–829

Stotz SC, Vriens J, Martyn D, Clardy J, Clapham DE (2008) Citral sensing by transient [corrected] receptor potential channels in dorsal root ganglion neurons. PLoS One 3:e2082

Straub I, Krugel U, Mohr F, Teichert J, Rizun O, Konrad M, Oberwinkler J, Schaefer M (2013) Flavanones that selectively inhibit TRPM3 attenuate thermal nociception in vivo. Mol Pharmacol 84:736–750

Strotmann R, Harteneck C, Nunnenmacher K, Schultz G, Plant TD (2000) OTRPC4, a nonselective cation channel that confers sensitivity to extracellular osmolarity. Nat Cell Biol 2:695–702

Sukumar P, Beech DJ (2010) Stimulation of TRPC5 cationic channels by low micromolar concentrations of lead ions ($Pb2^{+}$). Biochem Biophys Res Commun 393:50–54

Szallasi A, Appendino G (2004) Vanilloid receptor TRPV1 antagonists as the next generation of painkillers. Are we putting the cart before the horse? J Med Chem 47:2717–2723

Szallasi A, Blumberg PM (2007) Complex regulation of TRPV1 by vanilloids. In: Liedtke WB, Heller S (eds) TRP ion channel function in sensory transduction and cellular signaling cascades. Boca Raton, CRC Press

Tabuchi K, Suzuki M, Mizuno A, Hara A (2005) Hearing impairment in TRPV4 knockout mice. Neurosci Lett 382:304–308

Takaishi M, Fujita F, Uchida K, Yamamoto S, Sawada M, Hatai C, Shimizu M, Tominaga M (2012) 1,8-cineole, a TRPM8 agonist, is a novel natural antagonist of human TRPA1. Mol Pain 8:86

Tamayo NA, Bo Y, Gore V, Ma V, Nishimura N, Tang P, Deng H, Klionsky L, Lehto SG, Wang W, Youngblood B, Chen J, Correll TL, Bartberger MD, Gavva NR, Norman MH (2012) Fused Piperidines as a Novel Class of Potent and Orally Available Transient Receptor Potential Melastatin Type 8 (TRPM8) Antagonists. J Med Chem 55:1593–1611

Taylor-Clark TE, Undem BJ, MacGlashan DW Jr, Ghatta S, Carr MJ, McAlexander MA (2008) Prostaglandin-induced activation of nociceptive neurons via direct interaction with transient receptor potential A1 (TRPA1). Mol Pharmacol 73:274–281

Thiel G, Muller I, Rossler OG (2013) Signal transduction via TRPM3 channels in pancreatic beta-cells. J Mol Endocrinol 50:R75–R83

Thorneloe KS, Sulpizio AC, Lin Z, Figueroa DJ, Clouse AK, McCafferty GP, Chendrimada TP, Lashinger ES, Gordon E, Evans L, Misajet BA, Demarini DJ, Nation JH, Casillas LN, Marquis RW, Votta BJ, Sheardown SA, Xu X, Brooks DP, Laping NJ, Westfall TD (2008) N-((1 S)-1-{[4-((2 S)-2-{[(2,4-dichlorophenyl)sulfonyl]amino}-3-hydroxypropanoyl)-1 -piperazinyl] carbonyl}-3-methylbutyl)-1-benzothiophene-2-carboxamide (GSK1016790A), a novel and potent transient receptor potential vanilloid 4 channel agonist induces urinary bladder contraction and hyperactivity: part I. J Pharmacol Exp Ther 326:432–442

Tominaga M, Wada M, Masu M (2001) Potentiation of capsaicin receptor activity by metabotropic ATP receptors as a possible mechanism for ATP-evoked pain and hyperalgesia. Proc Natl Acad Sci U S A 98:6951–6956

Tsavaler L, Shapero MH, Morkowski S, Laus R (2001) Trp-p8, a novel prostate-specific gene, is up-regulated in prostate cancer and other malignancies and shares high homology with transient receptor potential calcium channel proteins. Cancer Res 61:3760–3769

Ueda K, Tsuji F, Hirata T, Takaoka M, Matsumura Y (2008) Preventive effect of TRPV1 agonists capsaicin and resiniferatoxin on ischemia/reperfusion-induced renal injury in rats. J Cardiovasc Pharmacol 51:513–520

Umezu T, Sakata A, Ito H (2001) Ambulation-promoting effect of peppermint oil and identification of its active constituents. Pharmacol Biochem Behav 69:383–390

Valdes-Rodriguez R, Kaushik SB, Yosipovitch G (2013) Transient receptor potential channels and dermatological disorders. Curr Top Med Chem 13:335–343

Valente P, Fernandez-Carvajal A, Camprubi-Robles M, Gomis A, Quirce S, Viana F, Fernandez-Ballester G, Gonzalez-Ros JM, Belmonte C, Planells-Cases R, Ferrer-Montiel A (2011) Membrane-tethered peptides patterned after the TRP domain (TRPducins) selectively inhibit TRPV1 channel activity. FASEB J 25:1628–1640

Valero ML, de Queiroz FM, Stuehmer W, Viana F, Pardo LA (2012) TRPM8 ion channels differentially modulate proliferation and cell cycle distribution of normal and cancer prostate cells. PLoS One 7(12):e51825

Vallin KSA, Sterky KJ, Nyman E, Bernstrom J, From R, Linde C, Minidis ABE, Nolting A, Narhi K, Santangelo EM, Sehgelmeble FW, Sohn D, Strindlund J, Weigelt D (2012) N-1-Alkyl-2-oxo-2-aryl amides as novel antagonists of the TRPA1 receptor. Bioorg Med Chem Lett 22:5485–5492

Van Buren JJ, Bhat S, Rotello R, Pauza ME, Premkumar LS (2005) Sensitization and translocation of TRPV1 by insulin and IGF-I. Mol Pain 1:17

Van Der Stelt M, Di MV (2004) Endovanilloids. Putative endogenous ligands of transient receptor potential vanilloid 1 channels. Eur J Biochem 271:1827–1834

Varga A, Bolcskei K, Szoke E, Almasi R, Czeh G, Szolcsanyi J, Petho G (2006) Relative roles of protein kinase A and protein kinase C in modulation of transient receptor potential vanilloid type 1 receptor responsiveness in rat sensory neurons in vitro and peripheral nociceptors in vivo 59. Neuroscience 140:645–657

Vellani V, Mapplebeck S, Moriondo A, Davis JB, McNaughton PA (2001) Protein kinase C activation potentiates gating of the vanilloid receptor VR1 by capsaicin, protons, heat and anandamide. J Physiol 534:813–825

Venkatachalam K, Montell C (2007) TRP channels. Annu Rev Biochem 76:387–417

Vennekens R, Owsianik G, Nilius B (2008) Vanilloid transient receptor potential cation channels: an overview. Curr Pharm Des 14:18–31

Vidal-Mosquera M, Fernandez-Carvajal A, Moure A, Valente P, Planells-Cases R, Gonzalez-Ros JM, Bujons J, Ferrer-Montiel A, Messeguer A (2011) Triazine-based vanilloid 1 receptor open channel blockers: design, synthesis, evaluation, and SAR analysis. J Med Chem 54:7441–7452

Vincent F, Duncton MA (2011) TRPV4 agonists and antagonists. Curr Top Med Chem 11:2216–2226

Vincent F, Acevedo A, Nguyen MT, Dourado M, DeFalco J, Gustafson A, Spiro P, Emerling DE, Kelly MG, Duncton MA (2009) Identification and characterization of novel TRPV4 modulators. Biochem Biophys Res Commun 389:490–494

Vogt-Eisele AK, Weber K, Sherkheli MA, Vielhaber G, Panten J, Gisselmann G, Hatt H (2007) Monoterpenoid agonists of TRPV3. Br J Pharmacol 151:530–540

Vriens J, Watanabe H, Janssens A, Droogmans G, Voets T, Nilius B (2004) Cell swelling, heat, and chemical agonists use distinct pathways for the activation of the cation channel TRPV4. Proc Natl Acad Sci U S A 101:396–401

Vriens J, Appendino G, Nilius B (2009) Pharmacology of vanilloid transient receptor potential cation channels. Mol Pharmacol 75:1262–1279

Vriens J, Owsianik G, Hofmann T, Philipp SE, Stab J, Chen X, Benoit M, Xue F, Janssens A, Kerselaers S, Oberwinkler J, Vennekens R, Gudermann T, Nilius B, Voets T (2011) TRPM3 is a nociceptor channel involved in the detection of noxious heat. Neuron 70:482–494

Wagner TF, Loch S, Lambert S, Straub I, Mannebach S, Mathar I, Dufer M, Lis A, Flockerzi V, Philipp SE, Oberwinkler J (2008) Transient receptor potential M3 channels are ionotropic steroid receptors in pancreatic beta cells. Nat Cell Biol 10:1421–1430

Wahl P, Foged C, Tullin S, Thomsen C (2001) Iodo-resiniferatoxin, a new potent vanilloid receptor antagonist. Mol Pharmacol 59:9–15

Walker KM, Urban L, Medhurst SJ, Patel S, Panesar M, Fox AJ, McIntyre P (2003) The VR1 antagonist capsazepine reverses mechanical hyperalgesia in models of inflammatory and neuropathic pain. J Pharmacol Exp Ther 304:56–62

Wang X, Miyares RL, Ahern GP (2005) Oleoylethanolamide excites vagal sensory neurones, induces visceral pain and reduces short-term food intake in mice via capsaicin receptor TRPV1. J Physiol 564:541–547

Wang Y, Yang Z, Meng Z, Cao H, Zhu G, Liu T, Wang X (2014) Knockdown of TRPM8 suppresses cancer malignancy and enhances epirubicin-induced apoptosis in human osteosarcoma cells. Int J Biol Sci 10:90–102

Watanabe H, Davis JB, Smart D, Jerman JC, Smith GD, Hayes P, Vriens J, Cairns W, Wissenbach U, Prenen J, Flockerzi V, Droogmans G, Benham CD, Nilius B (2002a) Activation of TRPV4 channels (hVRL-2/mTRP12) by phorbol derivatives. J Biol Chem 277:13569–13577

Watanabe H, Vriens J, Suh SH, Benham CD, Droogmans G, Nilius B (2002b) Heat-evoked activation of TRPV4 channels in a HEK293 cell expression system and in native mouse aorta endothelial cells. J Biol Chem 277:47044–47051

Watanabe H, Vriens J, Prenen J, Droogmans G, Voets T, Nilius B (2003) Anandamide and arachidonic acid use epoxyeicosatrienoic acids to activate TRPV4 channels. Nature 424:434–438

Wei H, Koivisto A, Pertovaara A (2010) Spinal TRPA1 ion channels contribute to cutaneous neurogenic inflammation in the rat. Neurosci Lett 479:253–256

Wes PD, Chevesich J, Jeromin A, Rosenberg C, Stetten G, Montell C (1995) TRPC1, a human homolog of a *Drosophila* store-operated channel. Proc Natl Acad Sci U S A 92:9652–9656

Wong CO, Huang Y, Yao X (2010) Genistein potentiates activity of the cation channel TRPC5 independently of tyrosine kinases. Br J Pharmacol 159:1486–1496

Wu LJ, Sweet TB, Clapham DE (2010) International union of basic and clinical pharmacology. LXXVI. Current progress in the mammalian TRP ion channel family. Pharmacol Rev 62:381–404

Wuensch T, Thilo F, Krueger K, Scholze A, Ristow M, Tepel M (2010) High glucose-induced oxidative stress increases transient receptor potential channel expression in human monocytes. Diabetes 59:844–849

Xing H, Chen M, Ling J, Tan W, Gu JG (2007) TRPM8 mechanism of cold allodynia after chronic nerve injury. J Neurosci 27:13680–13690

Xu H, Ramsey IS, Kotecha SA, Moran MM, Chong JA, Lawson D, Ge P, Lilly J, Silos-Santiago I, Xie Y, DiStefano PS, Curtis R, Clapham DE (2002) TRPV3 is a calcium-permeable temperature-sensitive cation channel. Nature 418:181–186

Xu H, Blair NT, Clapham DE (2005a) Camphor activates and strongly desensitizes the transient receptor potential vanilloid subtype 1 channel in a vanilloid-independent mechanism. J Neurosci 25:8924–8937

Xu SZ, Zeng F, Boulay G, Grimm C, Harteneck C, Beech DJ (2005b) Block of TRPC5 channels by 2-aminoethoxydiphenyl borate: a differential, extracellular and voltage-dependent effect. Br J Pharmacol 145:405–414

Xu H, Delling M, Jun JC, Clapham DE (2006a) Oregano, thyme and clove-derived flavors and skin sensitizers activate specific TRP channels. Nat Neurosci 9:628–635

Xu SZ, Muraki K, Zeng F, Li J, Sukumar P, Shah S, Dedman AM, Flemming PK, McHugh D, Naylor J, Cheong A, Bateson AN, Munsch CM, Porter KE, Beech DJ (2006b) A sphingosine-1-phosphate-activated calcium channel controlling vascular smooth muscle cell motility. Circ Res 98:1381–1389

Xu SZ, Sukumar P, Zeng F, Li J, Jairaman A, English A, Naylor J, Ciurtin C, Majeed Y, Milligan CJ, Bahnasi YM, Al-Shawaf E, Porter KE, Jiang LH, Emery P, Sivaprasadarao A, Beech DJ (2008) TRPC channel activation by extracellular thioredoxin. Nature 451:69–72

Yamamura H, Ugawa S, Ueda T, Morita A, Shimada S (2008) TRPM8 activation suppresses cellular viability in human melanoma. Am J Physiol Cell Physiol 295:C296–C301

Yang XR, Lin MJ, McIntosh LS, Sham JS (2006) Functional expression of transient receptor potential melastatin- and vanilloid-related channels in pulmonary arterial and aortic smooth muscle. Am J Physiol Lung Cell Mol Physiol 290:L1267–L1276

Yee NS, Zhou W, Lee M (2010) Transient receptor potential channel TRPM8 is over-expressed and required for cellular proliferation in pancreatic adenocarcinoma. Cancer Lett 297:49–55

Zamudio-Bulcock PA, Everett J, Harteneck C, Valenzuela CF (2011) Activation of steroid-sensitive TRPM3 channels potentiates glutamatergic transmission at cerebellar Purkinje neurons from developing rats. J Neurochem 119:474–485

Zeng F, Xu SZ, Jackson PK, McHugh D, Kumar B, Fountain SJ, Beech DJ (2004) Human TRPC5 channel activated by a multiplicity of signals in a single cell. J Physiol 559:739–750

Zhang X1, Huang J, McNaughton PA (2005) NGF rapidly increases membrane expression of TRPV1 heat-gated ion channels. EMBO J 24(24):4211–23.

Zhang X, Mak S, Li L, Parra A, Denlinger B, Belmonte C, McNaughton PA (2012) Direct inhibition of the cold-activated TRPM8 ion channel by G alpha(q). Nat Cell Biol 14:850–168

Zhu B, Xia M, Xu X, Ludovici DW, Tennakoon M, Youngman MA, Matthews JM, Dax SL, Colburn RW, Qin N, Hutchinson TL, Lubin ML, Brandt MR, Stone DJ, Flores CM, Macielag MJ (2013) Arylglycine derivatives as potent transient receptor potential melastatin 8 (TRPM8) antagonists. Bioorg Med Chem Lett 23:2234–2237

Zufall F, Ukhanov K, Lucas P, Liman ER, Leinders-Zufall T (2005) Neurobiology of TRPC2: from gene to behavior. Pflugers Arch 451:61–71

Chapter 3
Modulation of TRP Channels by N-glycosylation and Phosphorylation

María Pertusa and Rodolfo Madrid

Abstract Transient receptor potential (TRP) are cation permeable channels found in eukaryotic organisms, from yeast to human. These channels are involved in several physiological processes ranging from Ca^{2+} and Mg^{2+} homeostasis to sensory functions, including vision, pheromone signalling, taste, nociception, mechanotransduction and temperature sensing, among others. Most of these channels show covalent post-translational modifications (PTMs) that have a profound effect on their physiological function, by influencing their subcellular trafficking and/or their biophysical properties. Among these modifications, N-linked glycosylation and phosphorylation are two of the most relevant PTMs determining the functional expression of TRP channels. In this chapter we summarize their role in modulating the functional properties of TRP channels, with a critical role in sensory transduction.

Keywords N-glycosylation · Phosphorylation · Post-translational modifications · TRP channels

3.1 Introduction

In eukaryotic cells, proteins can be modified after translation by several reversible and irreversible modifications. These post-translational modifications (PTMs) result in the diversification of the protein functions beyond what is dictated by gene transcripts. Through these PTMs, eukaryotic cells can dynamically alter the structure, stability and function of a wide spectrum of proteins. Cleavage, addition of sugars, lipids, small proteins or chemical groups such as methyl, nitric oxide, phosphate and acetate (Fig. 3.1), are well known PTMs that regulate the functional expression of ion channels in different physiological and physiopathological con-

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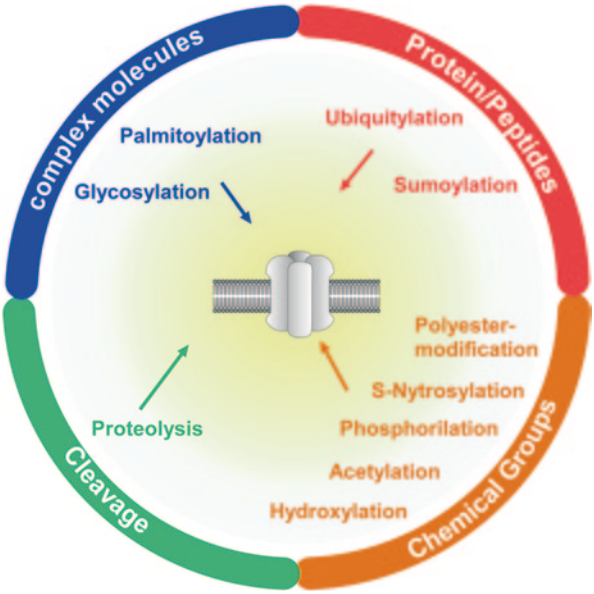

Fig. 3.1 Schematic representation of some post-translational modifications in ion channels. Addition of complex molecules, small proteins, chemical groups and cleavage is depicted. In this chapter we discuss the role of N-glycosylation and phosphorylation in TRP channels. (Inspired in Fig. 1 of (Wang et al. 2014))

texts. The regulation of ion channels function occurs either modulating their subcellular trafficking or altering their biophysical properties. TRP channels are not the exception, and among the PTMs that exert an enormous effect on their physiological role, N-glycosylation and phosphorylation stand out. In sensory transduction, where several members of this superfamily of ion channels have a critical role, these PTMs regulate their destination to the specialized structures where transduction take place, and tune their contribution to the net response of the sensory cell to different physiologically relevant stimuli.

In this chapter, we summarize the contribution of N-glycosylation and phosphorylation to the functional expression of some TRP channels (Fig. 3.2) with a relevant role in sensory transduction.

3.2 Modulation of TRP Channels by N-glycosylation

3.2.1 The N-linked Glycosylation Process

N-glycosylation is one of the most common PTMs influencing the function of TRP channels. This PTM consists of the covalent addition of sugar residues to asparagines that are part of Asn-**X**-Ser/Thr consensus sequence, where **X** represents any amino acid except proline (Apweiler et al. 1999). The sugar composition of this modification results from the activity of different enzymes in the endoplasmic reticulum (ER) and the Golgi apparatus (reviewed by Varki et al. 1999). This process

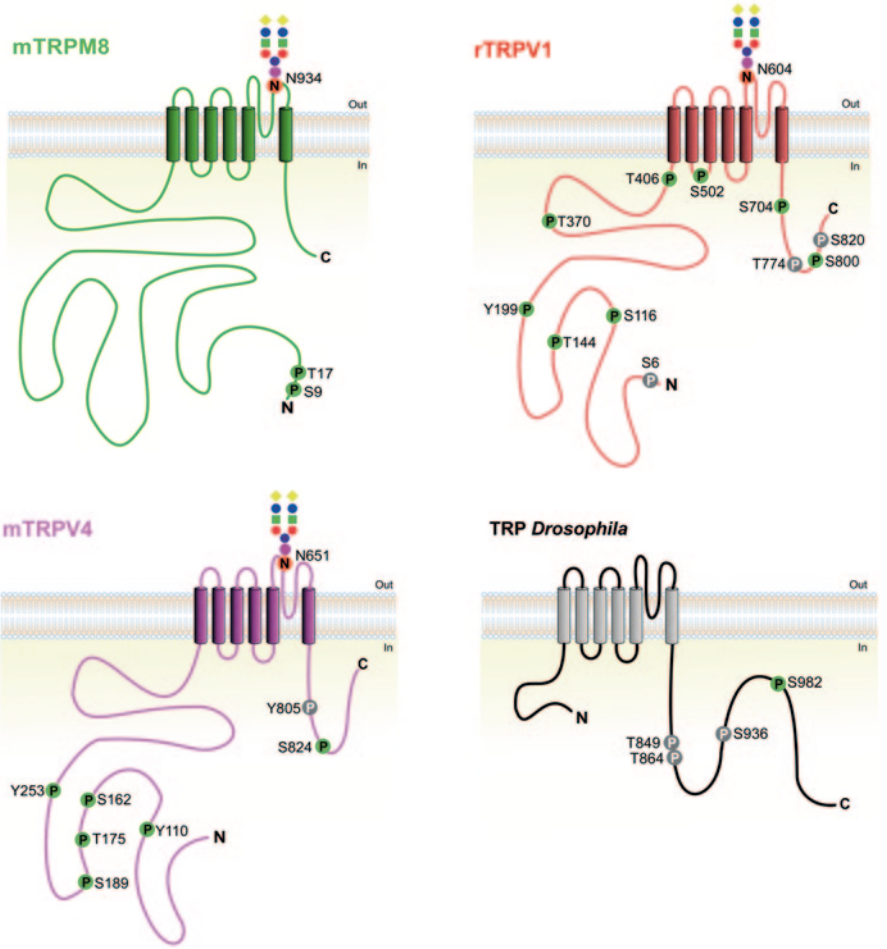

Fig. 3.2 Schematic representation of the different monomers of *mouse TRPM8*, *rat TRPV1*, *mouse TRPV4* and *Drosophila TRP*. Phosphorylated residues, identified with specific antibodies, site directed mutagenesis and amino acid sequencing are represented by *P*. Other phosphorylated residues identified by mass spectrometry are included in Table 3.1. *Green* and *grey* backgrounds in *P* represent phosphorylation with or without reported effects in channel function, respectively. N-glycosylation is shown as a branched chain of sugar moieties in asparagine residues (*N*)

begins with the co-translational transference of the oligosaccharide precursor Glc3Man9GlcNAc2 to the nascent polypeptide (reviewed by Varki et al. 1999). The acceptor asparagine has to be accessible to the oligosaccharyltransferase complex, which catalyzes the transference of the oligosaccharide precursor to the polypeptide on the luminal side of the ER membrane. The resulting glycoprotein undergoes enzymatic removal of three terminal glucose moieties in the ER, resulting in the "high-mannose" state, and is then transported to the Golgi apparatus where additional modifications occur (reviewed by Varki et al. 1999). In this compart-

ment, different enzymes may modify the early/immature glycosylation, generating a complex mature N-glycosylation. Unlike the high mannose glycosylation that takes place in the ER, which is the same for all the proteins, the composition of the N-glycosylation tree displayed at the end of the transit of the proteins through the Golgi is quite diverse, and depends on the specificities of Golgi enzymes involved and their tissue-specific expression patterns (Schachter et al. 1983; Comelli et al. 2006).

Several reports have shown that glycans can modify functional properties of ion channels (see (Scott and Panin 2014) for a review). One of the mechanisms by which N-glycosylation regulates ion channels function is modulating their trafficking to the cell surface. The role of this PTM in processes such as protein folding, stability and subcellular trafficking are critical to determine the final density of ion channels in the plasma membrane (reviewed by Helenius and Aebi 2001). N-glycosylation can also alter diverse biophysical properties of the channel, tuning their physiological function. Both modulatory mechanisms can be found in TRP channels (reviewed by Cohen 2006). For instance, the hydrolysis of some sugar residues of TRPV5 N-glycosylation by *klotho*, a glucuronidase enzyme, results in the stabilization of the channel at the apical plasma membrane of kidney epithelial cells, increasing the expression of TRPV5 in this compartment (Chang et al. 2005). This mechanism is used to regulate reabsorption of Ca^{2+} in the nephron, that maintains the normal Ca^{2+} levels in the blood (Chang et al. 2005). On the other hand, in the case of TRPC6 and TRPC3, differences in N-glycosylation pattern are responsible for basal channel activity (Dietrich et al. 2003). Mutation of one of the two N-glycosylation sites of TRPC6 turns this tightly regulated channel in a constitutively active channel, similar to TRPC3 (Dietrich et al. 2003).

3.2.2 TRPV1 N-glycosylation

Transient **R**eceptor **P**otential **V**anilloid **1** (**TRPV1**) is a non-selective, calcium-permeable cation channel, activated by capsaicin, heat, voltage and acidic pH expressed in nociceptors (Caterina et al. 1997). This channel is involved in heat thermosensation and has a critical role in inflammatory pain (Caterina et al. 1997; Davis et al. 2000; Zhang et al. 2005) (see Chap. 8 by McNaughton and Tan in this book). The predicted mass of the TRPV1 monomer is ~95 kDa, however, western blot experiments of transfected cell lines reveal the presence of higher molecular weight bands, suggesting that TRPV1 is a N-glycosylated protein (Kedei et al. 2001; Jahnel et al. 2001). Treatment of protein extracts with different glycosidases corroborated the existence of an immature high mannose type glycosylation that takes place in the ER, and a mature (or complex) glycosylation that requires protein transit through the different Golgi compartments and generates a diffuse band at ~115 kDa (Kedei et al. 2001; Jahnel et al. 2001; Veldhuis et al. 2012). Using biotinylation in a recombinant system, the analysis of TRPV1 channels located in the plasma membrane also evidenced the presence of multiple bands, suggesting that the channel at the cell surface could exist in different states of glycosylation (Veldhuis et al. 2012).

While the N-glycosylation of TRPV1 in transfected cell lines, such as **H**uman **E**mbryonic **K**idney (**HEK**293) cells and the dorsal root ganglia derived cell line F11, has been reported by several laboratories (Kedei et al. 2001; Jahnel et al. 2001; Veldhuis et al. 2012), the N-glycosylation of the native TRPV1 is more controversial. This is not a trivial point, since this kind of PTM is strongly dependent on the cellular context and it must be considered in order to select the appropriate expression system to study the functional properties of an ion channel of interest, and especially to extrapolate these findings to a physiological function of native channels. Using western blot analysis, Kedei and co-workers have reported the lack of mature N-glycosylation in purified membrane extracts of dorsal root ganglia (DRG) (Kedei et al. 2001). In contrast, Veldhuis and co-workers showed that N-glycosidase activity affected the apparent molecular weight of TRPV1 from rat DRG and sciatic nerve, suggesting that this post-translational modification occurs in vivo (Veldhuis et al. 2012).

The N-glycosylation of TRPV1 channels takes place at residue Asn604, located in the third extracellular loop near the pore domain (Jahnel et al. 2001). Although the lack of this PTM does not affect TRPV1 trafficking to the plasma membrane (Veldhuis et al. 2012), it significantly alters some of its biophysical properties (Wirkner et al. 2005; Veldhuis et al. 2012). Electrophysiological experiments revealed that mutation of N604T, that prevents the N-glycosylation, causes a significant reduction in the EC_{50} value for capsaicin and in the maximal response of the channel to this agonist (Wirkner et al. 2005). The response to 1 μM capsaicin of the unglycosylated channel is much more sensitive to changes in extracellular proton concentration, suggesting that this PTM plays a role in the regulation of the TRPV1 pH sensitivity (Wirkner et al 2005). Interestingly, compared to the wild type channel, in the N604T mutant the antagonist capsazepine was a more effective blocker of capsaicin-induced responses at physiological pH (Wirkner et al. 2005). Thus, these data correlate the presence of N-glycosylation with a decrease in TRPV1 sensitivity to agonists such as capsaicin and protons, and antagonists like capsazepine. During inflammatory responses, alterations in extracellular pH are common, and acidic pH changes predominate. In these conditions, N-glycosylation of TRPV1 dampens the effect of acidic pH on channel activation by other physiologically relevant stimuli, such as heat or endogenous modulators of its function in nociceptors. Thus, thermal hyperalgesia that results of an inflammatory state could be reduced if the channels are mainly in the N-glycosylated form in nociceptive free nerve endings.

The role of N-glycosylation in the capsaicin-evoked pore dilation, that has been recently described in TRPV1 channels (Chung et al. 2008), has also been investigated. Chung and co-workers described how activation of TRPV1 with capsaicin leads to an increase in relative permeability to large cations and Ca^{2+} through agonist-induced pore dilation process, a structural change originally described in ATP-gated P2Xs channels, which has also been studied in other TRP channels (Chen et al. 2009). The effect of N-glycosylation of TRPV1 in the pore dilation mechanism was assessed by Veldhuis and co-workers, measuring the capsaicin-dependent uptake of the large fluorescent cationic dye YO-PRO-1. Cells transfected with the unglycosylated mutant showed a decrease in the uptake of this molecule, suggesting that the pore dilation state induced by capsaicin is in part dependent on the N-glycosylation

status of TRPV1 (Veldhuis et al. 2012). Thus, capsaicin-induced pore dilation and the subsequent enhanced permeability to Ca^{2+} ions could be critical for the selective death of TRPV1-expressing neurons. Prolonged exposure to this vanilloid is one of the most effective treatments used to reduce pain in several neuropathies, and pore dilation may contribute to the paradoxical analgesic effect of capsaicin though death of nociceptive sensory nerve fibers (Bautista and Julius 2008).

The N-glycosylation of TRPV1 has also been connected with channel desensitization. Analysis of the decay of the responses to sustained stimulation with capsaicin has shown that the desensitization rate of the response in cells expressing TRPV1 displayed a large variability. In contrast, the response of the cells transfected with the unglycosylated N604T mutant always showed a constant relaxation kinetic (Veldhuis et al. 2012). This observation suggests that desensitization depends, at least to some extent, on the glycosylation status of the channel. As mentioned above, TRPV1 located in the plasma membrane shows different states of glycosylation, that could account for the observed cell-to-cell variability (Veldhuis et al. 2012). How this variability may influence the physiological net response of TRPV1 channels in native membranes is still unclear.

3.2.3 TRPM8 N-glycosylation

Transient **R**eceptor **P**otential **M**elastatine **8** (**TRPM8**) is a thermoTRP channel activated by cold, cooling compounds such as menthol and by voltage. This channel is the main molecular entity responsible for detection of cold temperatures in the somatosensory system (recently reviewed by McCoy et al. 2011; Almaraz et al. 2014; Madrid and Pertusa 2014); see also Chap. 9 by González et al. in this Book). Like TRPV1, TRPM8 is also a glycoprotein (Dragoni et al. 2006; Erler et al. 2006; Tsuruda et al. 2006). In TRPM8, the N-glycosylation takes place at residue Asn934, located in the extracellular loop between transmembrane domains five and six, near the pore domain (Dragoni et al. 2006; Erler et al. 2006). Analysis of protein extracts of HEK293 cells transfected with TRPM8 shows three distinguishable bands corresponding to different glycosylation states of TRPM8: the unglycosylated protein, an early N-glycosylated form produced in the ER and the mature N-glycosylated form that contains terminal sialic acid residues (Morenilla-Palao et al. 2009; Pertusa et al. 2012). The N934Q mutant, which has been used to evaluate the effect of this PTM in TRPM8 activity, shows a reduced response to cold and menthol (Dragoni et al. 2006; Erler et al. 2006; Pertusa et al. 2012). Different mechanisms have been proposed to explain this N-glycosylation-dependent effect on TRPM8. Whereas some authors suggest that it is mainly the result of a drop in the number of TRPM8 channels in the plasma membrane (Dragoni et al. 2006; Erler et al. 2006), others have suggested a direct effect on N-glycosylation on the biophysical properties of the channel (Pertusa et al. 2012). It has been demonstrated that the absence of this PTM causes a shift in the voltage-dependence of the channel of almost 60 mV to more positive voltages, decreasing its open probability at physiologically relevant

membrane potentials (Pertusa et al. 2012), therefore influencing its cold and menthol responses.

Protein extracts from DRG and trigeminal ganglia (TG) revealed that TRPM8 N-glycosylation also takes place in vivo. In fact, the treatment of TG neurons with the N-glycosylation inhibitor tunicamycin shifts the mean temperature threshold of cold responses from ~29 to ~23 °C (Pertusa et al. 2012). This shift in recombinant systems is from ~26 to ~23 °C, suggesting a larger impact of this PTM in temperature-dependent responses in native channels (Pertusa et al. 2012)

N-glycosylation also plays a role in the segregation of TRPM8 to lipid rafts (Morenilla-Palao et al. 2009), facilitating its association to these plasma membrane microdomains that contain high concentrations of cholesterol and glycosphingolipids (Simons and Ikonen 1997). It has been reported that within the plasma membrane TRPM8 is mainly localized in lipid rafts, in both native and recombinant systems (Morenilla-Palao et al. 2009). Interestingly, the association of TRPM8 to lipid rafts decreases both menthol- and cold-mediated activation of the channel. This observation suggests that TRPM8 can be affected by its lipid environment and that menthol- and cold-mediated responses of this channel are facilitated when the protein is outside these domains (Morenilla-Palao et al. 2009). Therefore, unglycosylated channels, which show an important reduction in their association to lipid rafts (Morenilla-Palao et al. 2009), should display higher responses to agonists than the N-glycosylated protein. Nevertheless, as mentioned before, the non-glycosylated mutant shows a reduced response to cold and menthol stimulation, indicating that N-glycosylation *per se*, independent of the lipid raft association, has an important influence on biophysical properties of TRPM8 (Pertusa et al. 2012).

Lipid rafts have been linked to vital cell processes such as trafficking and sorting of membrane proteins (reviewed by Pristera et al. 2012). Although the roles of lipid rafts and N-glycosylation in protein sorting of TRPM8 still remains unexplored, this association may be critical for the correct targeting and trafficking dynamics in native membranes. Moreover, it is also important to consider that by promoting TRPM8 association with lipid rafts, N-glycosylation may also be favoring protein-protein interactions that could modulate channel function in the nerve endings of primary somatosensory neurons, where cold transduction occurs. Thus, N-glycosylation of TRPM8 emerges as an important mechanism that can modulate channel function, affecting its biophysical properties and determining the association of the channel with lipid rafts.

Altogether, these results illustrate the relevance of N-glycosylation in the physiological function of TRPM8 and suggest that in native systems it could be critical in the fine tuning of cold sensitivity in cold thermoreceptor neurons.

3.2.4 TRPV4 N-glycosylation

As TRPM8 and TRPV1, **T**ransient **R**eceptor **P**otential **V**anilloid **4** (**TRPV4**) is also a polymodal TRP channel activated by a plethora of stimuli, including cell swelling,

innocuous heat, arachidonic acid, the endogenous cannabinoid neurotransmitter anandamide and several exogenous ligands. TRPV4 is a broadly expressed channel with important roles in osmosensation and thermosensation, and mutations of the gene encoding this protein are related to diverse diseases (see Nilius and Voets 2013 for a complete review).

As other glycosylated TRP channels, immunoblots from heterologously expressed and native TRPV4 show multiple bands (Xu et al. 2006; Arniges et al. 2006). In agreement with the presence of N-glycosylation, treatment of cell lysates with endoglycosidase F results in a single band at 96 kDa, corresponding to the non-glycosylated form of the channel (Xu et al. 2003; Arniges et al. 2006). Like in TRPV1 and TRPM8, western blots of TRPV4 show two additional higher molecular weight bands with apparent molecular masses of 100 and 110 kDa, representing respectively the high mannose and complex glycosylation states of the channel (Arniges et al. 2006). Site-directed mutagenesis of potential glycosylation sites revealed that N-glycosylation of TRPV4 occurs at residue Asn651, located in the pore loop (Xu et al. 2006). The unglycosylated mutant channel N651Q, expressed in HEK293 cells, exhibited greater responsiveness to hypotonicity (Xu et al. 2006). The mechanism behind this effect was revealed using biotinylation assays, where a higher expression level of this mutant channel at the cell surface was detected. Therefore, unlike the previous examples in the other thermoTRP channels mentioned above, the alteration in the response observed in the unglycosylated mutant is most likely due to enhanced trafficking of the protein to the plasma membrane.

Thus, N-glycosylation emerges as a critical PTM influencing the physiological role of TRP channels that are key elements in thermotransduction, inflammatory pain and transduction of osmotic stimuli.

3.3 Regulation of TRP Channels by Phosphorylation

3.3.1 Protein Phosphorylation

Protein phosphorylation consists in the transferring of a phosphate to the hydroxyl group of a serine, threonine or tyrosine of a substrate protein, usually producing a modification in its functional properties. This reversible and covalent modification is catalyzed by different kinases, and dephosphorylation is carried out by phosphoprotein phosphatases. Ion channels are targets of these enzymes, and the addition or removal of phosphate groups can modify the channel activity determining the net electrophysiological properties of the cell (reviewed by Swope et al. 1992). Typically, the phosphorylation pattern of ion channels involves several sites with consensus sequences for different protein kinases. TRP channels are modulated by phosphorylation, with quite diverse consequences. For example, TRPC3 and TRPC6 are inhibited by PKC-mediated phosphorylation, in contrast with the enhancement observed after Src family tyrosine kinases (SFK)-dependent phosphorylation (reviewed by Voolstra and Huber 2014).

In the next section we will discuss some examples of modulation by phosphorylation in TRPs involved in sensory transduction, with especial attention to TRPV1 channel, whose modulation by this PTM has been extensively studied.

3.3.2 Phosphorylation in TRPV1

3.3.2.1 Role of Phosphorylation in TRPV1 Channel Sensitization

Most of the nociceptive neurons show an increase in their responses under repetitive noxious stimulation. In these neurons, TRPV1 plays a key role in the generation of hyperalgesia, defined as exacerbated pain in response to normally painful stimulation, occurring under pathological states such as inflammation (Caterina et al. 1997; Davis et al. 2000). TRPV1 is directly responsible for the hyperalgesia triggered by several proinflammatory mediators including bradykinin, nerve growth factor (NGF) and ATP (Caterina et al. 1997; Cesare et al. 1999; Shu and Mendell 1999; Premkumar and Ahern 2000; Chuang et al. 2001; Vellani et al. 2001; Prescott and Julius 2003; Bonnington and McNaughton 2003). The interaction of these proinflammatory factors with their receptors leads a downstream activation of different kinases, such as PKC, PKA or SFKs. These kinases phosphorylate specific residues in TRPV1, causing an enhanced response of this channel to heat, capsaicin, protons or anandamide (Premkumar and Ahern 2000; Vellani et al. 2001; Numazaki et al. 2002; Zhang et al. 2005). Thus, phosphorylation of TRPV1 enhances its responses to endogenous and exogenous agonists, either by modifying its biophysical properties or by increasing the number of channels in the plasma membrane (Zhang et al. 2005, 2008; Studer and McNaughton 2010).

PKC is essential to the enhanced response of TRPV1 during inflammatory hyperalgesia (Premkumar and Ahern 2000; Vellani et al. 2001). Among the different putative targets of PKC in the protein, the phosphorylation of Ser502 and Ser800 are critical for this effect (Numazaki et al. 2002; Bhave et al. 2003). Mutation of Ser502 and Ser800 to alanine prevents both channel phosphorylation and the potentiation of the TRPV1-dependent responses caused by the PKC activator **p**horbol 12-**m**yristate 13-**a**cetate (PMA) (Numazaki et al. 2002; Bhave et al. 2003). Electrophysiological evidence obtained at single-channel level of the double mutant S502A/S801A of human TRPV1 has been useful to understand the mechanism behind this potentiation (Studer and McNaughton 2010). Activation of PKC enhances the response of TRPV1 to capsaicin, increasing the open probability of the channel with no alterations in single-channel conductance (Studer and McNaughton 2010). One possible explanation for this effect is that Ser502/Ser801 phosphorylation increases the capsaicin binding affinity. However, phosphorylation of TRPV1 by PKC also potentiates the response to heat, protons and anandamide (Premkumar and Ahern 2000; Vellani et al. 2001) The intracellular capsaicin binding site is also implicated in anandamide activation (Jordt and Julius 2002), while protons target an

extracellular site (Jordt et al. 2000). Given the distance between these two activation sites, it seems unlikely that the increased TRPV1 activity following PKC phosphorylation is only due to changes in the conformation of the capsaicin binding site. This suggests that phosphorylation of these residues affects common downstream steps that lead to channel opening (Studer and McNaughton 2010). On the other hand, it is important to point out that in basal conditions the double mutant S502A/S801A and the wild type channel are similarly activated by capsaicin. This suggests that the phosphorylation of these two serines are only involved in the potentiating effect of PKC (Studer and McNaughton 2010). In addition, mentioned before, agonist stimulation induces dynamic changes in the ionic selectivity of TRPV1 that could be enhanced by phosphorylation of Ser800 by PKC (Chung et al. 2008). This mechanism may contribute to the modulatory effects of different inflammatory mediators on TRPV1 function in nociceptors (Chung et al. 2008).

Nevertheless, other mechanisms could also be involved in the potentiation of PKC stimulation of TRPV1-dependent responses. TRPV1 channels located in vesicles can be rapidly recruited to the plasma membrane by regulated exocytosis upon PKC stimulation, increasing their density at the plasma membrane (Morenilla-Palao et al. 2004). In fact, it has been proposed that the interaction of the channel with AKAP79/150 (see below), together with the phosphorylation of TRPV1 in Ser502 by PKC or PKA, is required to promote TRPV1 trafficking to the cell surface (Zhang et al. 2008).

NGF also causes an enhanced trafficking of TRPV1 to the plasma membrane. The interaction of NGF with the TrkA receptor initiate a signalling pathway that activates the Src kinase, which directly phosphorylates TRPV1 at Tyr200, promoting the insertion of channel in the plasma membrane (Zhang et al. 2005). As a consequence, the responses of TRPV1 to agonists after NGF exposure are larger, influencing the net response of the nociceptive neuron to endogenous and exogenous activators of TRPV1 in hyperalgesic events (Zhang et al. 2005).

3.3.2.2 Basal Phosphorylation of TRPV1 and Desensitization

Phosphorylation of TRPV1 can also occur in basal conditions. In fact, several studies have reported that TRPV1 transfected cells display basal ^{32}P incorporation, indicating that this channel is constitutively phosphorylated (Numazaki et al. 2002; Bhave et al. 2002; Jung et al. 2004; Jeske et al. 2006). In agreement with these results, the general kinase inhibitor staurosporine reduces single-channel activity, supporting the relevance of basal phosphorylation on TRPV1 activity (Studer and McNaughton 2010).

One remarkable feature of TRPV1 is the pronounced desensitization during sustained agonist exposure (acute desensitization), or upon repetitive agonist applications (tachyphylaxis). Interestingly, both effects are correlated with a robust dephosphorylation of the protein after activation (Docherty et al. 1996; Bhave et al. 2002). The desensitization of capsaicin-evoked currents is dependent on external Ca^{2+} (Docherty et al. 1996; Koplas et al. 1997) and requires Ca^{2+}- and calmodulin-

dependent protein phosphatase 2B (calcineurin) (Docherty et al. 1996; Jung et al. 2004; Mohapatra and Nau 2005). Among the residues constitutively phosphorylated involved in the desensitization, Ser116 was the first identified (Bhave et al. 2002). Bhave and colleagues demonstrated that this position is dephosphorylated after capsaicin exposure, leading TRPV1 desensitization. Tachyphylaxis could be prevented by PKA activation that re-phosphorylates Ser116 (Bhave et al. 2002). However, dephosphorylation and phosphorylation of Ser 116 do not represent desensitized or sensitized states of the channel. In contrast, a mechanism was suggested where a dephosphorylated Ser116 allows the entry of TRPV1 into a desensitized state, which is prevented by the phosphorylation of the channel in this position (Bhave et al. 2002).

PKA also phosphorylate Thr370 and Thr144 in TRPV1 (Bhave et al. 2002). Interestingly, mutants T370D, T370A, T144D T144A exhibited a significantly reduced acute desensitization and tachyphylaxis. However, treatments with the PKA activator forskolin, or calcineurin inhibitors, used to reduce these desensitization processes, had different effects in these mutants. In the Thr144 mutants, both strategies caused a decrease in tachyphylaxis, whereas in Thr370 mutants, these treatments where ineffective. These results suggest that only Thr370 is involved in the reduction of the desensitization induced by PKA activation (Mohapatra and Nau 2003, 2005). The phosphorylation of residues Thr370 and Thr144 of TRPV1 has also been related with the anti-hyperalgesic effect displayed by some cannabinoid agonists such as WIN 55,212-2 (WIN). These residues are constitutively phosphorylated in native TRPV1 from DRG neurons (Jeske et al. 2006). In the subset of somatosensory neurons that coexpress TRPV1 and TRPA1 channels, activation of TRPA1 by WIN causes a Ca^{2+} influx that activates calcineurin, leading to the dephosphorylation of these residues and desensitization of TRPV1 (Jeske et al. 2006).

It has also been reported that native TRPV1 can be directly phosphorylated at position Thr407 by **c**yclin-**d**ependent **k**inase **5** (**cdk5**) (Pareek et al. 2007). When this kinase is inhibited, capsaicin responses of the channel are significantly smaller (Pareek et al. 2007). Thus, cdk5 in DRG neurons would be responsible for a basal phosphorylation of TRPV1 that increase its sensitivity to agonists in a native system (Pareek et al. 2007).

Finally, it has been described that Thr705 is phosphorylated in the subset of DRG neurons that coexpress TRPV1 and PKCβII. The direct binding of the PKCβII to the channel activates this kinase, which in turn phosphorylates Thr705 producing an increase in the sensitivity of TRPV1 to heat and capsaicin. Thus, in basal conditions, DRG neurons display different temperature thresholds depending on whether TRPV1 is expressed simultaneously with PKCβII or not (Li et al. 2014).

In addition, dephosphorylation of Thr704 has been related with TRPV1 desensitization. Experiments with the double mutant S502A/T704I have suggested that the dephosphorylation of both positions causes a reduction in capsaicin binding (Jung et al. 2004).

Altogether, these results suggest that in a non-stimulated cell, TRPV1 displays a basal phosphorylation that depends on cellular context and strongly determines the functional properties of the channel in basal conditions, influencing its physiological role in native membranes.

3.3.2.3 TRPV1 Phosphorylation and Scaffolding Proteins

The speed and specificity of the action of kinases is favoured in many cases by scaffolding proteins, which assemble these enzymes into signalling complexes with their substrate proteins. The scaffolding protein AKAP (**A** **K**inase **A**nchoring **P**rotein) integrate a large number of kinases and phosphatases into functional signalling complexes (Reviewed by (Smith et al. 2006)). AKAP79/150 has three orthologs: human AKAP79, rodent AKAP150 and bovine AKAP75. AKAP79/150 links PKA, PKC and calcineurin to different ion channels, including glutamate receptors, calcium channels, and M-type potassium channels (Gao et al. 1997; Colledge et al. 2000; Altier et al. 2002; Hoshi et al. 2003; Sandoz et al. 2006; Oliveria et al. 2007; Chai et al. 2007). Early works suggested that an AKAP protein may be involved in the modulation of TRPV1 by PKA (Rathee et al. 2002; Distler et al. 2003), and more recent evidence demonstrated a direct interaction of TRPV1 and AKAP79/150 in native systems (Zhang et al. 2008; Schnizler et al. 2008; Jeske et al. 2008).

Zhang and co-workers showed that overexpression of AKAP79 enhanced the sensitivity of TRPV1 to capsaicin. AKAP deletions without PKC binding site shows an important diminution in the responses to capsaicin, suggesting that PKC phosphorylation is important to modulate TRPV1 in basal conditions. However, a deleted version of AKAP79 protein lacking the PKA binding site has a smaller effect, suggesting that phosphorylation by PKA is less important than that of PKC at resting conditions (Zhang et al. 2008). AKAP79 also participates in the sensitization of TRPV1 by proinflammatory mediators that modulate the channel via PKA and PKC (Zhang et al. 2008; Schnizler et al. 2008; Jeske et al. 2008). In fact, the simple activation of these kinases is not enough to cause potentiation of TRPV1; the generation of a scaffolding complex between TRPV1, AKAP79, and PKC (or PKA) is required for sensitization (Zhang et al. 2008). In addition, AKAP79/150 is also implicated in TRPV1 desensitization through the binding site for the calcium-dependent phosphatase calcineurin (Zhang et al. 2008).

Summarizing, phosphorylation of the heat- and capsaicin-activated TRPV1 channel plays a critical role in fine tuning its response to thermal and chemical stimulation in basal conditions, or mediating the reversible regulation that occurs during sensitization triggered by proinflammatory mediators in nociceptive neurons.

3.3.3 Phosphorylation in TRPM8

In the case of the cold- and menthol-activated channel TRPM8, its function can be modulated by different protein kinases. In contrast to TRPV1, the activation of PKC by bradykinin or PMA produces a downregulation of TRPM8 (Premkumar et al. 2005; Abe et al. 2006; Linte et al. 2007), together with a decrease in serine-phosphorylation (Premkumar et al. 2005), suggesting that in basal conditions is phosphorylated. However the kinases that participate in this constitutive

phosphorylation, the residues where it takes place and its physiological relevance remain unresolved.

Different studies have also correlated a decrease in TRPM8 phosphorylation with a reduction in its sensitivity to chemical stimulation. Bavencoffe et al. (2010) reported a PKA-dependent basal phosphorylation of Ser9 and Thr17 residues of TRPM8. In this work, activation of the G_i protein and subsequent inhibition of the adenylate cyclase (AC)/cAMP/PKA pathway results in a decrease in the PKA-dependent phosphorylation, causing a reduction of the channel responses (Bavencoffe et al. 2010). However, the effects of G protein-PKA activation on TRPM8 function are diverse. Other authors have reported the opposite effect: activators of the AC/cAMP/PKA pathway, such as forskolin and 8-Br-cAMP, reduced the responses to agonists of the channel (De Petrocellis et al. 2007; Linte et al. 2007). Moreover, PKA is involved in TRPM8 inhibition by prostaglandin E2 (Linte et al. 2007).

Thus, although several reports suggest a relevant modulation of TRPM8 by phosphorylation, the precise mechanism behind this regulation is still poorly understood.

3.3.4 Phosphorylation in TRPV4

The osmo- and thermo-sensitive channel TRPV4 is also modulated by phosphorylation. PKC and PKA direct phosphorylation of TRPV4 causes a potentiation of the responses to hypotonic stimuli (Fan et al. 2009). The residues involved in the enhanced responses of the channel induced by PKC activation are Ser162, Thr175 and Ser189 in the N-terminal domain, and Ser824 in the C-terminal domain. The phosphorylation of the latter position seems to be involved not only in PKC but also in PKA-dependent potentiation (Fan et al. 2009; Peng et al. 2010). Substitution of Ser824 by an aspartic acid, mimicking a phosphorylation in this site, increases TRPV4-mediated calcium influx in basal conditions and under hyposmotic stimulation, stressing the relevance of this residue in TRPV4 regulation (Peng et al. 2010). Like in TRPV1, the scaffolding protein AKAP79 recruits PKC, PKA and TRPV4, facilitating the phosphorylation of the channel required to channel sensitization (Fan et al. 2009). In addition, TRPV4 is also modulated by SFKs (Xu et al. 2003; Wegierski et al. 2009). An early study showed that hypotonic stress results in a rapid tyrosine phosphorylation of TRPV4 channel in both native and heterologous systems (Xu et al. 2003). In this work, it was suggested that this phosphorylation is carried out by Lyn, a kinase activated by hypotonic stress (Xu et al. 2003). The site of tonicity-dependent tyrosine phosphorylation was identified by mutagenesis as Tyr253 (Xu et al. 2003). The Y253F mutant lost the hypotonicity-dependent phosphorylation and the TRPV4 response to hypotonic stimuli (Xu et al. 2003). However, other evidence has shown that this mutant displays similar responses to hypotonic stress and to other TRPV4-activating stimuli than in the wild type, questioning the role of Tyr253 phosphorylation in TRPV4 activation by hypotonic stress (Vriens et al. 2004).

Other studies support a relevant role of SFKs in the regulation of TRPV4. Two phosphorylation sites in the cytosolic N- and C-terminal domains of this channel were identified by mass spectrometry, using TRPV4 purified from HEK293 and MCDK at normal cell culture conditions. The analysis showed that in unstimulated cells, tyrosines Tyr110 and Tyr805 are phosphorylated by endogenous SFKs (Wegierski et al. 2009). However, calcium imaging experiments using Y110F and Y805F mutants, revealed that only phosphorylation of Tyr110 would be involved in the modulation of the responses of TRPV4 to heat, mechanical stress, and hypotonic cell swelling (Wegierski et al. 2009). As in TRPV1, direct phosphorylation of TRPV4 by PKC, PKA and SFKs produces sensitization of the channel, that could be relevant in inflammatory hyperalgesia (Fan et al. 2009; Wegierski et al. 2009). Thus, understanding how the phosphorylation pathways affect TRPV4 channel function is critical to clarify the role of this polymodal channel in pathological pain conditions.

3.3.5 Phosphorylation in Drosophila TRP

The founding member of the superfamily of TRP channels, the *Drosophila* TRP channel (see Chap. 5, by Bacigalupo et al. in this book), is expressed in the photosensitive rhabdomeral membrane of photoreceptor cells. This channel is the target of the phospholipase C-mediated visual transduction cascade, together with a second channel, termed TRPL (TRP-like) (reviewed by Minke and Parnas 2006). Light-activated rhodopsin couples to an heterotrimeric G protein, activating phospholipase Cβ (PLC), which hydrolyses PIP_2 (phosphatidylinositol 4,5-bisphophate) generating inositol 1,3,5-triphosphate (IP_3), 1,2-diacylglycerol (DAG) and a proton. The production of DAG also activates the eye-specific protein kinase C (eye-PKC), essential for deactivating the light response (reviewed by Minke and Parnas 2006). *Drosophila* visual signalling is one of the fastest transduction cascades (Zuker 1996), partly due to the formation of a macromolecular complex that increases the efficiency of the signal transduction. This complex is organized by the scaffolding protein INAD that holds together the TRP channel, PLC and an eye-specific PKC (Huber et al. 1996). This protein complex is also required for the rapid termination of the light response (Popescu et al. 2006). In fact, the fast component of the deactivation process depends on the direct phosphorylation of TRP channel at Ser982 by eye-PKC, facilitated by the interaction of both proteins with INAD (Popescu et al. 2006). However comparison of transgenic flies expressing a modified *trp*, trp^{S982A} or a mutant lacking eye-PKC, revealed that the deactivation defect in the latter is more complex than displayed by trp^{S982A}, which only became evident upon application of very bright light stimulus. This suggests that phosphorylation of additional PKC sites in TRP or other targets may also contribute to the fast deactivation of the visual response (Popescu et al. 2006).

To identify other TRP phosphorylation sites that can also modulate the light response, a recent study used a quantitative mass spectrometry approach where samples from light- and dark- adapted flies were analyzed (Voolstra et al. 2010,

2013). Twenty-eight phosphorylation sites were identified, one serine (S15) in the N-terminus and 27 other sites within the C-terminal domain (Voolstra et al. 2010, 2013). Among the C-terminal phosphorylation sites, 15 displayed enhanced phosphorylation light conditions, whereas position Ser936 exhibited an increase in the phosphorylation in the dark (Voolstra et al. 2010). Among the positions presenting an enhanced phosphorylation state with light, Thr849 and Thr864 stand out (Voolstra et al. 2013). Experiments with different mutants of the phototransduction cascade that exhibit impaired vision show that this phosphorylation is triggered by the calcium entry through TRP channels. Experiments using a constitutively active mutant of TRP channel showed an increased phosphorylation even in dark conditions. However, mutants with a completely functional phototransduction cascade but expressing a non-conducting TRP variant show total lack of phosphorylation, regardless of the light conditions (Voolstra et al. 2013). Despite the identification of these phosphorylation sites of the *Drosophila* TRP channel, more studies are needed to fully understand the role of these post-translational modifications in vivo.

3.4 Concluding Remarks

In summary, multiple lines of evidence show that several TRP channels are regulated N-glycosylation and phosphorylation, and that these PTMs are part of the molecular determinants involved in setting their function. N-glycosylation and basal phosphorylation modulate their responses to agonists, tuning their activity or directing these channels to specific membrane compartments in different cells. Inducible phosphorylation, in contrast, works as a dynamic and reversible mechanism responsible for modifications of TRP channel function, a modulation that could be relevant in pathological states such as inflammatory pain. Although our knowledge on the role of these PTMs in TRP channel function has progressed significantly, there are several questions that remain unclear. For example, some residues where phosphorylation actually occurs have been identified, but their contribution to channel function remains elusive (Table 3.1). There is also evidence suggesting that these channels are modulated by a kinase/phosphatase activity, but the position/s of putative phosphorylations have not been identified. More efforts are needed to fully understand the real relevance of these modulations in vivo under physiological and physiopathological conditions.

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Table 3.1 Phosphorylated and N-glycosylated residues identified in depicted TRP channels

Residue	*Species*	*Method*	*Effect*	*Reference*
TRPV1				
Ser6 (P)	r	S	NRE	(Bhave et al. 2002)
Ser116 (P)	r	S	Dephosphorylation of Ser 116 allows desensitization of TRPV1 response	(Bhave et al. 2002; Mohapatra and Nau 2003, 2005)
Thr144 (P)	r	S	Minor phosphorylation site. T144A mutation causes a decreased desensitization of TRPV1	(Bhave et al. 2002; Mohapatra and Nau 2003, 2005; Jeske et al. 2006)
Tyr200 (P)	h	S	The phosphorylation of Tyr200, induced by NFG, increased the plasma membrane expression of TRPV1	(Zhang et al. 2005)
Tyr310 (P)	h	MS	NC	(Rikova et al. 2007)
Thr370 (P)	r	S	Thr370 Minor phosphorylation site. Dephosphorylation of Thr370 is involved in the desensitization of TRPV1 response	(Bhave et al. 2002; Mohapatra and Nau 2003, 2005; Jeske et al. 2006)
Thr407 (P)	m	S	Phosphorylation of Thr407 by Cdk5 in DRG neurons, increases capsaicin responses	(Pareek et al. 2007)
Ser502 (P)	h,r	S	Phosphorylation of Ser502 and interaction with the scaffolding protein AKAP79/150 promotes trafficking to the plasma membrane. Phosphorylation of Ser502 by PKC causes an enhanced response of TRPV1 to agonists	(Zhang et al. 2008) (Numazaki et al. 2002; Bhave et al. 2003; Studer and McNaughton 2010)
Asn604 (N)	r	S	N-glycosylation affects some biophysical properties of the channel	(Wirkner et al. 2005; Veldhuis et al. 2012)
Thr 705 (P)	h	S	PKCβII phosphorylates TRPV1 in Thr7045 causing an increase in its temperature sensitivity	(Li et al. 2014)
Thr704 (P)	r	S	Basal phosphorylation of this residue is required to capsaicin response	(Jung et al. 2004)
Thr773 (P)	h	MS	NC	(Hornbeck et al. 2004; Kettenbach et al. 2011)
Thr 774 (P)	r	S	NRE	(Bhave et al. 2002, 2003)

Table 3.1 (continued)

Residue	*Species*	*Method*	*Effect*	*Reference*
Thr 775 (P)	h	MS	NC	(Kettenbach et al. 2011)
Ser780 (P)	h	MS	NC	(Kettenbach et al. 2011)
Ser800 (P)	r	S	Phosphorylation of Ser800 by PKC causes an enhanced response of TRPV1 to agonists	(Numazaki et al. 2002; Bhave et al. 2003)
Ser801 (P)	h	S	Phosphorylation of Ser801 by PKC causes an enhanced response of TRPV1 to agonists	(Studer and McNaughton 2010)
Ser 820 (P)	r	S	NRE	(Bhave et al. 2002, 2003)
TRPM8				
Ser17 (P)	h	S	Phosphorylation of Ser17 by PKA causes an increase in TRPM8 response	(Bavencoffe et al. 2010)
Asn934 (N)	m,h	S	Mutation of N934 causes a reduction in the responses of TRPM8 to cold and menthol	(Dragoni et al. 2006; Erler et al. 2006; Pertusa et al. 2012)
TRPV4				
Tyr91 (P)	h	MS	NC	(Hornbeck et al. 2004; Rikova et al. 2007)
Tyr110 (P)	m	S	Phosphorylation in Tyr 110 is involved in modulation of TRPV4 responses to heat, mechanical stress and hypotonic swelling	(Wegierski et al. 2009)
Tyr113 (P)	h	MS	NC	(Hornbeck et al. 2004; Rikova et al. 2007)
Ser162 (P)	h	S	Phosphorylation of Ser162 is involved in the enhanced responses of the channel induced by PKC activation	(Fan et al. 2009)
Thr175 (P)	h	S	Phosphorylation of Ser175 is involved in the enhanced responses of the channel induced by PKC activation	(Fan et al. 2009)
Thr181 (P)	m	MS	NC	(Huttlin et al. 2010)
Ser189 (P)	h	S	Phosphorylation of Ser189 is involved in the enhanced responses of the channel induced by PKC activation	(Fan et al. 2009)
Tyr253 (P)	m	S	Phosphorylation of Tyr253 mediates TRPV4 response to hypotonic stress	(Xu et al. 2003)

Table 3.1 (continued)

Residue	*Species*	*Method*	*Effect*	*Reference*
Ser505 (P)	h	MS	NC	(Beausoleil et al. 2006)
Tyr508 (P)	h	MS	NC	(Beausoleil et al. 2006)
Asn651 (N)	m	S	Mutation of N-glycosylation sites produces an increase in the expression of TRPV4 in the plasma membrane	(Xu et al. 2006)
Tyr805 (P)	m	S	NRE	(Wegierski et al. 2009)
Ser823	m	MS	NC	(Zanivan et al. 2008)
Ser824	h,m	S	Phosphorylation of Ser824 is involved in PKA and PKC-dependent potentiation	(Fan et al. 2009; Peng et al. 2010)
Drosophila TRP				
Ser15	d	MS	LIP. NC	(Voolstra et al. 2010, 2013)
Ser717	d	MS	NC	(Voolstra et al. 2010, 2013)
Ser721	d	MS	LDP. NC	(Voolstra et al. 2010, 2013)
Ser726	d	MS	LDP. NC	(Voolstra et al. 2010, 2013)
Ser828	d	MS	NC	(Voolstra et al. 2010, 2013)
Thr849	d	S	LDP. The relevance of this phosphorylation has to be determined	(Voolstra et al. 2013)
Thr864	d	S	LDP. The relevance of this phosphorylation has to be determined	(Voolstra et al. 2013)
Ser867	d	MS	NC	(Voolstra et al. 2013)
Ser872	d	MS	LDP. NC	(Voolstra et al. 2010, 2013)
Ser875	d	MS	NC	(Voolstra et al. 2010, 2013)
Ser876	d	MS	LDP. NC	(Voolstra et al. 2010, 2013)
Ser881	d	MS	LIP. NC	(Voolstra et al. 2010, 2013)
Ser884	d	MS	LIP. NC	(Voolstra et al. 2010, 2013)
Ser936	d	S	Phosphorylation of Ser936 occurs in dark conditions. The relevance of this phosphorylation has to be determined	(Voolstra et al. 2010)
Ser956	d	MS	NC	(Voolstra et al. 2010, 2013)
Ser958	d	MS	LDP. NC	(Voolstra et al. 2013)

Table 3.1 (continued)

Residue	*Species*	*Method*	*Effect*	*Reference*
Ser961	d	MS	LDP. NC	(Voolstra et al. 2010, 2013)
Thr963	d	MS	LIP. NC	(Voolstra et al. 2010, 2013)
Ser964	d	MS	LDP. NC	(Voolstra et al. 2010, 2013)
Ser982	d	S	Phosphorylation of Ser982 is required to the rapid termination of the light response	(Popescu et al. 2006)
Ser990	d	MS	LIP. NC	(Voolstra et al. 2010, 2013)
Thr998	d	MS	LDP. NC	(Voolstra et al. 2013)
Thr1036	d	MS	LDP. NC	(Voolstra et al. 2013)
Thr1049	d	MS	NC	(Voolstra et al. 2013)
Ser1056	d	MS	LDP. NC	(Voolstra et al. 2010, 2013)
Ser1123	d	MS	LDP. NC	(Voolstra et al. 2013)
Ser1253	d	MS	NC	(Voolstra et al. 2013)
Ser1254	d	MS	LDP. NC	(Voolstra et al. 2010, 2013)

MS phosphorylation determined using only a mass spectrometry approach, *Specific* phosphorylation determined by site directed mutagenesis, specific antibody, and amino acid sequencing, (*P*) Phosphorylation, (*N*) N-Glycosylation, *h* human, *r* rat, *m* mouse, *d Drosophila*, *NC* No characterized, *NRE* No reported effect, *LIP* Light independent phosphorylation, *LDP* Light dependent phosphorylation

References

Abe J, Hosokawa H, Sawada Y, Matsumura K, Kobayashi S (2006) Ca2+-dependent PKC activation mediates menthol-induced desensitization of transient receptor potential M8. Neurosci Lett 397:140–144

Almaraz L, Manenschijn JA, de la Peña E, Viana F (2014) Trpm8. Handb Exp Pharmacol 222:547–579

Altier C, Dubel SJ, Barrere C, Jarvis SE, Stotz SC, Spaetgens RL, Scott JD, Cornet V, De Waard M, Zamponi GW, Nargeot J, Bourinet E (2002) Trafficking of L-type calcium channels mediated by the postsynaptic scaffolding protein AKAP79. J Biol Chem 277:33598–33603

Apweiler R, Hermjakob H, Sharon N (1999) On the frequency of protein glycosylation, as deduced from analysis of the SWISS-PROT database. Biochim Biophys Acta 1473:4–8

Arniges M, Fernandez-Fernandez JM, Albrecht N, Schaefer M, Valverde MA (2006) Human TRPV4 channel splice variants revealed a key role of ankyrin domains in multimerization and trafficking. J Biol Chem 281:1580–1586

Bautista D, Julius D (2008) Fire in the hole: pore dilation of the capsaicin receptor TRPV1. Nat Neurosci 11:528–529

Bavencoffe A, Gkika D, Kondratskyi A, Beck B, Borowiec AS, Bidaux G, Busserolles J, Eschalier A, Shuba Y, Skryma R, Prevarskaya N (2010) The transient receptor potential channel TRPM8 is inhibited via the alpha 2A adrenoreceptor signaling pathway. J Biol Chem 285:9410–9419

Beausoleil SA, Villen J, Gerber SA, Rush J, Gygi SP (2006) A probability-based approach for high-throughput protein phosphorylation analysis and site localization. Nat Biotechnol 24:1285–1292

Bhave G, Zhu W, Wang H, Brasier DJ, Oxford GS, Gereau RW (2002) cAMP-dependent protein kinase regulates desensitization of the capsaicin receptor (VR1) by direct phosphorylation. Neuron 35:721–731

Bhave G, Hu HJ, Glauner KS, Zhu W, Wang H, Brasier DJ, Oxford GS, Gereau RW (2003) Protein kinase C phosphorylation sensitizes but does not activate the capsaicin receptor transient receptor potential vanilloid 1 (TRPV1). Proc Natl Acad Sci U S A 100:12480–12485

Bonnington JK, McNaughton PA (2003) Signalling pathways involved in the sensitisation of mouse nociceptive neurones by nerve growth factor. J Physiol 551:433–446

Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389:816–824

Cesare P, Dekker LV, Sardini A, Parker PJ, McNaughton PA (1999) Specific involvement of PKC-epsilon in sensitization of the neuronal response to painful heat. Neuron 23:617–624

Chai S, Li M, Lan J, Xiong ZG, Saugstad JA, Simon RP (2007) A kinase-anchoring protein 150 and calcineurin are involved in regulation of acid-sensing ion channels ASIC1a and ASIC2a. J Biol Chem 282:22668–22677

Chang Q, Hoefs S, van der Kemp AW, Topala CN, Bindels RJ, Hoenderop JG (2005) The beta-glucuronidase klotho hydrolyzes and activates the TRPV5 channel. Science 310:490–493

Chen J, Kim D, Bianchi BR, Cavanaugh EJ, Faltynek CR, Kym PR, Reilly RM (2009) Pore dilation occurs in TRPA1 but not in TRPM8 channels. Mol Pain 5:3

Chuang HH, Prescott ED, Kong H, Shields S, Jordt SE, Basbaum AI, Chao MV, Julius D (2001) Bradykinin and nerve growth factor release the capsaicin receptor from PtdIns(4,5)P2-mediated inhibition 7. Nature 411:957–962

Chung MK, Guler AD, Caterina MJ (2008) TRPV1 shows dynamic ionic selectivity during agonist stimulation. Nat Neurosci 11:555–564

Cohen DM (2006) Regulation of TRP channels by N-linked glycosylation. Semin Cell Dev Biol 17:630–637

Colledge M, Dean RA, Scott GK, Langeberg LK, Huganir RL, Scott JD (2000) Targeting of PKA to glutamate receptors through a MAGUK-AKAP complex. Neuron 27:107–119

Comelli EM, Head SR, Gilmartin T, Whisenant T, Haslam SM, North SJ, Wong NK, Kudo T, Narimatsu H, Esko JD, Drickamer K, Dell A, Paulson JC (2006) A focused microarray approach to functional glycomics: transcriptional regulation of the glycome. Glycobiology 16:117–131

Davis JB, Gray J, Gunthorpe MJ, Hatcher JP, Davey PT, Overend P, Harries MH, Latcham J, Clapham C, Atkinson K, Hughes SA, Rance K, Grau E, Harper AJ, Pugh PL, Rogers DC, Bingham S, Randall A, Sheardown SA (2000) Vanilloid receptor-1 is essential for inflammatory thermal hyperalgesia. Nature 405:183–187

Dietrich A, Schnitzler M, Emmel J, Kalwa H, Hofmann T, Gudermann T (2003) N-linked protein glycosylation is a major determinant for basal TRPC3 and TRPC6 channel activity. J Biol Chem 278:47842–47852

Distler C, Rathee PK, Lips KS, Obreja O, Neuhuber W, Kress M (2003) Fast Ca2+-induced potentiation of heat-activated ionic currents requires cAMP/PKA signaling and functional AKAP anchoring. J Neurophysiol 89:2499–2505

Docherty RJ, Yeats JC, Bevan S, Boddeke HW (1996) Inhibition of calcineurin inhibits the desensitization of capsaicin-evoked currents in cultured dorsal root ganglion neurones from adult rats. Pflugers Arch 431:828–837

Dragoni I, Guida E, McIntyre P (2006) The cold and menthol receptor TRPM8 contains a functionally important double cysteine motif. J Biol Chem 281:37353–37360

Erler I, Al-Ansary DM, Wissenbach U, Wagner TF, Flockerzi V, Niemeyer BA (2006) Trafficking and assembly of the cold-sensitive TRPM8 channel. J Biol Chem 281:38396–38404

Fan HC, Zhang X, McNaughton PA (2009) Activation of the TRPV4 ion channel is enhanced by phosphorylation. J Biol Chem 284:27884–27891

Gao T, Yatani A, Dell'Acqua ML, Sako H, Green SA, Dascal N, Scott JD, Hosey MM (1997) cAMP-dependent regulation of cardiac L-type Ca2+ channels requires membrane targeting of PKA and phosphorylation of channel subunits. Neuron 19:185–196

Helenius A, Aebi M (2001) Intracellular functions of N-linked glycans. Science 291:2364–2369

Hornbeck PV, Chabra I, Kornhauser JM, Skrzypek E, Zhang B (2004) PhosphoSite: a bioinformatics resource dedicated to physiological protein phosphorylation. Proteomics 4:1551–1561

Hoshi N, Zhang JS, Omaki M, Takeuchi T, Yokoyama S, Wanaverbecq N, Langeberg LK, Yoneda Y, Scott JD, Brown DA, Higashida H (2003) AKAP150 signaling complex promotes suppression of the M-current by muscarinic agonists. Nat Neurosci 6:564–571

Huber A, Sander P, Gobert A, Bahner M, Hermann R, Paulsen R (1996) The transient receptor potential protein (Trp), a putative store-operated Ca2+ channel essential for phosphoinositide-mediated photoreception, forms a signaling complex with NorpA, InaC and InaD. EMBO J 15:7036–7045

Huttlin EL, Jedrychowski MP, Elias JE, Goswami T, Rad R, Beausoleil SA, Villen J, Haas W, Sowa ME, Gygi SP (2010) A tissue-specific atlas of mouse protein phosphorylation and expression. Cell 143:1174–1189

Jahnel R, Dreger M, Gillen C, Bender O, Kurreck J, Hucho F (2001) Biochemical characterization of the vanilloid receptor 1 expressed in a dorsal root ganglia derived cell line. Eur J Biochem 268:5489–5496

Jeske NA, Patwardhan AM, Gamper N, Price TJ, Akopian AN, Hargreaves KM (2006) Cannabinoid WIN 55,212-2 regulates TRPV1 phosphorylation in sensory neurons. J Biol Chem 281:32879–32890

Jeske NA, Diogenes A, Ruparel NB, Fehrenbacher JC, Henry M, Akopian AN, Hargreaves KM (2008) A-kinase anchoring protein mediates TRPV1 thermal hyperalgesia through PKA phosphorylation of TRPV1. Pain 138:604–616

Jordt SE, Julius D (2002) Molecular basis for species-specific sensitivity to "hot" chili peppers. Cell 108:421–430

Jordt SE, Tominaga M, Julius D (2000) Acid potentiation of the capsaicin receptor determined by a key extracellular site. Proc Natl Acad Sci U S A 97:8134–8139

Jung J, Shin JS, Lee SY, Hwang SW, Koo J, Cho H, Oh U (2004) Phosphorylation of vanilloid receptor 1 by Ca2+/calmodulin-dependent kinase II regulates its vanilloid binding. J Biol Chem 279:7048–7054

Kedei N, Szabo T, Lile JD, Treanor JJ, Olah Z, Iadarola MJ, Blumberg PM (2001) Analysis of the native quaternary structure of vanilloid receptor 1. J Biol Chem 276:28613–28619

Kettenbach AN, Schweppe DK, Faherty BK, Pechenick D, Pletnev AA, Gerber SA (2011) Quantitative phosphoproteomics identifies substrates and functional modules of Aurora and Polo-like kinase activities in mitotic cells. Sci Signal 4:rs5

Koplas PA, Rosenberg RL, Oxford GS (1997) The role of calcium in the desensitization of capsaicin responses in rat dorsal root ganglion neurons. J Neurosci 17:3525–3537

Li L, Hasan R, Zhang X (2014) The basal thermal sensitivity of the TRPV1 ion channel is determined by PKCβII. J Neurosci 34:8246–8258

Linte RM, Ciobanu C, Reid G, Babes A (2007) Desensitization of cold- and menthol-sensitive rat dorsal root ganglion neurones by inflammatory mediators. Exp Brain Res 178:89–98

Madrid R, Pertusa M (2014) Intimacies and physiological role of the polymodal cold-sensitive ion channel TRPM8. Curr Top Membr 74:293-324

McCoy DD, Knowlton WM, McKemy DD (2011) Scraping through the ice: uncovering the role of TRPM8 in cold transduction. Am J Physiol Regul Integr Comp Physiol 300:R1278–R1287

Minke B, Parnas M (2006) Insights on TRP channels from in vivo studies in *Drosophila*. Annu Rev Physiol 68:649–684

Mohapatra DP, Nau C (2003) Desensitization of capsaicin-activated currents in the vanilloid receptor TRPV1 is decreased by the cyclic AMP-dependent protein kinase pathway. J Biol Chem 278:50080–50090

Mohapatra DP, Nau C (2005) Regulation of Ca2+-dependent desensitization in the vanilloid receptor TRPV1 by calcineurin and cAMP-dependent protein kinase. J Biol Chem 280:13424–13432

Morenilla-Palao C, Planells-Cases R, Garcia-Sanz N, Ferrer-Montiel A (2004) Regulated exocytosis contributes to protein kinase C potentiation of vanilloid receptor activity. J Biol Chem 279:25665–25672

Morenilla-Palao C, Pertusa M, Meseguer V, Cabedo H, Viana F (2009) Lipid raft segregation modulates TRPM8 channel activity. J Biol Chem 284:9215–9225

Nilius B, Voets T (2013) The puzzle of TRPV4 channelopathies. EMBO Rep 14:152–163

Numazaki M, Tominaga T, Toyooka H, Tominaga M (2002) Direct phosphorylation of capsaicin receptor VR1 by protein kinase Cepsilon and identification of two target serine residues. J Biol Chem 277:13375–13378

Oliveria SF, Dell'Acqua ML, Sather WA (2007) AKAP79/150 anchoring of calcineurin controls neuronal L-type Ca2+ channel activity and nuclear signaling. Neuron 55:261–275

Pareek TK, Keller J, Kesavapany S, Agarwal N, Kuner R, Pant HC, Iadarola MJ, Brady RO, Kulkarni AB (2007) Cyclin-dependent kinase 5 modulates nociceptive signaling through direct phosphorylation of transient receptor potential vanilloid 1. Proc Natl Acad Sci U S A 104:660–665

Peng H, Lewandrowski U, Muller B, Sickmann A, Walz G, Wegierski T (2010) Identification of a Protein Kinase C-dependent phosphorylation site involved in sensitization of TRPV4 channel. Biochem Biophys Res Commun 391:1721–1725

Pertusa M, Madrid R, Morenilla-Palao C, Belmonte C, Viana F (2012) N-glycosylation of TRPM8 ion channels modulates temperature sensitivity of cold thermoreceptor neurons. J Biol Chem 287:18218–18229

De Petrocellis L, Starowicz K, Moriello AS, Vivese M, Orlando P, Di M V (2007) Regulation of transient receptor potential channels of melastatin type 8 (TRPM8): effect of cAMP, cannabinoid CB(1) receptors and endovanilloids. Exp Cell Res 313:1911–1920

Popescu DC, Ham AJ, Shieh BH (2006) Scaffolding protein INAD regulates deactivation of vision by promoting phosphorylation of transient receptor potential by eye protein kinase C in *Drosophila*. J Neurosci 26:8570–8577

Premkumar LS, Ahern GP (2000) Induction of vanilloid receptor channel activity by protein kinase C. Nature 408:985–990

Premkumar LS, Raisinghani M, Pingle SC, Long C, Pimentel F (2005) Downregulation of transient receptor potential melastatin 8 by protein kinase C-mediated dephosphorylation. J Neurosci 25:11322–11329

Prescott ED, Julius D (2003) A modular PIP2 binding site as a determinant of capsaicin receptor sensitivity. Science 300:1284–1288
Pristera A, Baker MD, Okuse K (2012) Association between tetrodotoxin resistant channels and lipid rafts regulates sensory neuron excitability. PLoS One 7:e40079
Rathee PK, Distler C, Obreja O, Neuhuber W, Wang GK, Wang SY, Nau C, Kress M (2002) PKA/AKAP/VR-1 module: a common link of Gs-mediated signaling to thermal hyperalgesia. J Neurosci 22:4740–4745
Rikova K et al (2007) Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. Cell 131:1190–1203
Sandoz G, Thummler S, Duprat F, Feliciangeli S, Vinh J, Escoubas P, Guy N, Lazdunski M, Lesage F (2006) AKAP150, a switch to convert mechano-, pH- and arachidonic acid-sensitive TREK K(+) channels into open leak channels. EMBO J 25:5864–5872
Schachter H, Narasimhan S, Gleeson P, Vella G (1983) Control of branching during the biosynthesis of asparagine-linked oligosaccharides. Can J Biochem Cell Biol 61:1049–1066
Schnizler K, Shutov LP, Van Kanegan MJ, Merrill MA, Nichols B, McKnight GS, Strack S, Hell JW, Usachev YM (2008) Protein kinase A anchoring via AKAP150 is essential for TRPV1 modulation by forskolin and prostaglandin E2 in mouse sensory neurons. J Neurosci 28:4904–4917
Scott H, Panin VM (2014) The role of protein N-glycosylation in neural transmission. Glycobiology 24:407–417
Shu X, Mendell LM (1999) Nerve growth factor acutely sensitizes the response of adult rat sensory neurons to capsaicin. Neurosci Lett 274:159–162
Simons K, Ikonen E (1997) Functional rafts in cell membranes. Nature 387:569–572
Smith FD, Langeberg LK, Scott JD (2006) The where's and when's of kinase anchoring. Trends Biochem Sci 31:316–323
Studer M, McNaughton PA (2010) Modulation of single-channel properties of TRPV1 by phosphorylation. J Physiol 588:3743–3756
Swope SL, Moss SJ, Blackstone CD, Huganir RL (1992) Phosphorylation of ligand-gated ion channels: a possible mode of synaptic plasticity. FASEB J 6:2514–2523
Tsuruda PR, Julius D, Minor DL Jr (2006) Coiled coils direct assembly of a cold-activated TRP channel. Neuron 51:201–212
Varki A, Cummings R, Esko J, Freeze H, Hart G, Marth J (1999) Essentials of glycobiology. Cold Spring Harbor Laboratory Press, Cold Spring Harbor
Veldhuis NA, Lew MJ, Abogadie FC, Poole DP, Jennings EA, Ivanusic JJ, Eilers H, Bunnett NW, McIntyre P (2012) N-glycosylation determines ionic permeability and desensitization of the TRPV1 capsaicin receptor. J Biol Chem 287:21765–21772
Vellani V, Mapplebeck S, Moriondo A, Davis JB, McNaughton PA (2001) Protein kinase C activation potentiates gating of the vanilloid receptor VR1 by capsaicin, protons, heat and anandamide. J Physiol 534:813–825
Voolstra O, Huber A (2014) Post-translational modifications of TRP Channels. Cells 3:258–287
Voolstra O, Beck K, Oberegelsbacher C, Pfannstiel J, Huber A (2010) Light-dependent phosphorylation of the *Drosophila* transient receptor potential ion channel. J Biol Chem 285:14275–14284
Voolstra O, Bartels JP, Oberegelsbacher C, Pfannstiel J, Huber A (2013) Phosphorylation of the *Drosophila* transient receptor potential ion channel is regulated by the phototransduction cascade and involves several protein kinases and phosphatases. PLoS One 8:e73787
Vriens J, Watanabe H, Janssens A, Droogmans G, Voets T, Nilius B (2004) Cell swelling, heat, and chemical agonists use distinct pathways for the activation of the cation channel TRPV4. Proc Natl Acad Sci U S A 101:396–401
Wang YC, Peterson SE, Loring JF (2014) Protein post-translational modifications and regulation of pluripotency in human stem cells. Cell Res 24:143–160
Wegierski T, Lewandrowski U, Muller B, Sickmann A, Walz G (2009) Tyrosine phosphorylation modulates the activity of TRPV4 in response to defined stimuli. J Biol Chem 284:2923–2933

Wirkner K, Hognestad H, Jahnel R, Hucho F, Illes P (2005) Characterization of rat transient receptor potential vanilloid 1 receptors lacking the N-glycosylation site N604. Neuroreport 16:997–1001

Xu H, Zhao H, Tian W, Yoshida K, Roullet JB, Cohen DM (2003) Regulation of a transient receptor potential (TRP) channel by tyrosine phosphorylation. SRC family kinase-dependent tyrosine phosphorylation of TRPV4 on TYR-253 mediates its response to hypotonic stress. J Biol Chem 278:11520–11527

Xu H, Fu Y, Tian W, Cohen DM (2006) Glycosylation of the osmoresponsive transient receptor potential channel TRPV4 on Asn-651 influences membrane trafficking. Am J Physiol Renal Physiol 290:F1103–F1109

Zanivan S, Gnad F, Wickstrom SA, Geiger T, Macek B, Cox J, Fassler R, Mann M (2008) Solid tumor proteome and phosphoproteome analysis by high resolution mass spectrometry. J Proteome Res 7:5314–5326

Zhang X, Huang J, McNaughton PA (2005) NGF rapidly increases membrane expression of TRPV1 heat-gated ion channels. EMBO J 24:4211–4223

Zhang X, Li L, McNaughton PA (2008) Proinflammatory mediators modulate the heat-activated ion channel TRPV1 via the scaffolding protein AKAP79/150. Neuron 59:450–461

Zuker CS (1996) The biology of vision of *Drosophila*. Proc Natl Acad Sci U S A 93:571–576

Chapter 4
TRP Channels in Visual Transduction

Juan Bacigalupo, Ricardo Delgado, Yorka Muñoz and Peter O'Day

Abstract The original members of the superfamily of transient receptor potential channels are the TRP and TRPL channels underlying *Drosophila* light transduction. Light transduction takes place in the photosensitive microvilli within the photoreceptor cells; it is initiated by photon absorption which leads ultimately to the generation of a depolarizing receptor potential caused by TRP and TRPL channel opening. Channel opening is mediated by a phospholipase C pathway where the membrane second messenger diacylglycerol appears to be the channel activator. TRP is a Ca^{2+} selective channel responsible for nearly 95 % of the net transduction current, whereas TRPL, a poorly-selective Ca^{2+} channel, accounts for the difference. The scaffolding protein INAD forms a complex with TRP and other transduction proteins, offering an extremely fast transduction mechanism. TRP and TRPL are also found in the synaptic terminals of the photoreceptors, where they play a role in presynaptic Ca^{2+} increments during synaptic transmission.

Keywords Light · Transduction · Vision · Retina · Photoreceptor · Lipids · TRP channels

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4.1 Introduction

The ability of animals to see is remarkable. Even the early steps in vision that involve light detection by photoreceptor cells are very complex and not fully understood in invertebrates and vertebrates. Light detection begins with absorption of photons by photopigments embedded in specialized membranes, and this event triggers biochemical signaling cascades. The nature of these signaling cascades varies widely across species. The biochemical cascades generate transmembrane neural electrical signals that travel to the photoreceptor cell synapse, where the neural information is relayed to the next level of signaling in the visual pathways. The process by which light is coded into an electrical neural signal is called phototransduction, because light energy is transduced into electrochemical energy.

Photoreceptor cell organization and complexity also varies widely, with photoreceptors organized as functional units of simple multicellular tissues or as components of complex eyes that focus light directly to the transducing regions. Some photoreceptors exist as isolated individual cells only serving to detect changes in illumination, forming part of simple photosensitive multicellular arrangements with different degrees of complexity; others are integrated into highly sophisticated organs, such as the compound eyes of insects and the camera eyes of cephalopods and vertebrates. Depending on the photosensitive structure, the animal can distinguish shadows, shapes, colors and even images of great complexity. Visible light represents a small fraction, 400–700 nm, of the vast spectrum of electromagnetic waves that reach our planet, 10^{-11}–10^{3} cm!

Photoreceptors emerged late in evolution relative to more primitive sensory receptors, such as chemo and mechanotransducing cells. The fact that the number of species boosted dramatically after phototransduction evolved hundreds of million years ago clearly suggests a considerable adaptive value of vision (Arendt 2008; Arendt and Wittbrodt 2001; Lamb 2011).

Photoreceptors are extremely sensitive and remarkably efficient photon detectors. In fact, they can generate discernable electrical responses to single photons and transmit single photon responses to secondary neurons, eventually reaching the central processing regions of the nervous system.

In this chapter we will briefly discuss vertebrate photoreceptors and then shift the focus to *Drosophila melanogaster* photoreceptors, the best characterized of all invertebrate visual cells, which employ TRP channels in phototransduction. Notably, these are the founding members of the extensive TRP channel superfamily, with key roles in a wide variety of sensory transduction mechanisms in invertebrates and vertebrates, such as mechano, thermo, taste, olfactory, pain and osmotransduction.

4.2 Vertebrate Photoreceptors

Vertebrate photoreceptors, rods and cones, are widely conserved in function, morphology and biochemical signaling components across species. They are exquisitely arranged and aligned in outer layers of the retina, and they transmit information

in the form of neural signals to higher order cells in more proximal layers. Each cell possesses a clearly distinguishable light transducing region, called the outer segment. This region contains about 2000 internal membrane structures (discs) containing the photopigment rhodopsin, a G-protein coupled membrane receptor, and several other transduction proteins that form the metabotropic signaling cascades that generate and modulate the electrical responses to light. These light responses are mediated by the closing of plasma membrane ion channels, often called transduction channels. These cationic non-selective channels reside exclusively in the outer segment plasma membrane, allowing Ca^{2+} and Na^{+} ions to enter the outer segment. These are the only ion channel types present in the outer segment. Because these channels are open in the dark, they sustain an inward current in the outer segment that keeps the membrane depolarized. This current is counterbalanced by an outward current at the inner segment membrane through K^{+} channels responsible of the receptor potential in most cell types and the membrane potential in darkness is set around −35 mV. As a consequence, there is an extracellular current flow from the inner to the outer segment. Light causes the closure of the transduction channels and the hyperpolarization of the cell due to the reduction of the inward current, shifting the membrane potential towards the K^{+} equilibrium potential, reaching nearly −70 mV if challenged with a saturating bright light (Lou et al. 2008).

These transduction channels are gated by cyclic GMP and belong to the small family of the cyclic nucleotide-gated channels, CNGs. cGMP levels are governed by the balance between cGMP production by guanylate cyclase (GC) and cGMP degradation by phosphodiesterase (PDE) that hydrolyzes cGMP to GMP. This balance maintains stable cGMP levels in the dark within the outer segment. Upon absorbing a photon, rhodopsin couples to a GTP-binding protein (G-protein) that activates the PDE; the resulting drop in cGMP levels leads to closure of the transduction channels (Fig. 4.1).

There is another class of vertebrate photoreceptor in the higher order layers of the retina, called the ipRGCs (intrinsically photosensitive retinal ganglion cells). Phototransduction in these cells appears to involve biochemistry similar to that found in invertebrate photoreceptors, as we explain below.ipRGCs contribute to non-image forming vision, including pupillary responses (Xue et al. 2011) and photoperiodic processes (Provencio et al. 2002). Although the underlying mechanisms of phototransduction in ipRGCs have not been elucidated, there is a growing body of evidence so far consistent with the idea that light responses in ipRGCs are mediated by pathways including melanopsin as photopigment activating a G_q type G-protein, leading ultimately to the opening of TRP channels.

Remarkably, melanopsin initiated phototransduction is found in photoreceptors from the earliest chordate, amphioxus, and evidence suggests the involvement of a PLC-dependent signaling cascade, as in *Drosophila* (see below) (Ferrer et al. 2012; Pulido et al. 2012).

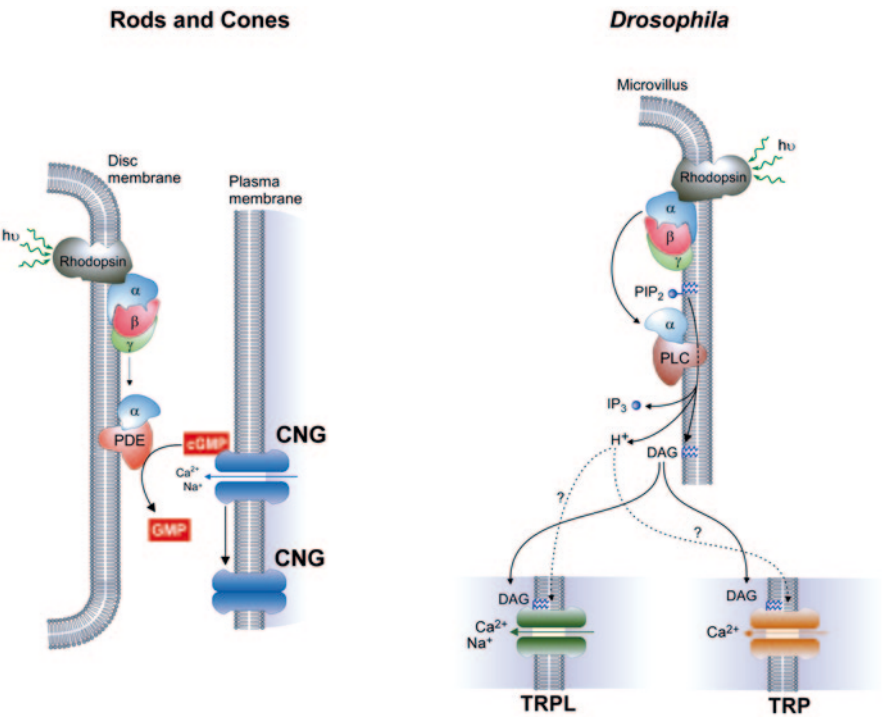

Fig. 4.1 Light transduction in vertebrate and *Drosophila* photoreceptors. *Left*. In vertebrates (*rods and cones*), when a photon hits rhodopsin in the disc membrane it undergoes a photoisomerization and couples to a heterotrimeric G-protein ("transducin"), whose Gα subunit is released and binds to phosphodiesterase (PDE), activating this enzyme. PDE hydrolyses cGMP, the ligand that opens a cationic non-selective cyclic-nucleotide-gated channel (CNG) in the plasma membrane, as a consequence of which the channel closes, generating a hyperpolarizing receptor potential. *Right*. In the microvilli of *Drosophila* photoreceptors, photoactivated rhodopsin couples to a G-protein which activates the enzyme phospholipase C. This enzyme cleaves the membrane phospholipid phosphatidylinositol bisphosphate (PIP_2), generating inositol trisphosphate IP_3, diacylglycerol (DAG) and a proton. DAG somehow opens the TRP and TRPL channels, giving rise to a depolarizing receptor potential. Protons may also contribute to channel opening

4.3 *Drosophila* Photoreceptors

Invertebrate photoreceptors are very different from rods and cones in many ways, biochemically, physiologically and morphologically. There is also great variation among invertebrate photoreceptors, but their photosensitive organelles generally consist of microvilli instead of discs and, with few exceptions, most invertebrate photoreceptors, such as crustaceans, molluscs, arachnids and insects, respond to light with depolarizing receptor potentials resulting from opening of transduction channels, but involving a diversity of signaling cascades.

Drosophila melanogaster photoreceptors have been the most extensively studied among invertebrates because they are most experimentally tractable for biochemical, genetic, molecular, physiological and morphological approaches. A diversity of genetic tools and mutants defective in phototransduction have been developed providing an incomparable advantage over the use of other animal species. These tools, in combination with the other techniques, have been effectively utilized for unraveling phototransduction to a remarkable degree.

Studies of *Drosophila* vision led to the discovery of the TRP class of channels, (Montell and Rubin 1989; Cosens and Manning 1969; Hardie and Minke 1992) which has proven to be a central and widespread theme in neural signaling in biology, with particular relevance for many modalities of sensory transduction. Here we review the properties of the two *Drosophila* light-dependent channels, the transient receptor potential (TRP) and the transient receptor potential-like (TRPL), in the context of phototransduction.

The fruit fly compound eye contains some 800 units known as ommatidia, each possessing eight photoreceptors, or retinula cells. Each photoreceptor has a slender cell body (40 μm × 2 μm), with a clearly distinguishable longitudinal narrow ribbon-like organelle called the rhabdomere, which consists of roughly 30,000 highly packed photosensitive microvilli. The outer six cells of each ommatidium are positioned circularly beside one another, with two cells positioned centrally; all eight rhabdomeres face each other, oriented towards the inner core matrix of the ommatidium (Fig. 4.2). The axons of the outer six photoreceptors project to the lamina while the axons of the other two central photoreceptors project to the medulla, where they synapse.

Within the microvilli, the phototransduction "machinery" is tightly organized in scaffolded macromolecular assemblies, variously called transducisomes or signaplexes (Tsunoda et al. 1997). The scaffolding protein, INAD, encoded by the *inaD* gene, binds key transduction proteins: TRP, PLC, the regulatory enzyme protein kinase C (PKC) and the myosin NINAC, which anchors the transducing complex to the actin cytoskeleton (Hicks et al. 1996; Wes et al. 1999). INAD scaffolding molecules also bind one another, presumably forming a large supercomplex in the microvillus. Normally, one photon induces only one of these events, termed quantum bump and. Under dim light, it is possible to distinguish the individual quantum bumps, which present obvious differences in size and shape. Using a fly deficient in the regulatory Ca^{2+}-binding protein calmodulin, which is necessary for the deactivation of photoactivated rhodopsin, it was observed that a single photon hit triggered multiple repetitive unitary responses (Scott and Zucker 1998). Remarkably, the size and shape of the repetitive events were almost identical, in contrast to those observed in wild type photoreceptors. This result suggests that in the wild type cell every quantum bump originated in a different microvillus, whereas in the mutant the bumps were generated in the only photoactivated organelle where the unitary delivered photon hit. The fact that the single photon response in the mutant consisted of a sequence of individual bumps rather than a continuous depolarization occurred in response to a single photon, is thought to reflect a refractory period following each bump during which the supply of PIP_2, exhausted during the response,

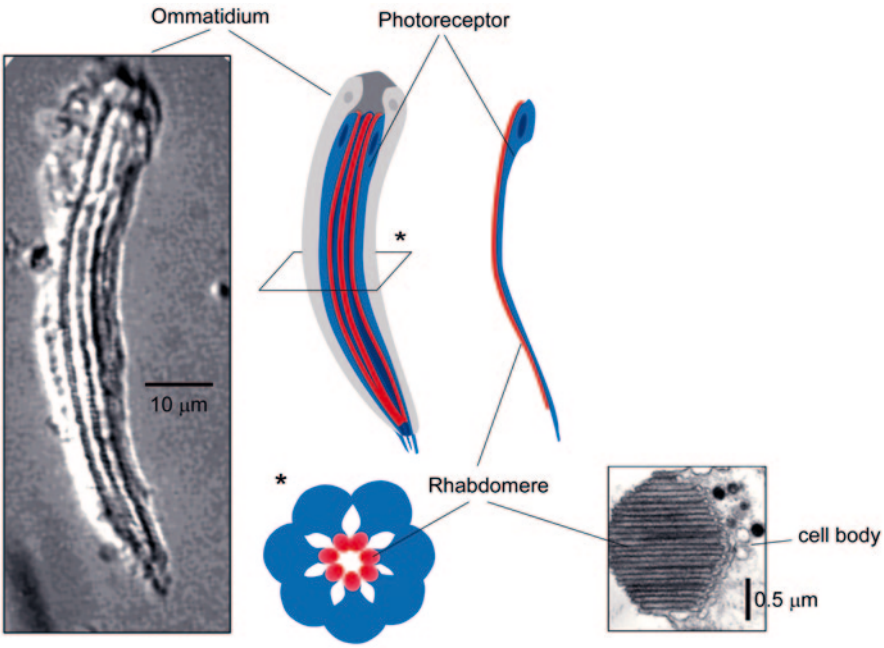

Fig. 4.2 *Drosophila* photoreceptor. Photograph of a dissociated ommatidium (*left*). Schematic representation of an ommatidium depicting the cell bodies of the individual photoreceptors forming part of it and their rhabdomeres (*center*). Individual photoreceptor (*center, right*). Cross section of an ommatidium illustrating the arrangement of the photoreceptors (*center, bottom*); the rhabdomeres of each cell is oriented to the core of the ommatidium. Electron micrograph of the cross section of a rhabdomere, where the highly packed microvilli are appreciated (*right, bottom*) (modified from Hardie 2014)

needed to recover in the microvillus. It is implied that every unitary event would recruit the whole transduction machinery of the respective microvillus, consistent with an all-or-none response. It is concluded that rhabdomeral microvilli are the structural and functional units of phototransduction and contain the proteins responsible for light absorption, signaling cascade, channel opening, and response modulation (Scott and Zuker 1998).

Interestingly, in addition to their rhabdomeral location, TRP and TRPL are also found in photoreceptor axonal terminals in the lamina and the medulla neuropiles of *Drosophila*. Astorga et al. (2012) reported that both channels coexist with the voltage-dependent Ca^{2+} channel, called cacophony (Cac), in those cellular regions and they contribute to the transient increase in Ca^{2+} required for neurotransmitter release, reinforcing the Ca^{2+} influx through Cac. In this synapse, TRP and TRPL work as store-operated channels, but their opening appears to be also mediated by PLC as in the rhabdomere (see below), although opening of these channels is not triggered by rhodopsin as this receptor protein is absent at the synapse. There is additionally IP_3 -dependent release of Ca^{2+} from the endoplasmic reticulum. The contributions from all these Ca^{2+} sources allows a massive Ca^{2+} elevation that is crucial for the

particularly fast synaptic transmission from the photoreceptors that follows the fast kinetics of phototransduction. Indeed, this synapse is of the ribbon type, characteristically associated to rapid transduction events, also occurring in vestibular hair cells (Lenzi et al. 2002).

4.4 Phototransduction

Light-activated rhodopsin couples to a heterotrimeric GTP-binding protein of the G_q class. The $G_{q\alpha}$ catalytic subunit, activated by activated rhodopsin, directly activates inositol-specific phospholipase C (PLC), which is bound in the transducisome to the INAD scaffold. PLC cleaves phosphatidylinositol bisphosphate (PIP_2), a low abundance plasma membrane phospholipid of importance in signaling, giving three products, inositol trisphosphate (IP_3), diacylglycerol (DAG), and a proton (Fig. 4.1). The critical requirement for PLC in phototransduction was revealed by the discovery that a null mutation in the gene coding for this enzyme completely abolished the light response (Bloomquist et al. 1988).

It was initially believed that IP_3 released Ca^{2+} from an intracellular reservoir, possibly the subrhabdomeral cisternae, close to the microvilli somehow caused the opening of the transduction channels in the microvilli. However, two lines of evidence rule out IP_3 as a required messenger in phototransduction. First, elevating IP_3 in the photoreceptors artificially in the dark by photoreleasing caged IP_3 did not affect subsequent responses to light; and second, mutants lacking IP_3 receptors nonetheless respond normally to light. This leaves the substrate PIP_2 of PLC and the two other products, DAG and H^+, as possible activators.

The first demonstration that lipids could activate the light-dependent channels was provided by Hardie and collaborators (Chyb et al. 1999). They showed that polyunsaturated fatty acids (PUFAs) added extracellularly to *Drosophila* photoreceptors in darkness generated an inward current with the same current-voltage relation as that activated by light, suggesting that light and PUFAs activated the TRP/TRPL-dependent current. PUFAs are products of the enzymatic activity of DAG lipase, suggesting this enzyme as a key component of the transduction mechanism and therefore present in the microvilli. Nevertheless, immunohistochemical studies that have detected DAG lipase in photoreceptor cell bodies failed to find it in rhabdomeres (Leung et al. 2008). This result, however, must be taken with caution because of the limitation in the resolution of the technique. Several PUFAs were tested by exogenous application to photoreceptors and found to differ in their potency to activate the channels (Chyb et al. 1999). The profile of lipids present in the rhabdomere was unknown at the time, but the presence of ten DAG species with different fatty acid chains, 11 of which are polyunsaturated, namely FUFAs, was recently reported (Muñoz et al. 2013). It was also found that PUFAs not existing endogenously could open TRP channels. Arachidonic acid, which is absent in *Drosophila* rhabdomeres (Muñoz et al. 2013), was able to open the TRP channels. PUFAs exhibit limited solubility in aqueous solutions and partition into the lipid

bilayer, where presumably they induce channel opening. This action could in principle be exerted by directly interacting with the channel proteins or another protein associated with them, or indirectly through transient modifications the structure of the bilayer; however, the mechanism of TRP channel activation is unknown. Subsequent works addressing the role of PUFA in opening the channels have produced conflicting results (Hardie 2014; Muñoz et al. 2013; Delgado et al. 2014).

The other two lipid products of PLC catalytic activity, PIP_2 and DAG, were extensively investigated by several laboratories. PIP_2 was first considered on the basis of study on TRPL ectopically expressed in Sf9 insect cells (Estacion et al. 2001). Adding PLC to inside-out excised membrane patches stimulated TRPL activity, while applying PIP_2 had the opposite effect. These conditions resembled the light and dark situations in the rhabdomere; so, presumably PIP_2 levels are low and channels are open in light, and PIP_2 levels are high and channels are closed in dark. However, no direct evidence regarding the actual PIP_2 levels in the Sf9 cells or of the presumed PLC enzymatic activity in those experiments was reported. Subsequent work on PIP_2 from different laboratories did not give a consistent picture, as they ranged from inhibitory to excitatory action of the lipid, including absence of an effect on TRPL (Lev et al. 2012). A recent report suggested that PIP_2 might lead to channel opening in a combined action with a drop of pH that accompanies its hydrolysis by PLC, although the observations done in photoreceptors and excised patches from S2 cells expressing TRPL in this paper are contradictory (Huang et al. 2010).

A role for DAG in TRP and TRPL channel opening has also been explored, in cells heterologously expressing TRPL and in inside-out patches from *Drosophila* rhabdomeres and from expression systems. The results, however, varied among the different experimental preparations (Lev et al. 2012). It is noteworthy that the barrier for DAG to flip from the extracellular to the intracellular membrane leaflet, where is normally located, is much larger than for PUFAs, therefore it is not surprising to fail to see a DAG effect on the channels when applied extracellularly, as opposed to PUFAs. Delgado and Bacigalupo (2009) first showed that DAG applied to inside-out excised rhabdomeral membrane patches of the native membrane was a potent activator of TRP and TRPL. PUFAs could activate the channels as well. A later, more detailed investigation also conducted in excised rhabdomeric patches provided strong support for DAG as the physiological activator of the light-dependent channels (Delgado et al. 2014). With this preparation, the biochemical steps in phototransduction could be reconstituted and manipulated. This study focused on TRP, which is responsible for nearly 95% of the transduction current, whereas TRPL only contributes with 5% (Reuss et al. 1997). When rhabdomeral patches were excised in the dark, TRP remained closed. However, when patches were excised under illumination, TRP was found to be constitutively active, behaving as if the transduction biochemistry in the patch was suspended and the molecular constitution of the patch remained locked in the condition existing at excision. Importantly in this preparation, ATP closed the channels and subsequent application of DAG reopened them; subsequent reintroduction of ATP once more closed the channel. This observation suggested that DAG kinase (DGK), an important component

of phototransduction, was retained in the isolated microvilli membranes and that manipulating its enzymatic activity with ATP mimicked the physiological situation, as this enzyme phosphorylates DAG, generating phosphatidic acid. Although DGK was not detected in rhabdomeres by immunostaining (Masai et al. 1997), the physiological evidence suggests that it is present and functions in DAG turnover (Delgado et al. 2014). This ATP effect was suppressed when DGK was inhibited, either pharmacologically or in the mutant deficient in DGK (*rdgA*). These results strongly implicate DAG as endogenous TRP activator. Evidence militated against the notion of PUFAs as channel activators. If PUFAs were to function as channel activators in phototransduction, functional DAG lipase, which generates PUFA from DAG, would be necessary. If this were the case, one would predict that DGL inhibition would affect TRP opening and that PUFA levels would be light dependent. However, pharmacological inhibition of DGL did not affect the opening of TRP by DAG, showing that conversion of DAG into PUFA was not a necessary step (Delgado et al. 2014). Furthermore, ultra high resolution measurements with liquid-chromatography/mass-spectrometry of DAG and PUFAs levels in a membrane fraction enriched in rhabdomere revealed light-dependent increments in DAG, but no change in PUFAs (Delgado et al. 2014). In this work, PIP_2 and protons were also examined as potential channel activators. Application of PIP_2 to constitutively active excised rhabdomeric membranes patches, in which this lipid presumably was at low level as in darkness, did not alter the channel behavior. Insertion of PIP_2 in the membrane was confirmed by a bioassay in patched from rhabdomeres ectopically expressing the PIP_2-sensitive Kir2.1 potassium channel. In contrast, introduction of protons did open the channels from the cytoplasmic side of the excised patches. Nevertheless, the effect of acidification from pH 7.15 to 6.4 proved to be irreversible, suggesting that the effect was non-physiological. A previous study had shown that a similar pH change reversible enhanced S2-expressed TRPL basal activity in excised patches, although this was not consistent with experiments in photoreceptors, where pH decrements with bright light were minimal (Huang et al. 2010).

Aside from *Drosophila*, very few other invertebrate photoreceptors have been studied. Scallops have two separate retinas, one containing microvillar and the other ciliated photoreceptors. Although the molecular nature of their light-dependent channels remains unknown, the transduction cascade in the former seems to involve PLC, as in *Drosophila* and their receptor potential is also depolarizing (Nasi and Gomez 1992), whereas in the latter there is a cGMP cascade that leads to the activation of K^+-selective light-dependent channels (Gomez and Nasi 1994), In the ventral eye of the horshoe crab Limulus, which are also rhabdomeric, the light-dependent channels differ substantially from *Drosophila* and scallop in their relatively large and non-selective cation conductance and ion selectivity; additionally, the underlying signaling mechanism appears to be more complex and is under debate, as there is evidence for the involvement of IP_3-Ca^{2+} and cGMP (Garger et al. 2004; Bacigalupo et al. 1991; Payne and Fein 1986).Summarizing, there is strong evidence indicating that DAG serves as endogenous activator of the light-dependent channels, while PIP_2 and H^+ are not involved directly, and PUFAs do not participate.

Interestingly, mammalian homologs of TRP such as TRPC2, 3, 6 and 7 have been shown to activate by DAG independently of protein kinase C, its most common target (Estacion et al. 2006; Hofmann et al. 1999; Lucas et al. 2003; Okada et al. 1999; Trebak et al. 2003).

4.5 Mechanism of Activation of TRP and TRPL

Although it is well established that lipids related to PLC activity are constituents of the phototransduction cascade and that DAG seems to be the most likely activator of the TRP and TRPL channels, the biophysical basis of the gating of these channels has been scarcely investigated. One obvious possibility is that DAG binds to the channels in one or more specific sites within the membrane. Another alternative is that lipids may lead to channel gating by means of altering the membrane fluidity or curvature, thereby modifying the lipid-channel interaction resulting in a mechanical gating of the channel mechanism, as shown in a mechanotransduction channel (Perozo et al. 2002). A recent report supports this possibility. In a set of elegant experiments Hardie's group (Hardie and Franze 2012) observed a change in photoreceptor length associated with the light response. Notably, the time course of the light-activated current and the photomechanical response measured with atomic force microscopy were similar. The mutant lacking PLC (*norpA*) was unresponsive to light and did not present mechanical responses as well, indicating that the enzymatic activity of this enzyme was crucial. The mechanical response took place even in the mutant devoid of both light-dependent channels (*trp;trpl*), suggesting that it did not involve the channel activity. Furthermore, incorporating the mechanosensitive channel gramicidin in this double mutant restored the ability to generate electrical light responses to these cells, mediated by the truncated phototransduction cascade.

The light response involves a rapid and massive inward current mainly relying on the TRP channel. However, the fact that this channel is normally largely blocked by Ca^{2+} like other Ca^{2+}-selective channels poses a paradox. Remarkably, it was recently shown that this divalent open channel block can be alleviated upon light activating the PLC enzymatic activity (Parnas et al. 2009), suggesting that the light-induced transient changes in the lipid composition of the membrane moiety of the channels would somehow unblock the channels allowing dramatic transient increment in its Ca^{2+} permeability, being rapidly inactivated in a Ca^{2+}-calmodulin dependent fashion.

4.6 Molecular and Biophysical Characteristics of TRP and TRPL

Four protein subunits form the TRP and TRPL light-dependent channels, each possessing six transmembrane domains. In this aspect they resemble K^+ rather than Ca^{2+} channels, which possess a subunit having four identical six transmembrane

domains. The *Drosophila* channels share 40 % homology between one another and also have a significant homology with mammalian TRPC channels, which are members of the same "canonical" TRP subfamily. The subunit composition of the light-dependent channels was recently shown to be strictly homomeric (Katz et al. 2013).

TRP and TRPL differ in their cation-selectivity. TRP is highly Ca^{2+} selective (~ 100 Ca^{2+} : 1 Na^{+}) while TRPL is poorly selective for Ca^{2+} (4 Ca^{2+} : 1 Na^{+}). Around 90 % of the phototransduction current flows through TRP. The relevance of TRPL is still mysterious. Under physiological conditions noise analysis of light-dependent currents recorded from dissociated photoreceptors estimated conductances of 8 for TRP and 35 pS for TRPL (Reuss et al. 1997), whereas they respectively have 58 and 40 pS under low divalent cations conditions.

4.7 Concluding Remarks

TRP channels in visual transduction have been under investigation for quite some time, and many channel properties and roles have been revealed that have been of broad importance. The mechanism underlying TRP gating has been elusive, however. Recent evidence points to DAG as activator of TRP channels in the *Drosophila* retina. This finding will have significance for many signaling systems, beyond phototransduction and it will have even greater significance when the molecular mechanisms by which DAG activates TRP are elucidated.

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References

Arendt D (2008) The evolution of cell types in animals: emerging principles from molecular studies. Nat Rev Genet 9:868–882

Arendt D, Wittbrodt J (2001) Reconstructing the eyes of Urbilateria. Philos Trans R Soc Lond B Biol Sci 356:1545–1563

Astorga G, Härtel S, Sanhueza M, Bacigalupo J (2012) TRP, TRPL and cacophony channels mediate Ca^{2+} influx and exocytosis in photoreceptors axons in *Drosophila*. PLoS One 7:e44182

Bacigalupo J, Johnson EC, Vergara C, Lisman JE (1991) Light-dependent channels from excised patches of Limulus ventral photoreceptors are opened by cGMP. Proc Natl Acad Sci U S A 88:7938–7942

Bloomquist BT, Shortridge RD, Schneuwly S, Perdew M, Montell C, Steller H, Rubin G, Pak WL (1988) Isolation of a putative phospholipase C gene of *Drosophila*, norpA, and its role in phototransduction. Cell 54:723–733

Chyb S, Hevers W, Forte M, Wolfgang WJ, Selinger Z, Hardie RC (1999) Modulation of the light response by cAMP in *Drosophila* photoreceptors. J Neurosci 19:8799–8807

Cosens DJ, Manning A (1969) Abnormal electroretinogram from a *Drosophila* mutant. Nature 224:285–287

Delgado R, Bacigalupo J (2009) Unitary recordings of TRP and TRPL channels from isolated *Drosophila* retinal photoreceptor rhabdomeres: activation by light and lipids. J Neurophysiol 101:2372–2379

Delgado R, Muñoz Y, Pena-Cortes H, Giavalisco P, Bacigalupo J (2014) Diacylglycerol activates the light-dependent channel TRP in the photosensitive microvilli of *Drosophila* melanogaster photoreceptors. J Neurosci 34:6679–6686

Estacion M, Sinkins WG, Schilling WP (2001) Regulation of *Drosophila* transient receptor potential-like (TrpL) channels by phospholipase C-dependent mechanisms. J Physiol 530:1–19

Estacion M, Sinkins WG, Jones SW, Applegate MA, Schilling WP (2006) Human TRPC6 expressed in HEK 293 cells forms non-selective cation channels with limited Ca^{2+} permeability. J Physiol 572:359–377

Ferrer C, Malagón G, Gomez MP, Nasi E (2012) Dissecting the determinants of light sensitivity in amphioxus microvillar photoreceptors: possible evolutionary implications for melanopsin signaling. J Neurosci 32:17977–17987

Garger AV, Richard EA, Lisman JE (2004) The excitation cascade of Limulus ventral photoreceptors: guanylate cyclase as the link between InsP3-mediated Ca^{2+} release and the opening of cGMP-gated channels. BMC Neurosci 5:7

Gomez MP, Nasi E (1994) The light-sensitive conductance of hyperpolarizing invertebrate photoreceptors: a patch-clamp study. J Gen Physiol 103:939–956

Hardie RC (2014) Photosensitive TRPs. Handb Exp Pharmacol 223:795–826

Hardie RC, Franze K (2012) Photomechanical responses in *Drosophila* photoreceptors. Science 338:260–263

Hardie RC, Minke B (1992) The *trp* gene is essential for a light activated Ca^{2+} channel in *Drosophila* photoreceptors. Neuron 8:643–651

Hicks JL, Liu X, Williams DS (1996) Role of the ninaC proteins in photoreceptor cell structure: ultrastructure of ninaC deletion mutants and binding to actin filaments. Cell Motil Cytoskeleton 35:367–379

Hofmann T, Obukhov AG, Schaefer M, Harteneck C, Gudermann T, Schultz G (1999) Direct activation of human TRPC6 and TRPC3 channels by diacylglycerol. Nature 397:259–263

Huang J, Liu CH, Hughes SA, Postma M, Schwiening CJ, Hardie RC (2010) Activation of TRP channels by protons and phosphoinositide depletion in *Drosophila* photoreceptors. Curr Biol 20:189–197

Katz B, Oberacker T, Richter D, Tzadok H, Peters M, Minke B, Huber A (2013) *Drosophila* TRP and TRPL are assembled as homomultimeric channels in vivo. J Cell Sci 126:3121–3133

Lamb TD (2011) Evolution of the eye. Scientists now have a clear vision of how our notoriously complex eye came to be. Sci Am 305:64–69

Lenzi D, Crum J, Ellisman MH, Roberts WM (2002) Depolarization redistributes synaptic membrane and creates a gradient of vesicles on the synaptic body at a ribbon synapse. Neuron 36:649–659

Leung HT, Tseng-Crank J, Kim E, Mahapatra C, Shino S, Zhou Y, An L, Doerge RW, Pak WL (2008) DAG lipase activity is necessary for TRP channel regulation in *Drosophila* photoreceptors. Neuron 58:884–896

Lev S, Katz B, Minke B (2012) The activity of the TRP-like channel depends on its expression system. Channels (Austin) 6:86–93

Lou D-G, Xue T, Yau K-W (2008) How vision begins: an odyssey. Proc Natl Acad Sci U S A 105:9855–9862

Lucas P, Ukhanov K, Leinders-Zufall T, Zufall F (2003) A diacylglycerol-gated cation channel in vomeronasal neuron dendrites is impaired in TRPC2 mutant mice: mechanism of pheromone transduction. Neuron 40:551–561

Masai I, Suzuki E, Yoon CS, Kohyama A, Hotta Y (1997) Immunolocalization of *Drosophila* eye-specific diacylgylcerol kinase, rdgA, which is essential for the maintenance of the photoreceptor. J Neurobiol 32:695–706

Montell C, Rubin GM (1989) Molecular characterization of the *Drosophila* trp locus: a putative integral membrane protein required for phototransduction. Neuron 2:1313–1323

Muñoz Y, Fuenzalida K, Bronfman M, Gatica A, Sepulveda M, Bacigalupo J, Roth AD, Delgado R (2013) Fatty acid composition of *Drosophila* photoreceptor light-sensitive microvilli. Biol Res 46:289–294

Nasi E, Gomez MP (1992) Light-activated ion channels in solitary photoreceptors of the scallop Pecten irradians. J Gen Physiol 99:747–769

Okada T, Inoue R, Yamazaki K, Maeda A, Kurosaki T, Yamakuni T, Tanaka I, Shimizu S, Ikenaka K, Imoto K, Mori Y (1999) Molecular and functional characterization of a novel mouse transient receptor potential protein homologue TRP7. Ca^{2+} -permeable cation channel that is constitutively activated and enhanced by stimulation of G protein-coupled receptor. J Biol Chem 274:27359–27370

Parnas M, Katz B, Lev S, Tzarfaty V, Dadon D, Gordon-Shaag A, Metzner H, Yaka R, Minke B (2009) Membrane lipid modulations remove divalent open channel block from TRP-like and NMDA channels. J Neurosci 29:2371–2383

Payne R, Fein A (1986) The initial response of Limulus ventral photoreceptors to bright flashes. Released calcium as a synergist to excitation. J Gen Physiol 87:243–269

Perozo EI, Kloda A, Cortes DM, Martinac B (2002) Physical principles underlying the transduction of bilayer deformation forces during mechanosensitive channel gating. Nat Struct Biol 9:696–703

Provencio I, Rollag MD, Castrucci AM (2002) Photoreceptive net in the mammalian retina. This mesh of cells may explain how some blind mice can still tell day from night. Nature 415:493

Pulido C, Malagón G, Ferrer C, Chen JK, Angueyra JM, Nasi E, Gomez MP (2012) The light-sensitive conductance of melanopsin-expressing Joseph and Hesse cells in amphioxus. J Gen Physiol 139:19–30

Reuss H, Mojet MH, Chyb S, Hardie RC (1997) In vivo analysis of the *Drosophila* light-sensitive channels, TRP and TRPL. Neuron 19:1249–1259

Scott K, Zuker CS (1998) Assembly of the *Drosophila* phototransduction cascade into a signalling complex shapes elementary responses. Nature 395:805–808

Trebak M, St J Bird G, McKay RR, Birnbaumer L, Putney JW Jr (2003) Signaling mechanism for receptor-activated canonical transient receptor potential 3 (TRPC3) channels. J Biol Chem 278:16244–16252

Tsunoda S, Sierralta J, Sun Y, Bodner R, Suzuki E, Becker A, Socolich M, Zuker CS (1997) A multivalent PDZ-domain protein assembles signalling complexes in a G-protein-coupled cascade. Nature 388:243–249

Wes PD, Xu XZ, Li HS, Chien F, Doberstein SK, Montell C (1999) Termination of phototransduction requires binding of NINAC myosin III and the PDZ protein INAD. Nat Neurosci 2:447–453

Xue T, Do MT, Riccio A, Jiang Z, Hsieh J, Wang HC, Merbs SL, Welsbie DS, Yoshioka T, Weissgerber P, Stolz S, Flockerzi V, Freichel M, Simon MI, Clapham DE, Yau KW (2011) Melanopsin signalling in mammalian iris and retina. Nature 479:67–73

Chapter 5
TRP Channels in Transduction for Responses to Odorants and Pheromones

Diego Restrepo, Rona Delay, Weihong Lin, Fabián López and Juan Bacigalupo

Abstract Transient receptor potential cation channel, subfamily C, member 2 (TRPC2) is a TRP channel expressed by sensory neurons in the vomeronasal organ. Clearly the TRPC2 channel is critically important for transduction in the vomeronasal sensory neurons. However, it appears that TRPC2 is not the only channel that mediates chemical transduction in the VNO. In addition the transient receptor potential channel M5 (TRPM5) is expressed in approximately 5 % of the olfactory sensory neurons in the main olfactory epithelium where it plays a role in responding to intraspecific semiochemicals (chemicals involved in animal communication of the same species). Interestingly, TRPC2 is expressed in rodents but not in humans, whereas TRPM5 is expressed in the human raising the question for future work whether this TRP channel is involved in human semiochemical transduction.

Keywords Pheromones · Transduction · Olfaction · Vomeronasal · Semiochemical · Ordinary odorants

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5.1 Introduction

Significant work has determined that the mammalian olfactory and vomeronasal systems possess two chemosensory systems detecting chemicals in the nose and sending information to the main or accessory olfactory bulbs respectively (Fig. 5.1), that express two different TRP channels: TRPC2 and TRPM5 (Liman et al. 1999; Lin et al. 2007). Interestingly, in both systems the TRP channels participate in sensory signal transduction of pheromones and intraspecific semiochemicals. Pheromones were first defined by Karlson and Luscher as 'substances which are secreted to the outside by an individual and received by a second individual of the same

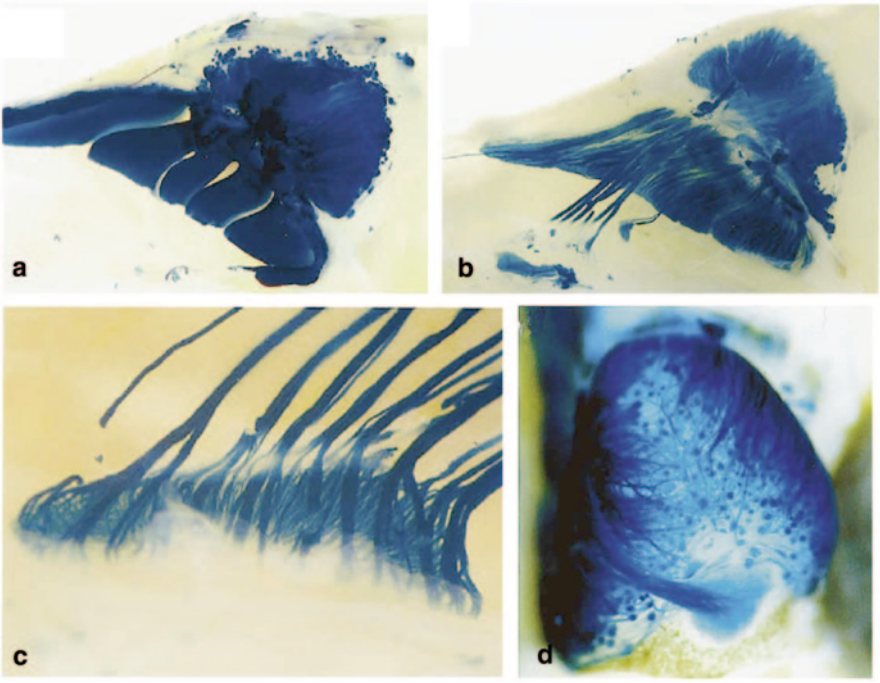

Fig. 5.1 Staining of olfactory and vomeronasal sensory neurons blue in the nose and olfactory bulb with X-Gal in OMP-tau-lacZ mice (OMP is olfactory marker protein, expressed in sensory neurons in both VNO and MOE). **a** Whole mount view of the wall of the nasal cavity and the medial aspect of the olfactory bulb. Cell bodies, dendrites and axons are stained intensely such that the epithelium (*left*) and the bulb (*top right*) appear deeply *blue*. **b** Whole mount view of the nasal septum and the medial aspect of the bulb. The nasal septum, which divides the nasal cavity along the midline, contains three olfactory structures: the main olfactory epithelium (*right*), the vomeronasal organ (*bottom left*), and the septal organ (visible as a small triangle in between the former two structures). The bulb is at the *top right*. Orientation of (**a**) and (**b**): anterior to the *left*, dorsal at *top*; the *right* half of a mouse head is shown. **c** Whole mount higher power view of the vomeronasal organ. **d** Whole mount view of the dorsal-posterior aspect of the olfactory bulb. Orientation of (**d**): medial to the *left*, anterior at *top*. Fascicles of *blue* axons from the main olfactory epithelium terminate in distinct glomeruli in the bulb. Details on the gene-targeted mouse and staining are in Mombaerts et al. (1996)

species, in which they release a specific reaction' (Karlson and Luscher 1959). However, defining mammalian pheromones in this way is problematic, as it is difficult to define what constitutes a 'specific reaction' (Brennan and Keverne 2004). In addition, "semiochemicals" is a broader definition of chemicals involved in animal communication (from the Greek semeion for "sign" that include pheromones) that do involve pheromones. Semiochemicals are different from 'ordinary' odorants.

Importantly, axons from the vomeronasal sensory epithelium whose receptor neurons express TRPC2 target the accessory olfactory bulb that was thought to exclusively send information on pheromones to the vomeronasal medial amygdale (Dulac and Torello 2003; Halpern and Martinez-Marcos 2003; Baum 2012; Baum and Bakker 2013). The medial amygdala then conveys information to certain hypothalamic areas where control of neuroendocrine functions and bisexual social behavior is regulated (Witt and Hummel 2006; Baum and Bakker 2013). However, studies of behavior and glomerular responsiveness indicated that the main olfactory system whose receptor neurons express TRMP5 (Lin et al. 2007) responded not only to ordinary odorants but also to pheromones (Teicher et al. 1980; Dorries et al. 1995; Lin et al. 2008b). Recently it has become clear that a subset of mitral cells in the main olfactory bulb send axons to the vomeronasal amygdala (Pro-Sistiaga et al. 2007; Kang et al. 2009, 2011; Thompson et al. 2012) (see review in (Baum 2012)), implying that a subset of main olfactory system olfactory sensory neurons (OSNs) detect pheromones to convey information to the vomeronasal amygdala.

Here we review recent work on the role of the vomeronasal TRPC2 and main olfactory TRPM5 channels in mediating responses to semiochemicals in the olfactory and vomeronasal systems with a focus on their role on transduction. The reader should refer to other reviews on regular and other aspects of main olfactory transduction (Schild and Restrepo 1998; Munger et al. 2009; Ma 2010) and olfactory and vomeronasal receptors (Axel 2005; Buck 2005; Liberles and Buck 2006; Zhang and Firestein 2009). Finally, this manuscript mentions, but is not focused on other chemosensory cells in the nose and vomeronasal organ that express TRPM5 but are not either OSNs or vomeronasal sensory neurons (VSNs) (Finger et al. 2003; Lin et al. 2008a; Ogura et al. 2011; Tizzano and Finger 2013; Kusumakshi et al. 2015). Importantly there are complementary roles in mediating pheromonal responses for the vomeronasal and main olfactory systems (Brennan and Keverne 2004) and we review below recent work indicating that TRPM5 in the main olfactory neurons responds to semiochemicals.

5.2 TRP Channels and the Vomeronasal Organ

In 1987, Nakamura and Gold showed that $[cAMP]_i$ directly gates a conductance in olfactory cilia (Nakamura and Gold 1987) and it was subsequently demonstrated that regular odors induce increments in cAMP (Breer et al. 1990) and that cAMP mediates the electrical response (Lowe et al. 1989), implicating that $[cAMP]_i$ directly gated a conductance that depolarized the olfactory sensory neuron (Nakamura and Gold 1987). Since then substantial work has shown that the activation

of conductance was mediated by a cyclic nucleotide-gated non-selective (CNG) channel (Schild and Restrepo 1998; Munger et al. 2009; Ma 2010). The vomeronasal organ is part of the accessory olfactory system and the VSNs are similar to olfactory sensory neurons in morphology, with the substantial exception that the knobs of the VSNs are crowned with microvilli rather than cilia (expressed by most of the sensory olfactory neurons) and their axons project to the accessory olfactory bulb rather than the main olfactory bulb. However they differ in their transduction pathways compared to sensory neurons from the main olfactory epithelium. Liman and Corey found no evidence for CNG channels in VSNs (Liman and Corey 1996), motivating further research implicating the TRPC2 channel (see below).

5.2.1 TRPC2

The TRPC2 channel is highly expressed in the vomeronasal organ although it has been found in lower levels in a few other tissues (i.e. sperm (Vannier et al. 1999; Jungnickel et al. 2001), for review see (Yildirim and Birnbaumer 2007)). TRPC2 was found to be a critical component of the odor transduction cascade in vomeronasal sensory neurons (VSNs) (Liman et al. 1999; Liman and Innan 2003; Brennan and Keverne 2004; Kiselyov et al. 2010). Interestingly, the gene for this channel has been lost in old world monkeys and human genomes but it is robust in most other mammals tested (Liman and Innan 2003; Zhang et al. 2003a; Bacigalupo 2014); moreover, in these animals the vomeronasal organ is vestigial.

The mechanism proposed for activation of TRPC2 in VSNs is similar to that involucrated for the signal cascade in photoreceptors of *Drosophila* that involves a cascade of diacylglycerol that leads to aperture of channels TRP y TRPL (Minke and Parnas 2006; Hardie 2007). In VSNs the canonical signaling pathway starts with a chemical cue binding to a specific G-protein coupled receptor on the microvillar membrane. Once bound, the receptor undergoes a conformational change, activating one of two tightly coupled G protein receptors with $G_{\alpha i}$ expressed in the VSNs with soma closer to the apical side of the epithelium (V1R) and $G_{\alpha o}$ expressed in VSNs with cell bodies closer to the basal side of the epithelium (V2R) (Berghard and Buck 1996). The G proteins then activate phospholipase C (PLC) releasing diacylglycerol (DAG) and inositol triphosphate (IP_3) (Cinelli et al. 2002; Runnenburger et al. 2002; Iwata et al. 2013). DAG directly gates the closely associated TRPC2 channel, allowing Na^+ and Ca^{2+} to enter the cell (Lucas et al. 2003; Zufall 2005). Ca^{2+} influx then activates a Ca^{2+}-dependent Cl^- current, leading to further depolarization of the VSN (Yang and Delay 2010; Kim et al. 2011). In a subset of VSNs, DAG is converted to arachidonic acid (AA) by DAG lipase and activates a Ca^{2+} permeable channel different to the CNG, not yet identified (Spehr et al. 2002; Zhang et al. 2010). Depending on the cell, the species and the location of the smooth ER, IP_3 can enhance the semiochemical-induced Ca^{2+} transient through release of Ca^{2+} from intracellular stores (Taniguchi et al. 1995; Inamura et al. 1997; Iwata et al. 2013) (Fig. 5.2).

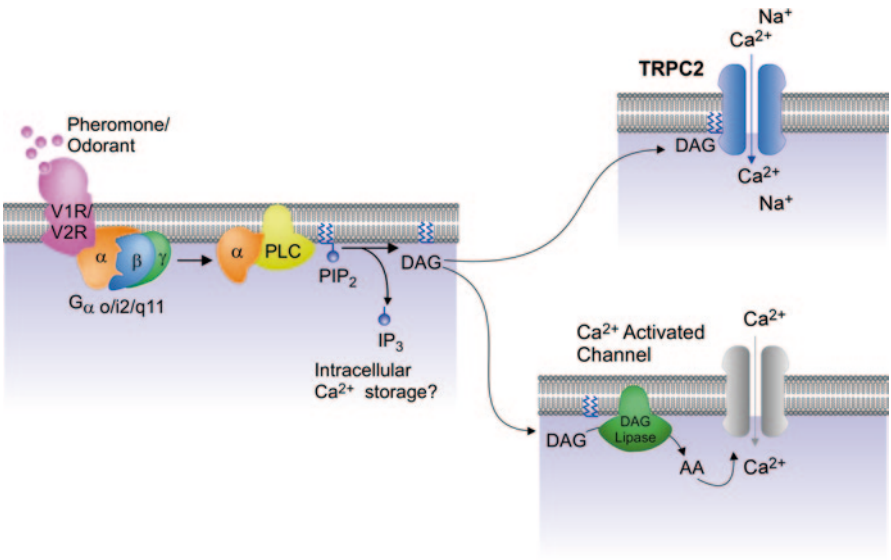

Fig. 5.2 Proposed signal transduction of odor responses in VSNs. Pheromone or Odorant (urine) stimulation activate the PLC pathway and elevates DAG, which is converted to AA by a DAG lipase. DAG activates TRPC2, whereas AA activates a Ca^{2+}-permeable channels. Both pathways play the excitatory role in the odor responses

The critical need for the TRPC2 channel in VSN signal transduction became clear in 2002 when two seminal papers, by Leypold et al. and Stowers et al. were published (Leypold et al. 2002; Stowers et al. 2002). Stowers et al. (2002) used genetic ablation to create a $TRPC2^{-/-}$ mouse (TRPC2 was then named TRP2). Using a sheet of the $TRPC2^{-/-}$VNO neuroepithelium and a multi-electrode array on the microvillar surface, they showed no apparent change in the firing pattern when dilute urine was applied in the knockout compared to baseline. Further, in behavioral studies of male mice, they observed a **decrease** in male/male aggression, using a resident male/intruder male paradigm. Briefly, under this testing procedure, an isolated male mouse established in its home cage has an intruder mouse introduced. Normally, under these conditions, the resident mouse attacks the intruder. When the resident male mouse was a $TRPC2^{-/-}$ such aggression did not occur. Rather the $TRPC2^{-/-}$ male would often mount the intruder instead. Leypold et al. (2002) deleted the fragment of the TRPC2 channel that contained the transmembrane domains 3–6 and replaced that with a *neo* gene. Their $TRPC2^{-/-}$ showed a similar loss on male aggression. In addition, $TRPC2^{-/-}$ lactating females showed a decrease in aggression towards an intruder male. They recorded local field potentials of the VNO sensory epithelium and they found the responses to stimuli such as dilute urine were greatly reduced **but not completely eliminated**. Both groups reported that the $TRPC2^{-/-}$ males still mated with females and their general behavior seemed normal.

At this point it was clear that TRPC2 was required for many of the VSN responses, but the need for DAG to activate the channel wasn't established until Lucas et al.

(2003) did experiments that showed it was DAG and not IP_3 that activated the channel (Lucas et al. 2003; Zufall et al. 2005). This was important since activation of the original TRP channels discovered was also mediated by DAG (Hardie and Postma 2008; Bacigalupo 2014; Delgado 2014). Further the TRPC2 channels are highly expressed in the dendritic knob/microvillar region of the VSNs (Zufall et al. 2005).

Further research with $TRPC2^{-/-}$ female mice has revealed that more than aggressive behavior has been altered. In addition to a reduction in maternal aggression and lactating behavior, these mice show more male type behavior in the presence of a male (Kimchi et al. 2007; Hasen and Gammie 2009). For example, $TRPC2^{-/-}$ females will mount males make ultrasound vocalizations and investigation of the anal region of other mice (Kimchi et al. 2007). Interestingly not all responses mediating through the VNO are abolished in $TRPC2^{-/-}$ mice (Baum 2012). In pregnant females, the introduction of a strange male results in pregnancy block, called the Bruce effect. If the VNO is surgically removed this effect is abolished (Bellringer et al. 1980). However $TRPC2^{-/-}$ females still exhibit the Bruce effect. Research by (Kelliher et al. 2006) suggests that in the basal zone of the VNO VSNs of $TRPC2^{-/-}$ mice are still functional and could account for the retention of the Bruce effect. Since only the TRPC2 channel was eliminated it is still possible for DAG to be broken down by DAG lipase to AA and AA to activate a second Ca^{2+} permeable channel (Spehr et al. 2002; Zhang et al. 2010). Finally, Liman found in VNO neurons a calcium-activated cation channel permeable for Na^{+} and Ca^{2+}, and blocked by ATP and cAMP, that may amplify the primary sensory response(Liman 2003).

Clearly the TPRC2 channel is **critically important** to the functioning of the VSNs, although it appears it is not the only channel that mediates chemical transduction in the VNO (Baum 2012).

5.2.2 TRPM5

The TRPM5 channel is a well-known component of taste transduction for Type 2 taste cells. When a tastant binds to its G-protein coupled receptor on the cell membrane it activates PLC leading to an increase in intracellular Ca^{2+}. Ca^{2+} in turn activates TRMP5 channels depolarizing the cell (Zhang et al. 2003b; Damak et al. 2006; Liman 2007). In the vomeronasal organ Kaske et al. (2007) reported that the microvillar region of the sensory epithelium appeared to bind antibodies directed at TRPM5 (Kaske et al. 2007). While the authors showed their antibodies were selective for TRPM5 in taste tissue, it wasn't clear if the antibody would bind to TRPC2 but they raised the interesting possibility that the TRPM5 might be involved in VSN transduction. Ogura et al. (2010) explored the role TRPM5 might play in the VNO using a TRPM5-GFP mouse model. They found expression of TRPM5 in the duct of the VNO that they showed to be in a distinct cell subpopulation termed solitary chemosensory cells. They further showed that these cells, when activated, restricted the access of chemicals into the lumen of the VNO (Ogura et al. 2010) Finally, Cre drives expression of tauGFP in TRPM5-IRES-Cre mice in a subset of VNO sensory neurons (Kasumakshi et al. 2015).

5.3 TRPM5 in the Main Olfactory Epithelium

5.3.1 Ciliary TRPM5 is Responsible for Olfactory Transduction of Responses to Semiochemicals

Olfactory sensory neurons in the main olfactory epithelium extend cilia (~200 nm wide, tens of μm long) into the mucus layer overlaying the epithelium (Menco 1997). In the cilia odorants bind to seven transmembrane spanning receptors (Zhang and Firestein 2002; Axel 2005; Buck 2005; Liberles and Buck 2006; Zhang and Firestein 2009) that trigger a G-protein (G_{olf}) (Jones and Reed 1989) to activate adenylyl cyclase III (Bakalyar and Reed 1990) (see Fig. 5.2). Rapidly (within tens of milliseconds (Breer et al. 1990)) the cyclase generates cAMP, which directly opens an unselective cationic cyclic nucleotide-gated channel (CNG) mediating a Ca^{2+} influx (Nakamura and Gold 1987; Frings et al. 1995; Baker et al. 1999). Ca^{2+} in turn gates a Ca^{2+}-activated Cl^- channel (Kleene and Gesteland 1991; Lowe and Gold 1993), suggested to be ANO2 (Stephan et al. 2009; Stohr et al. 2009; Rasche et al. 2010) (but see (Gonzalez-Silva et al. 2013)). The electrochemical gradient for Cl^- in the cilia favors an efflux of this anion through the Ca^{2+}-activated Cl^- channel, which significantly potentiates the depolarizing effect of the CNG channel (Kurahashi and Yau 1993; Lowe and Gold 1993) (Fig. 5.3) (but see (Billig et al. 2011)). See reviews in (Schild and Restrepo 1998; Munger et al. 2009; Ma 2012)). The expression of TRPM5 in a subset of OSNs has been suggested by immunohistochemistry, abolished by TRPM5 knockout, and TRPM5-promoter driven expression of GFP (Lin et al. 2007; Oshimoto et al. 2013; Kasumakshi et al. 2015).

Importantly, calcium imaging showed that response of the TRPM5-expressing OSNs to semiochemicals involves discrete increases in Ca^{2+} in microdomains (~0.4 μm) in the cilia, and complementary patch clamp showed that the cilia express Ca^{2+}-activated single channels apparently corresponding to TRPM5, as indicated by their sensitivity to TPPO, a specific inhibitor of TRPM5 (Lopez et al. 2014). Furthermore, loose patch experiments performed in this study indicate that in a small number of OSNs (< 5 %) semiochemicals elicit activation of OSNs mediated by TRPM5, and not by ANO2 (Fig. 5.4). Finally, the loose patch response of this subset of TRPM5-expressing OSNs to semiochemicals is abolished by knockout of TRPM5 and by the TRPM5 inhibitor TPPO (Lopez et al. 2014).

Incidentally, this explains why the TRPM5 knockout does not affect electroolfactogram to semiochemicals (Lin et al. 2007) because at the concentrations used they would stimulate both the OSNs not expressing TRPM5 and the small number of OSNs that do express TRPM5. Finally, it is interesting that the OSNs that do not express TRPM5 do respond to putative pheromones at high concentration. Likely what these sensory neurons do is to detect putative pheromones and semiochemicals at high concentration as regular odorants explaining why "pheromones" such as 2-heptanaone and 2,5-dimethylpyrazine are detected by mice as a regular odor at high concentration. Indeed, humans can smell these 'pheromones'.

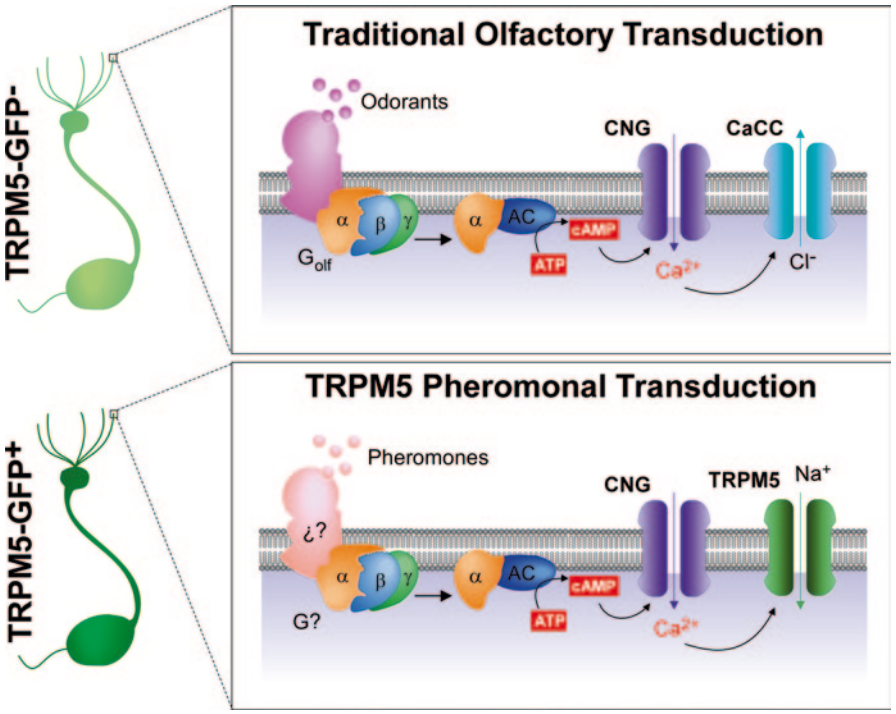

Fig. 5.3 Diagrams showing: **a** The traditional ciliary olfactory pathway found in OSNs devoid of GFP fluorescence in TRPM5-GFP mice (TRPM5-GFP^{-} cells). **b** In this work we show that CNGA2 and TRPM5 are involved in pheromonal transduction in the TRPM5-GFP^{+}OSNs. This diagram shows the pheromonal TRPM5 transduction pathway that we propose based on these experiments and the work of Wang and co-workers implicating the involvement of ACIII in pheromonal responses (Wang and Storm 2011). As in odorant transduction both pathways implicate the CNG and a role of Ca^{2+} entering through this channel activating a Ca^{2+} -activated Cl^{-} channel (principally ANO2) and TRPM5 in odorant and pheromone transduction, respectively. Whether G_{olf} is involved in pheromone transduction in these cells as well, and which proteins are the pheromone receptors remain to be determined. (Reproduced from Lopez et al. 2014)

5.3.2 Effect of Knockout of Both CNGA2 and TRPM5

The recent results by Lopez et al. (2014) demonstrate an important route of activating TRPM5 in the OSNs and its role in pheromone signal transduction, which also provides a logic explanation for the co-expression of TRPM5 and CNGA2 in a set of mature OSNs (Lin et al. 2007). However, in the absence of CNGA2 volatile pheromones 2-hepatanone and 2, 5-dimethylpyrazine can induce small EOG responses in the MOE and activate glomeruli in the olfactory bulb of CNGA2 knockout mice (Lin et al. 2004). This raises the question whether TRPM5 may be activated independently in the absence of CNGA2. TRPM5 knockout mouse lines were cross-bred with the CNGA2 heterozygous lines to generate offspring that are null to both TRPM5 and CNGA2 (Lin 2014). Clearly, as previously reported (Brunet et al.

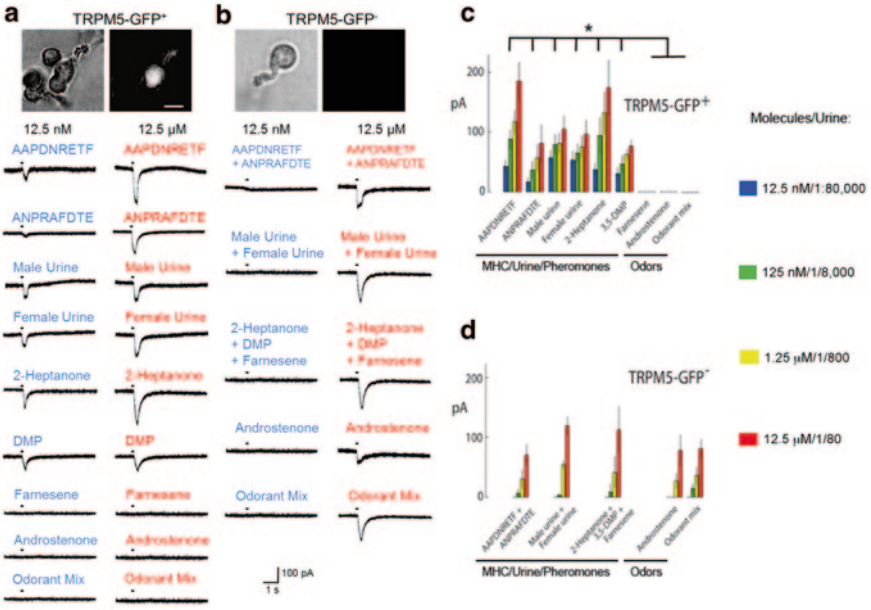

Fig. 5.4 Chemosensory neurons expressing GFP in TRPM5-GFP mice respond to MHC peptides, two pheromones and urine, but not to general odorants and E-β-farnesene. (*A* and *B*) Photographs of the TRPM5-GFP$^+$ (*A*, *top*) or TRPM5-GFP$^-$ (*B*, *top*) OSNs under phase contrast (*left*) and fluorescence optics (*right*) and results of loose-patch current recordings from TRPM5-GFP$^+$ (*A*) or TRPM5-GFP$^-$ (*B*) OSNs stimulated with 200 ms puffs of pheromones, MHC peptides, urine or odorants. (*C* and *D*) Average peak currents (±SEM) of the responses induced by stimuli at different concentrations in TRPM5-GFP$^+$ (*C*) or TRPM5-GFP$^-$ OSNs. (*D*). The number of OSNs responding to each odor were: TRPM5-GFP$^+$: AAPDNRETF (5 of 14), ANPRAFDTE (3 of 30), male C57BL/6 urine (7 of 18), female C57BL/6 urine (7 of 18), 2-heptanone (5 of 18), DMP (5 of 15), E-β-farnesene (0 of 15), androstenone (0 of 15) and odorant mixture (citralva, isoamyl acetate, lilial, cineol, pine, black pepper) (0 of 20). TRPM5-GFP$^-$: mixture of AAPDNRETF and ANPRAFDTE (6 of 17), mixture of male and female C57BL/6 urine (8 of 20), mixture of 2-heptanone, DMP and E-β-farnesene (10 of 26), androstenone (5 of 20) and odorant mixture (7 of 17). The asterisk denotes significant differences in responses to the highest concentration of the stimulus determined using rank sum $p < 0.0025$ with the p-value corrected by false discovery rate ($p < 0.03$). Odor concentration for g and h: 12.5 nM, 125 nM, 1.25 µM and 12.5 µM, except for the urine that was diluted 1/80, 1/800, 1/8000 and 1/80,000. Error bars are SEM . These experiments are from Lopez et al. 2014

1996; Baker et al. 1999; Zhao and Reed 2001) CNGA2 knockout negatively impacts the survival of newborn pups that are null to CNGA2, which results in fewer such males at the weaning age (27% of the total male offspring vs. predicted 50% of the males). The CNGA2 knockout pups are known to have difficulty in locating milk source and die at neonatal stage (Brunet et al. 1996). However, the outcome of TRPM5$^{-/-}$/CNGA2$^{+/-}$ female and TRPM5$^{-/-}$/CNGA2$^{+/y}$ male mating is drastically different (Tables 5.1 and 5.2). This raises the question whether the lack of survival of nulls for both TRPM5 and CNGA2 is due to a role of TRPM5 in olfactory signaling processing in the CNGA2 knockout mouse.

Table 5.1 Offspring (juvenile) genotyping results from CNGA2 mouse line (Mendel prediction in parentheses)

Mating pair genotypes	♂ CNGA2$^{+/y}$	♂ CNGA2$^{-/y}$	♀ CNGA2$^{+/+}$	♀ CNGA2$^{+/-}$
♀ CNGA2$^{+/+}$ X ♂ CNGA2$^{+/y}$	58 (42.75)	*21 (42.75)*	*35 (42.75)*	57 (42.75)

The genotyping results were obtained from juvenile mice after weaning at 3–4 weeks of age. Pups that died neonatally were not counted. The number of postnatal mice with different genotypes are given for mating of ♂ CNGA2$^{+/y}$ with ♀ CNGA2$^{+/-}$. The total number of postnatal mice was 171. Bold are different from ♂ CNGA2$^{+/y}$ or ♀CNGA2$^{+/-}$

Table 5.2 Offspring (juvenile) genotyping results from cross mating of TRPM5 and CNGA2lines (Mendel prediction in parentheses)

Mating pair genotypes	♂ TRPM5$^{-/-}$, CNGA2$^{+/y}$	♂ TRPM5$^{-/-}$, CNGA2$^{-/y}$	♀ TRPM5$^{-/-}$, CNGA2$^{+/+}$	♀ TRPM5$^{-/-}$, CNGA2$^{+/-}$
♀ TRPM5$^{-/-}$, CNGA2$^{+/-}$ X ♂ TRPM5$^{-/-}$, CNGA2$^{+/y}$	24 (16.75)	*0 (16.75)*	25 (16.75)	18 (16.75)

The genotyping results were obtained from juvenile mice after weaning at 3–4 weeks of age. Pups that died neonatally were not counted. The number of postnatal mice with different genotypes are given for mating of ♂ TRPM5$^{-/-}$, CNGA2$^{-/y}$ with ♀ TRPM5$^{-/-}$, CNGA2$^{+/y}$. The total number of postnatal mice was 67. Bold are different from ♂ TRPM5$^{-/-}$, CNGA2$^{-/y}$

5.4 Future Work on TRP Channels Involved in the Vomeronasal and Main Olfactory Epithelium

The findings have clearly shown an involvement for TRP channels in the VNO. However, there are future studies necessary. An important question is whether there are other TRP channels in the VNO (and the MOE). In a subset of VSNs, DAG lipase converts DAG to AA and activates a calcium permeable channel (Zhang et al. 2010). Is this channel another type of TRP channel? If so, which one? In addition as indicated above, there is expression of TRPM5 in the VNO. Is it just in the solitary chemosensory cells or is there a subset of VSNs that activate this channel rather than Ca^{2+}-dependent Cl^- channels? What is the underlying TRPM5 transduction mechanism in the VNO? What happens if TRPM5 is genetically removed from specific cells and in these knockouts which behavioral activities are affected? Are they activities for "vomeronasal" behaviors?

Interestingly TRPM5 activity is well known to be steeply dependent on temperatures between 15 and 35 °C, and heat activation of this channel is thought to underlie thermal sensitivity of sweet taste (Talavera et al. 2005) (See Chap. 6 by *Talavera* in this book). This raises the question whether the temperature of the nose decreases substantially affecting the detection of semiochemicals.

There has been considerable controversy on whether higher primates, including human, communicate using 'pheromones' and related semiochemicals (Wysocki and Preti 2004; Baum 2012; Baum and Bakker 2013). Nevertheless it is suspected that they respond to semiochemicals, and yet the vomeronasal system appears to be non-functional in humans (Witt and Hummel 2006; Trotier 2011). In addition, the transient receptor TRPC2 expressed in the vomeronasal sensory neurons in rodents is not expressed in primates (Liman and Innan 2003). Yet, there are data indicating that it is possible that OSNs respond to semiochemicals in humans. Thus, for example, putative human male axillary pheromone enhances females' rating of male sexual attractiveness as well as their sexual arousal (Wyart et al. 2007; Saxton et al. 2008). In addition, human tears contain a chemosignal suspected to be pheromonal (Gelstein et al. 2011). Finally, it is suspected, but not entirely proven, that androstenedione (AND), a putative pheromone that is detected by a particular chemosensory receptor (Keller et al. 2007), may facilitate the attractiveness of potential male sexual partners to women (reviewed in (Baum 2012; Baum and Bakker 2013)). Importantly, the detection of AND is differential for different sexes at certain but not other concentrations (Burke et al. 2012; Baum and Bakker 2013).

5.5 Concluding Remarks

Future work is necessary to determine whether human OSNs respond to semiochemicals and, if these cells do respond to semiochemicals, whether TRPM5 is involved in transduction. TRPM5 is expressed in solitary chemoreceptor cells in humans (Barham et al. 2013), but it has not been studied whether this channel is expressed in human OSNs. If it is demonstrated that humans are sensitive to semiochemicals, it will become important to determine whether social and sexual behaviors are affected by semiochemicals.

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References

Axel R (2005) Scents and sensibility: a molecular logic of olfactory perception (Nobel lecture). Angew Chem Int Ed Engl 44:6110–6127

Bacigalupo J (2014) Chapter this book

Bakalyar HA, Reed RR (1990) Identification of a specialized adenylyl cyclase that may mediate odorant detection. Science 250:1403–1406

Baker H, Cummings DM, Munger SD, Margolis JW, Franzen L, Reed RR, Margolis FL (1999) Targeted deletion of a cyclic nucleotide-gated channel subunit (OCNC1): biochemical and morphological consequences in adult mice. J Neurosci 19:9313–9321

Barham HP, Cooper SE, Anderson CB, Tizzano M, Kingdom TT, Finger TE, Kinnamon SC, Ramakrishnan VR (2013) Solitary chemosensory cells and bitter taste receptor signaling in human sinonasal mucosa. Int Forum Allergy Rhinol 3:450–457

Baum MJ (2012) Contribution of pheromones processed by the main olfactory system to mate recognition in female mammals. Front Neuroanat 6:20

Baum MJ, Bakker J (2013) Roles of sex and gonadal steroids in mammalian pheromonal communication. Front Neuroendocrinol 34:268–284

Bellringer JF, Pratt HP, Keverne EB (1980) Involvement of the vomeronasal organ and prolactin in pheromonal induction of delayed implantation in mice. J Reprod Fertil 59:223–228

Berghard A, Buck LB (1996) Sensory transduction in vomeronasal neurons: evidence for G alpha o, G alpha i2, and adenylyl cyclase II as major components of a pheromone signaling cascade. J Neurosci 16:909–918

Billig GM, Pal B, Fidzinski P, Jentsch TJ (2011) Ca(2+)-activated Cl(−) currents are dispensable for olfaction. Nat Neurosci 14:763–769

Breer H, Boekhoff I, Tareilus E (1990) Rapid kinetics of second messenger formation in olfactory transduction. Nature 345:65–68

Brennan PA, Keverne EB (2004) Something in the air? New insights into mammalian pheromones. Curr Biol 14:R81–89

Brunet LJ, Gold GH, Ngai J (1996) General anosmia caused by a targeted disruption of the mouse olfactory cyclic nucleotide-gated cation channel. Neuron 17:681–693

Buck LB (2005) Unraveling the sense of smell (Nobel lecture). Angew Chem Int Ed Engl 44:6128–6140

Burke SM, Veltman DJ, Gerber J, Hummel T, Bakker J (2012) Heterosexual men and women both show a hypothalamic response to the chemo-signal androstadienone. PLoS One 7:e40993

Cinelli AR, Wang D, Chen P, Liu W, Halpern M (2002) Calcium transients in the garter snake vomeronasal organ. J Neurophysiol 87:1449–1472

Damak S, Rong M, Yasumatsu K, Kokrashvili Z, Perez CA, Shigemura N, Yoshida R, Mosinger B Jr, Glendinning JI, Ninomiya Y, Margolskee RF (2006) Trpm5 null mice respond to bitter, sweet, and umami compounds. Chem Senses 31:253–264

Delgado R, Munoz Y, Pena-Cortez H, Givalisco P, Bacigalupo J (2014) Diacylglycerol activates the light-dependent channels TRP and TRPL in the photosensitive microvilli of *Drosophila melanogaster* photoreceptors. J Neurosci 34:6679–6686

Dorries KM, Adkins-Regan E, Halpern BP (1995) Olfactory sensitivity to the pheromone, androstenone, is sexually dimorphic in the pig. Physiol Behav 57:255–259

Dulac C, Torello AT (2003) Molecular detection of pheromone signals in mammals: from genes to behaviour. Nat Rev Neurosci 4:551–562

Finger TE, Bottger B, Hansen A, Anderson KT, Alimohammadi H, Silver WL (2003) Solitary chemoreceptor cells in the nasal cavity serve as sentinels of respiration. Proc Natl Acad Sci U S A 100:8981–8986

Frings S, Seifert R, Godde M, Kaupp UB (1995) Profoundly different calcium permeation and blockage determine the specific function of distinct cyclic nucleotide-gated channels. Neuron 15:169–179

Gelstein S, Yeshurun Y, Rozenkrantz L, Shushan S, Frumin I, Roth Y, Sobel N (2011) Human tears contain a chemosignal. Science 331:226–230

Gonzalez-Silva C, Vera J, Bono MR, Gonzalez-Billault C, Baxter B, Hansen A, Lopez R, Gibson EA, Restrepo D, Bacigalupo J (2013) Ca2+ -activated Cl− channels of the ClCa family express in the cilia of a subset of rat olfactory sensory neurons. PLoS One 8:e69295

Halpern M, Martinez-Marcos A (2003) Structure and function of the vomeronasal system: an update. Prog Neurobiol 70:245–318

Hardie RC (2007) Vision: dynamic platforms. Nature 450:37–39

Hardie RC, Postma PM (2008) Phototransduction in microvillar photoreceptors of *Drosophila* and other invertebrates. Elsevier, Cambridge

Hasen NS, Gammie SC (2009) Trpc2 gene impacts on maternal aggression, accessory olfactory bulb anatomy and brain activity. Genes Brain Behav 8:639–649

Inamura K, Kashiwayanagi M, Kurihara K (1997) Blockage of urinary responses by inhibitors for IP3-mediated pathway in rat vomeronasal sensory neurons. Neurosci Lett 233:129–132
Iwata T, Nakada T, Toyoda F, Yada T, Shioda S, Kikuyama S (2013) Responsiveness of vomeronasal cells to a newt peptide pheromone, sodefrin as monitored by changes of intracellular calcium concentrations. Peptides 45:15–21
Jones DT, Reed RR (1989) Golf: an olfactory neuron specific-G protein involved in odorant signal transduction. Science 244:790–795
Jungnickel MK, Marrero H, Birnbaumer L, Lemos JR, Florman HM (2001) Trp2 regulates entry of Ca2+ into mouse sperm triggered by egg ZP3. Nat Cell Biol 3:499–502
Kang N, Baum MJ, Cherry JA (2009) A direct main olfactory bulb projection to the 'vomeronasal' amygdala in female mice selectively responds to volatile pheromones from males. Eur J Neurosci 29:624–634
Kang N, Baum MJ, Cherry JA (2011) Different profiles of main and accessory olfactory bulb mitral/tufted cell projections revealed in mice using an anterograde tracer and a whole-mount, flattened cortex preparation. Chem Senses 36:251–260
Karlson P, Luscher M (1959) Pheromones': a new term for a class of biologically active substances. Nature 183:55–56
Kaske S, Krasteva G, Konig P, Kummer W, Hofmann T, Gudermann T, Chubanov V (2007) TRPM5, a taste-signaling transient receptor potential ion-channel, is a ubiquitous signaling component in chemosensory cells. BMC Neurosci 8:49
Keller A, Zhuang H, Chi Q, Vosshall LB, Matsunami H (2007) Genetic variation in a human odorant receptor alters odour perception. Nature 449:468–472
Kelliher KR, Spehr M, Li XH, Zufall F, Leinders-Zufall T (2006) Pheromonal recognition memory induced by TRPC2-independent vomeronasal sensing. Eur J Neurosci 23:3385–3390
Kim S, Ma L, Yu CR (2011) Requirement of calcium-activated chloride channels in the activation of mouse vomeronasal neurons. Nat Commun 2:365
Kimchi T, Xu J, Dulac C (2007) A functional circuit underlying male sexual behaviour in the female mouse brain. Nature 448:1009–1014
Kiselyov K, van Rossum DB, Patterson RL (2010) TRPC channels in pheromone sensing. Vitam Horm 83:197–213
Kleene SJ, Gesteland RC (1991) Calcium-activated chloride conductance in frog olfactory cilia. J Neurosci 11:3624–3629
Kusumakshi S, Voigt A, Hubner S, Hermans-Borgmeyer I, Ortalli A, Pyrski M et al. (2015) A binary genetic approach to characterize TRPM5 cells in mice. *Chem Senses* e-pub ahead of print.
Kurahashi T, Yau KW (1993) Co-existence of cationic and chloride components in odorant-induced current of vertebrate olfactory receptor cells. Nature 363:71–74
Leypold BG, Yu CR, Leinders-Zufall T, Kim MM, Zufall F, Axel R (2002) Altered sexual and social behaviors in trp2 mutant mice. Proc Natl Acad Sci U S A 99:6376–6381
Liberles SD, Buck LB (2006) A second class of chemosensory receptors in the olfactory epithelium. Nature 442:645–650
Liman ER (2003) Regulation by voltage and adenine nucleotides of a Ca2+ -activated cation channel from hamster vomeronasal sensory neurons. J Physiol 548:777–787
Liman ER (2007) TRPM5 and taste transduction. Handb Exp Pharmacol 179:287–298
Liman ER, Corey DP (1996) Electrophysiological characterization of chemosensory neurons from the mouse vomeronasal organ. J Neurosci 16:4625–4637
Liman ER, Innan H (2003) Relaxed selective pressure on an essential component of pheromone transduction in primate evolution. Proc Natl Acad Sci U S A 100:3328–3332
Liman ER, Corey DP, Dulac C (1999) TRP2: a candidate transduction channel for mammalian pheromone sensory signaling. Proc Natl Acad Sci U S A 96:5791–5796
Lin W, Margolskee R, Donnert G, Hell SW, Restrepo D (2007) Olfactory neurons expressing transient receptor potential channel M5 (TRPM5) are involved in sensing semiochemicals. Proc Natl Acad Sci U S A 104:2471–2476
Lin W, Ezekwe EA Jr, Zhao Z, Liman ER, Restrepo D (2008a) TRPM5-expressing microvillous cells in the main olfactory epithelium. BMC Neurosci 9:114

Lin W, Ogura T, Margolskee RF, Finger TE, Restrepo D (2008b) TRPM5-expressing solitary chemosensory cells respond to odorous irritants. J Neurophysiol 99:1451–1460
Lin W, Luo W, Restrepo D (2014) Lethal phenotype in newborns with knockout of both TRPM5 and CNGA2. Open Biology
Lopez F, Delgado R, Lopez R, Bacigalupo J, Restrepo D (2014) Transduction for pheromones in the main olfactory epithelium is mediated by the Ca2+- activated channel TRPM5. J Neurosci 34(9):3268–3278
Lowe G, Gold GH (1993) Nonlinear amplification by calcium-dependent chloride channels in olfactory receptor cells. Nature 366:283–286
Lowe G, Nakamura T, Gold GH (1989) Adenylate cyclase mediates olfactory transduction for a wide variety of odorants. Proc Natl Acad Sci U S A 86:5641–5645
Lucas P, Ukhanov K, Leinders-Zufall T, Zufall F (2003) A diacylglycerol-gated cation channel in vomeronasal neuron dendrites is impaired in TRPC2 mutant mice: mechanism of pheromone transduction. Neuron 40:551–561
Ma M (2010) Multiple olfactory subsystems convey various sensory signals. In: Menini A (ed) The neurobiology of olfaction. CRC Press, Boca Raton
Ma M (2012) Odor and pheromone sensing via chemoreceptors. Adv Exp Med Biol 739:93–106
Menco BP (1997) Ultrastructural aspects of olfactory signaling. Chem Senses 22:295–311
Minke B, Parnas M (2006) Insights on TRP channels from in vivo studies in *Drosophila*. Annu Rev Physiol 68:649–684
Mombaerts P, Wang F, Dulac C, Chao SK, Nemes A, Mendelsohn M, Edmondson J, Axel R (1996) Visualizing an olfactory sensory map. Cell 87:675–686
Munger SD, Leinders-Zufall T, Zufall F (2009) Subsystem organization of the mammalian sense of smell. Annu Rev Physiol 71:115–140
Nakamura T, Gold GH (1987) A cyclic nucleotide-gated conductance in olfactory receptor cilia. Nature 325:442–444
Ogura T, Krosnowski K, Zhang L, Bekkerman M, Lin W (2010) Chemoreception regulates chemical access to mouse vomeronasal organ: role of solitary chemosensory cells. PLoS One 5:e11924
Ogura T, Szebenyi SA, Krosnowski K, Sathyanesan A, Jackson J, Lin W (2011) Cholinergic microvillous cells in the mouse main olfactory epithelium and effect of acetylcholine on olfactory sensory neurons and supporting cells. J Neurophysiol 106:1274–1287
Oshimoto A, Wakabayashi Y, Garske A, Lopez R, Rolen S, Flowers M et al. (2013) Potential role of transient receptor potential channel M5 in sensing putative pheromones in mouse olfactory sensory neurons. *PLoS One* 8, e61990.
Pro-Sistiaga P, Mohedano-Moriano A, Ubeda-Banon I, Del Mar Arroyo-Jimenez M, Marcos P, Artacho-Perula E, Crespo C, Insausti R, Martinez-Marcos A (2007) Convergence of olfactory and vomeronasal projections in the rat basal telencephalon. J Comp Neurol 504:346–362
Rasche S, Toetter B, Adler J, Tschapek A, Doerner JF, Kurtenbach S, Hatt H, Meyer H, Warscheid B, Neuhaus EM (2010) Tmem16b is specifically expressed in the cilia of olfactory sensory neurons. Chem Senses 35:239–245
Runnenburger K, Breer H, Boekhoff I (2002) Selective G protein beta gamma-subunit compositions mediate phospholipase C activation in the vomeronasal organ. Eur J Cell Biol 81:539–547
Saxton TK, Lyndon A, Little AC, Roberts SC (2008) Evidence that androstadienone, a putative human chemosignal, modulates women's attributions of men's attractiveness. Horm Behav 54:597–601
Schild D, Restrepo D (1998) Transduction mechanisms in vertebrate olfactory receptor cells. Physiol Rev 78:429–466
Spehr M, Hatt H, Wetzel CH (2002) Arachidonic acid plays a role in rat vomeronasal signal transduction. J Neurosci 22:8429–8437
Stephan AB, Shum EY, Hirsh S, Cygnar KD, Reisert J, Zhao H (2009) ANO2 is the cilial calcium-activated chloride channel that may mediate olfactory amplification. Proc Natl Acad Sci U S A 106:11776–11781

Stohr H, Heisig JB, Benz PM, Schoberl S, Milenkovic VM, Strauss O, Aartsen WM, Wijnholds J, Weber BH, Schulz HL (2009) TMEM16B, a novel protein with calcium-dependent chloride channel activity, associates with a presynaptic protein complex in photoreceptor terminals. J Neurosci 29:6809–6818

Stowers L, Holy TE, Meister M, Dulac C, Koentges G (2002) Loss of sex discrimination and male-male aggression in mice deficient for TRP2. Science 295:1493–1500

Talavera K, Yasumatsu K, Voets T, Droogmans G, Shigemura N, Ninomiya Y, Margolskee RF, Nilius B (2005) Heat activation of TRPM5 underlies thermal sensitivity of sweet taste. Nature 438:1022–1025

Taniguchi M, Kashiwayanagi M, Kurihara K (1995) Intracellular injection of inositol 1,4,5-trisphosphate increases a conductance in membranes of turtle vomeronasal receptor neurons in the slice preparation. Neurosci Lett 188:5–8

Teicher MH, Stewart WB, Kauer JS, Shepherd GM (1980) Suckling pheromone stimulation of a modified glomerular region in the developing rat olfactory bulb revealed by the 2-deoxyglucose method. Brain Res 194:530–535

Thompson JA, Salcedo E, Restrepo D, Finger TE (2012) Second-order input to the medial amygdala from olfactory sensory neurons expressing the transduction channel TRPM5. J Comp Neurol 520:1819–1830

Tizzano M, Finger TE (2013) Chemosensors in the nose: guardians of the airways. Physiology (Bethesda) 28:51–60

Trotier D (2011) Vomeronasal organ and human pheromones. Eur Ann Otorhinolaryngol Head Neck Dis 128:184–190

Vannier B, Peyton M, Boulay G, Brown D, Qin N, Jiang M, Zhu X, Birnbaumer L (1999) Mouse trp2, the homologue of the human trpc2 pseudogene, encodes mTrp2, a store depletion-activated capacitative Ca2+ entry channel. Proc Natl Acad Sci U S A 96:2060–2064

Wang Z, Storm DR (2011) Maternal behavior is impaired in female mice lacking type 3 adenylyl cyclase. Neuropsychopharmacology 36:772–781

Witt M, Hummel T (2006) Vomeronasal versus olfactory epithelium: is there a cellular basis for human vomeronasal perception? Int Rev Cytol 248:209–259

Wyart C, Webster WW, Chen JH, Wilson SR, McClary A, Khan RM, Sobel N (2007) Smelling a single component of male sweat alters levels of cortisol in women. J Neurosci 27:1261–1265

Wysocki CJ, Preti G (2004) Facts, fallacies, fears, and frustrations with human pheromones. Anat Rec A Discov Mol Cell Evol Biol 281:1201–1211

Yang C, Delay RJ (2010) Calcium-activated chloride current amplifies the response to urine in mouse vomeronasal sensory neurons. J Gen Physiol 135:3–13

Yildirim E, Birnbaumer L (2007) TRPC2: molecular biology and functional importance. Handb Exp Pharmacol 179:53–57

Zhang X, Firestein S (2002) The olfactory receptor gene superfamily of the mouse. Nat Neurosci 5:124–133

Zhang X, Firestein S (2009) Genomics of olfactory receptors. Results Probl Cell Differ 47:25–36

Zhang JJ, Okutani F, Inoue S, Kaba H (2003a) Activation of the cyclic AMP response element-binding protein signaling pathway in the olfactory bulb is required for the acquisition of olfactory aversive learning in young rats. Neuroscience 117:707–713

Zhang Y, Hoon MA, Chandrashekar J, Mueller KL, Cook B, Wu D, Zuker CS, Ryba NJ (2003b) Coding of sweet, bitter, and umami tastes: different receptor cells sharing similar signaling pathways. Cell 112:293–301

Zhang P, Yang C, Delay RJ (2010) Odors activate dual pathways, a TRPC2 and a AA-dependent pathway, in mouse vomeronasal neurons. Am J Physiol Cell Physiol 298:C1253–C1264

Zhao H, Reed RR (2001) X inactivation of the OCNC1 channel gene reveals a role for activity-dependent competition in the olfactory system. Cell 104:651–660

Zufall F (2005) The TRPC2 ion channel and pheromone sensing in the accessory olfactory system. Naunyn Schmiedebergs Arch Pharmacol 371:245–250

Zufall F, Ukhanov K, Lucas P, Liman ER, Leinders-Zufall T (2005) Neurobiology of TRPC2: from gene to behavior. Pflugers Arch 451:61–71

Chapter 6
TRP Channels as Targets for Modulation of Taste Transduction

Karel Talavera

Abstract The sense of taste endows organisms with the ability to distinguish nutritious from potentially harmful food components and to orchestrate adaptive attractive or aversive behaviors. Several members of the Transient Receptor Potential (TRP) family of cation channels have been implicated in the perception of canonical and non-canonical taste modalities, TRPM5 for sweet, bitter, umami and fat, TRPP3/PKD1L3 for sour and TRPV1 for salt and metallic tastes. However, TRPM5 is the only one for which there is consensus on its contribution to taste transduction. Here I review recent findings on the role of this channel in taste perception at the peripheral level. Emphasis is made on reported mechanisms of TRPM5 channel modulation that may have an impact on gustatory transduction. Understanding these mechanisms allows learning about basic taste signaling processes and their modulation (e.g. by temperature and taste-taste interactions), and is essential for the design of optimal therapeutic strategies targeting the gustatory system.

Keywords Taste transduction · Sweet · Bitter · Umami · Salt · Sour · TRPM5 · TRPV1 · TRPP3/PKD1L3

6.1 Introduction

Animal adaptation requires constant monitoring of the chemical environment. In mammals, chemosensation is based on three main mechanisms, olfaction, taste and chemesthesis. The first two allow the detection and identification of chemical compounds that are airborne or dissolved in the saliva, respectively, whereas the third

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is based on detection at the level of the skin, mucosa and viscera. The initial step in these processes is the interaction of compounds with specialized receptors expressed in sensory cells. This event is then transduced into electric signals, which are carried by nerve fibers to high centers of the nervous system. In the case of taste, transduction takes place in taste buds located in the tongue and palate. These structures contain four types of taste cells (I–IV) and the endings of gustatory nerves (chorda tympani and glossopharyngeal). Type I cells seem to provide mechanical support to the rest of the structure (Pumplin et al. 1997), play buffering functions that maintain low levels of extracellular ATP (Bartel et al. 2006; Vandenbeuch et al. 2013) and K^+ (Dvoryanchikov et al. 2009) and are thought to contribute to salt taste transduction (Vandenbeuch et al. 2008). Type II cells express G protein-coupled receptors (GPCRs) for molecules that we perceive as sweet, bitter or umami (glutamate). Expression of these receptors is more or less exclusive, in the sense that GPCRs for bitter compounds (T2Rs) are not co-expressed with receptors for sweet and umami (heterodimers of T1R1, T1R2 and T1R3) (Chaudhari and Roper 2010). However, it is important to note that the taste quality of a particular taste receptor cell is not determined by the receptors they express, but rather the nerve fiber which they are connected to (Mueller et al. 2005). Type III cells are stimulated by sour and by salts. Type II and type III cells differ in the way they connect to the taste sensory nerves. Upon stimulation, type II cells release ATP and there is evidence indicating that this may occur through the passage of ATP through pannexin 1 (Huang et al. 2007; Romanov et al. 2007; Huang and Roper 2010) and through CALHM1 channels (Taruno et al. 2013a; Taruno et al. 2013b), but it remains unclear whether these mechanisms are mutually exclusive. Extracellular ATP activates P2X receptors in taste nerves and P2Y receptors in Type III cells (Kinnamon and Finger 2013). The latter are endowed with classical synaptic proteins, as well as with voltage-gated Ca^{2+} channels. Type III cells, also known as 'presynaptic', are thought to communicate with the gustatory nerves via vesicular release of GABA and serotonin (Chaudhari and Roper 2010). Finally, type IV cells, also called basal cells, are considered to be precursors of taste receptor cells. These structural and functional aspects of taste were covered by recent reviews (Feng et al. 2012; Liman et al. 2014; Roper 2014).

Several TRP channels have been implicated in distinct aspects of taste transduction. For instance, TRPV1 has been proposed in multiple studies as mediator of the amiloride-insensitive component of salt taste transduction (Lyall et al. 2004, 2005, 2009; Treesukosol et al. 2007; Katsumata et al. 2008). In addition, TRPP3/PKD2L3 were suggested as sour receptors (Ishimaru et al. 2006; LopezJimenez et al. 2006; Ishimaru and Matsunami 2009). However, negative or inconclusive evidence from knockout mice, and lack of definitive proof for the functional expression of these channels in taste receptor cells, have been raised as doubts for their classification as *bona fide* elements of taste transduction pathways (Roper 2014). In contrast, although many issues remain unclear, TRPM5 has drawn consensus about its role in the transduction of several taste qualities. For these reasons, and given the availability of very recent excellent reviews on this subject (Kinnamon 2012; Nilius and Appendino 2013; Liman 2014; Liman et al. 2014; Roper 2014), this chapter focuses on

the most salient basic properties of TRPM5 and how the mechanisms of modulation of this channel influence gustatory transduction at the level of taste receptor cells.

6.2 TRPM5 and its Role in Gustatory Transduction and Modulation

6.2.1 Basic Properties in Heterologous and Native Expression Systems

Of all TRP channels thought to be involved in taste transduction TRPM5 is the best characterized. TRPM5 was first identified in a differential screening of cDNAs from taste receptor cells and was shown to co-express with molecules known to participate in taste signaling, including α-gusducin and phospholipase C-β_2 (Perez et al. 2002, 2003). TRPM5 was originally described as a Ca^{2+}-permeable store-operated channel (Perez et al. 2002, 2003), but soon afterwards it was established that it is in fact a non-selective Ca^{2+}-impermeable cation channel activated by increase in intracellular Ca^{2+} concentration (Hofmann et al. 2003; Liu and Liman 2003; Prawitt et al. 2003).

The effective intracellular Ca^{2+} concentration (EC_{50}) for TRPM5 activation has been reported for recombinant channels between 0.7 and 32 μM (Hofmann et al. 2003; Liu and Liman 2003; Ullrich et al. 2005) for mouse and 0.84 μM for human (Prawitt et al. 2003). The reasons for these divergences seem to be related to the degree of desensitization of the channel in different recording conditions. Indeed, Ullrich et al. showed that in the whole-cell configuration the EC_{50} was 0.7 μM, whereas in inside-out patches it was 32 μM (Ullrich et al. 2005). This desensitization was found to be Ca^{2+}-dependent and partially reverted by phosphatidylinositol-4,5-bisphosphate (PIP_2) (Liu and Liman 2003). The mechanisms underlying the sensitivity of TRPM5 to intracellular Ca^{2+} remain poorly understood, although it has been discarded that it is mediated by calmodulin (Hofmann et al. 2003). Intracellular Ca^{2+} uncaging experiments revealed that TRPM5 is rapidly activated ($\tau < 2$ ms) upon a strong increase of intracellular Ca^{2+} concentration (Ullrich et al. 2005).

The characterization of the biophysical properties of TRPM5 has been limited. The single-channel conductance has been estimated between 16 and 25 pS (Hofmann et al. 2003; Liu and Liman 2003; Prawitt et al. 2003; Zhang et al. 2007), but detailed properties and modulation of single-channel kinetics have not been reported. It is known though that TRPM5 is a voltage-gated channel, activated by membrane depolarization. Like for other voltage-gated TRP channels, the voltage-dependence of TRPM5 activation is quite shallow, with a slope factor between 53 and 58 mV (Talavera et al. 2008c; Gees et al. 2014). This is suggestive of a low apparent gating valence of ~0.6 per channel (Talavera et al. 2005), similar to what has been directly estimated for TRPM8 (Voets et al. 2007). In regard to the kinet-

ics of voltage-dependent gating, it has been shown that current relaxation can be described with a single exponential time course. The resulting time constant has a bell-shaped voltage dependence, which is a classical indicative of faster channel closing and opening at very negative and very positive potentials, respectively (Talavera et al. 2008c; Gees et al. 2014). The relationship between the voltage and Ca^{2+} dependencies of gating is not yet known, and no gating models have been put forward. As detailed below, TRPM5 was shown to be also strongly stimulated by heat (Talavera et al. 2005).

Most of the properties of recombinant TRPM5 channels have been confirmed in the native environment of taste receptor cells. These include lack of selectivity between Na^+ and Cs^+ as charge carriers, impermeability to $NMDG^+$ and Ca^{2+}, stimulation by increase in intracellular Ca^{2+} concentration and membrane depolarization and high sensitivity to heat (Romanov et al. 2007; Zhang et al. 2007).

6.2.2 TRPM5 in the Perception of Sweet, Bitter and Umami: Two Knockout Mice Tell Slightly Different Stories

The role of TRPM5 in taste transduction has been directly tested by evaluating the responses of *Trpm5* knockout mice. The first report, by Zhang et al., utilized mice with a partial deletion of the *Trpm5* gene and retaining the promoter region, which suggest that a large part of the amino terminal of TRPM5 could be still transcribed (Zhang et al. 2003). Such mice were reported not to be different from wild type mice regarding the responses to salt and acid stimuli, but completely insensitive to sweet, bitter and umami stimuli at the level of behavior and gustatory nerve responses (Zhang et al. 2003). Thus, it was concluded that TRPM5 is absolutely required for the transduction of these taste modalities. However, other studies that used knockout mice fully lacking the TRPM5 protein consistently yielded different results. In several separate experimental series it was found that gustatory nerve responses to sweet, bitter and umami stimuli were significantly reduced, but not completely abolished in knockout mice (Talavera et al. 2005, 2008c; Damak et al. 2006). Damak et al. (2006) found that *Trpm5* knockout mice were sensitive to sweet, bitter and umami tastes in a long access two-bottle preference test but not in a brief-access licking test. It was argued that this difference could result from a lower sensitivity of the brief-access test and/or from the influence of post-ingestive taste-independent effects (Damak et al. 2006). Interestingly, it was later found that the sweet-blind *Trpm5* knockout mice from Zhang et al. displayed preference for sucrose in a long-term test due to post-ingestive reward (de Araujo et al. 2008). This preference was associated with calorie intake because the non-energetic sweetener sucralose failed to trigger preference over water in the same test.

The reasons underlying the differences in the results from studies with the two *Trpm5* knockout strains remain unknown. It has been suggested that the lack of response of knockout mice in the study of Zhang et al. could be due to a putative dominant-negative action of the TRPM5 N-terminal protein fragment that is

presumably produced in this mice on alternative transduction pathways (Damak et al. 2006). Additionally, differences in genetic background of these mice were also put forward (Damak et al. 2006). However, none of these hypotheses has been tested. Nevertheless, the observations of Damak et al. (2006) and several other studies (Devantier et al. 2008; Eddy et al. 2012 and see below) tilt the balance in favor of a model of transduction of sweet, bitter and umami featuring TRPM5-dependent as well as TRPM5-independent mechanisms. Not surprisingly, the relative importance of these mechanisms depends on the tastant, experimental paradigm and gustatory field being evaluated (Damak et al. 2006).

These and other studies served to establish a qualitative model for the role of TRPM5 in transduction of sweet, bitter and umami stimuli (Fig. 6.1). Activation of G-protein-coupled taste receptors by these tastants stimulates PLCβ2 with the consequent production of IP_3 and Ca^{2+} release from the endoplasmic reticulum through type 3 IP_3 receptors (IP_3R3) (Hisatsune et al. 2007). The increase in intracellular Ca^{2+} concentration activates TRPM5, leading to cation influx and depolarization of the taste receptor cell (Hofmann et al. 2003; Liu and Liman 2003; Prawitt et al. 2003; Zhang et al. 2003). This depolarization activates voltage-gated Na^+ channels leading to action potential firing (Gao et al. 2009; Vandenbeuch and Kinnamon 2009). It seems that the combination of increase in intracellular Ca^{2+} concentration and depolarization triggers the release of ATP through pannexin 1 (Huang et al. 2007; Huang and Roper 2010) and/or CALHM1 channels (Taruno et al. 2013a, b).

6.2.3 Role of TRPM5 in the Perception of Salts and Fats

Intriguingly, the *Trpm5* knockout mice from Damak et al. (2006) displayed significantly lower responses to NaCl than wild type mice. This again contrasts with the results of Zhang et al. who reported that their *Trpm5* knockout and wild type mice have the same sensitivity to NaCl, but are in agreement with reduced responses to NaCl in *α-gustducin* (Glendinning et al. 2005) and of *Plcβ2* (Dotson et al. 2005) knockout mice. Notably, if we consider TRPM5 to be exclusively expressed in cells transducing sweet, bitter or umami stimuli we should conclude that the TRMP5-dependent responses to NaCl do not elicit salty taste. Instead, they should elicit sweet, bitter and/or umami qualities. Alternatively, TRPM5 may participate in the transduction of saltiness of NaCl in a small proportion of taste receptor cells reported to express this channel but not α-gustducin (Damak et al. 2006) or T1R or T2R (Zhang et al. 2003). This latter possibility is supported by the findings that *Trpm5* knockout mice, but not *Plcβ2* knockout mice, display smaller chorda tympani responses to low concentrations (100 mM) of NaCl in the presence of benzamil, a potent blocker of the epithelial Na^+ channel ENaC (Ren et al. 2013). On the other hand, the responses of the chorda tympani to salts at high concentrations (500 mM KCl or 500 mM NaCl plus amiloride) were shown to be about 50% weaker in *Trpm5* knockout mice and *Plcβ2* knockout mice than in wild type animals (Oka et al. 2013). Interestingly, wild type mice clearly preferred solutions containing

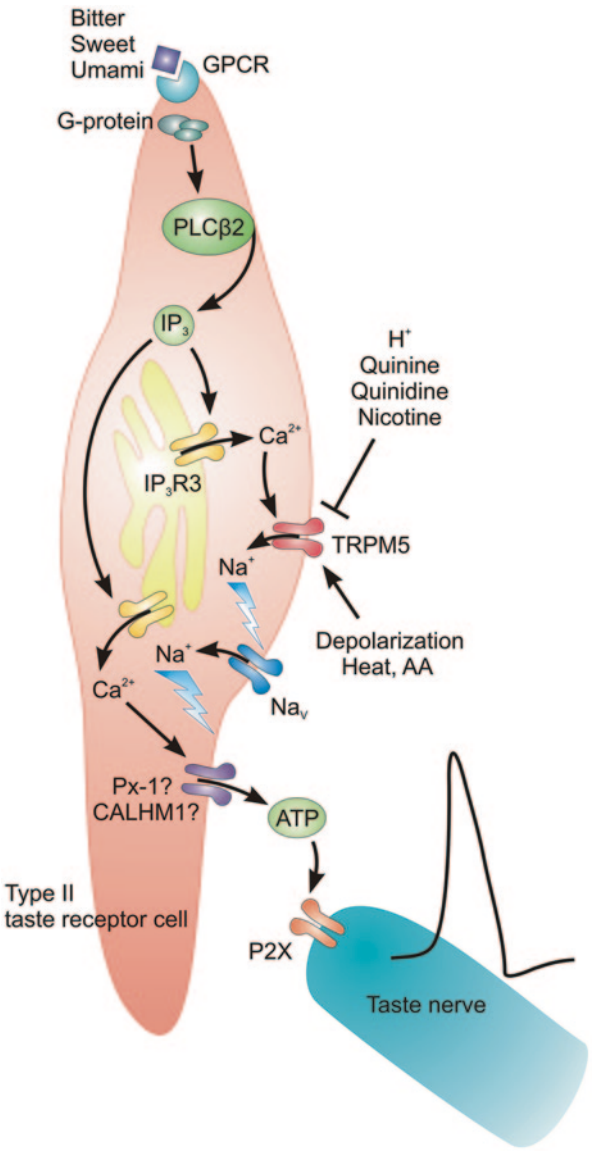

Fig. 6.1 Cartoon of the TRPM5-dependent signaling pathway for the detection of sweet, bitter and umami stimuli. Type II taste receptor cells express TRPM5 in the basolateral membrane. TRPM5 channels are activated by increase in intracellular Ca^{2+} that results from the activation of G-protein-coupled taste receptors sensitive to bitter, sweet and umami compounds. TRPM5 is inhibited by extracellular acidosis, quinine, quinidine and nicotine and stimulated by membrane depolarization, heat and arachidonic acid (*AA*)

low concentrations of divalent salts such as $FeSO_4$ and $ZnSO_4$ over water, but this was not observed in *Trpm5* knockout mice nor in *T1r3* knockout mice (Riera et al. 2009). Furthermore, recent evidence indicates that T1R3 is involved in the detection of calcium and magnesium (Tordoff et al. 2008, 2012).

Finally, there is one study showing that linoleic acid elicits non-selective monovalent cation currents in taste receptor cells that are reduced by application of the TRPM5 inhibitor triphenylphosphine oxide (TPPO) and by genetic ablation of

Trpm5 (Liu et al. 2011). However, it remains unclear why these maneuvers also reduced the increase in intracellular Ca^{2+} concentration triggered by linoleic acid. This is not expected because TRPM5 activation is supposed to be downstream the Ca^{2+} signal. Of note, *Trpm5* knockout mice were found to be deficient in the detection of linoleic acid in tests for long-term preference and brief-access licking recordings after conditioned taste aversion. This body of evidence indicates that TRPM5 is involved in the detection of salts and fats, but the precise underlying pathways remain to be fully characterized.

6.2.4 Modulators of TRPM5 and their Influence on Taste Transduction

The key role of TRPM5 in transduction mechanisms in the taste receptor cells implies that factors that modulate this channel are expected to affect taste. It is notable, however, that the influences of the Ca^{2+}-dependent desensitization and the modulation by PIP_2 and voltage on taste transduction remain completely unknown.

As other voltage-gated TRP channels (Nilius et al. 2005; Talavera et al. 2008a; b), TRPM5 displays a strong thermal sensitivity, being stimulated by heating. The underlying mechanism entails a shift of the voltage-dependence of channel activation to negative potentials (Talavera et al. 2005), very much like that described for activation of TRPV1 (Voets et al. 2004). Of note, channel stimulation by either depolarization or heating can not be observed at low intracellular Ca^{2+} concentration (Talavera et al. 2005). Notably, it was shown that heating strongly enhances chorda tympani responses to sweet compounds in wild type but not in *Trpm5* knockout mice. The strong sensitivity of TRPM5 to heat was therefore proposed to underlie the enhanced sweetness perception at high temperatures. It is also tempting to speculate that heat-induced stimulation of TRPM5 mediates "thermal taste", which consists in the trigger of taste sensations in the absence of tastants by warming the tongue from 20 to 30 °C (Cruz and Green 2000). This effect would require the stimulation of TRPM5 currents by heating at basal (low) intracellular Ca^{2+} concentrations. Alternatively, heating could induce on its own slight increase in the intracellular Ca^{2+} concentration and act as initial trigger for Ca^{2+}-induced activation of TRPM5. However, none of these possibilities have been addressed experimentally. It has been argued that, besides TRPM5, other elements of the taste transduction pathways could be sensitive to heat and contribute to enhanced taste response (Liman 2006, 2014). However, for any element to be significantly important, it would have to feature a thermal sensitivity similar to that of TRPM5 ($Q_{10} \sim 10$ between 25 and 35 °C). For any TRP channel lover who recognizes that the "TRP-centric" view of sensory biology has done more harm than good (Meseguer et al. 2011), it would be extremely interesting to identify such heat-sensitive element outside the TRP protein family. At the moment, TRPM5 remains the most plausible candidate (Talavera et al. 2007).

It has been also pointed out that, considering the role of TRPM5 in the transduction of bitter and umami, heat-induced activation of TRPM5 should result in enhanced responses to these stimuli (Liman et al. 2014; Roper 2014). However, this was not observed experimentally (Talavera et al. 2005). To explain this apparent contradiction, it is important to notice that only the responses that strongly rely on TRPM5 activation are expected to be significantly enhanced by heating. In the original report, which used the *Trpm5* knockout strain generated by Damak et al. (2006), only the chorda tympani responses to sweeteners were strongly reduced in the mice lacking TRPM5 (Talavera et al. 2005). As explained below, a similar discussion rose around the inhibitory effect of quinine on TRPM5.

Another potent modulator of TRPM5 is extracellular acidosis. Liu et al. showed that TRPM5 currents were sharply inhibited by drops in extracellular pH between 7 and 5.5 (Liu et al. 2005). This effect was voltage-independent, which is in accordance with the location of residues important for the inhibitory effect at the extracellular face of the channel. Notably, acidosis enhanced the desensitization of TRPM5 currents. Given the restricted localization of TRPM5 at the basolateral membrane of taste receptor cells, the relevance of its inhibition by extracellular protons has been taken with caution (Roper 2014). Also with reserves one should take the reports on stimulation of TRPM5 by arachidonic acid (Oike et al. 2006) and on the inhibition of this channel by extracellular Zn^{2+} (Uchida and Tominaga 2013). It is currently not clear whether and how these effects are relevant for taste transduction.

The rather extensive literature on interactions between bitter and sweet tastants motivated the study of the possible effects of membrane-permeable bitter compounds on TRPM5 (Talavera et al. 2008c; Gees et al. 2014). It was found that quinine, the bitter additive in tonic waters, and its stereoisomer quinidine inhibit TRPM5 at micromolar concentrations. Furthermore, both compounds inhibited the responses of the chorda tympani to sweet tastants in wild type mice, but did not alter the residual responses of sweet-sensitive gustatory fibers of *Trpm5* knockout mice (Talavera et al. 2008c) (Fig. 6.2). These findings can readily explain the long-known inhibitory effect of quinine on sweet taste perception (Öhrwall 1891) and its partial localization at the gustatory periphery (Formaker and Frank 1996). This phenomenon was considered as a bitter-sweet taste interaction, but it is now clear that the quinine-induced inhibition of the sweet pathway is at least in part dissociated from the detection and processing of the bitter signal because the bitter tastant inhibits directly the sweet transduction pathway.

TRPM5 currents were later found to be inhibited also by nicotine, a compound that has been usually linked to gustatory transduction (Grunberg 1982, 1985; Simons et al. 2006; Lyall et al. 2007; Tomassini et al. 2007). The exact way in which nicotine acts on the perception of these tastes is still not well understood, but very likely multiple different mechanisms are involved. These include nicotine-induced reduction of the size of fungiform papillae and the number of taste cells (Tomassini et al. 2007), inhibition of chorda tympani responses to KCl and NaCl (Lyall et al. 2007), suppression of gustatory responses of neurons in the nucleus of the solitary tract (Simons et al. 2006), inhibition of voltage-dependent sodium channels, sensitization of TRPV1 (Liu et al. 2004) and activation of TRPA1 (Talavera et al. 2009).

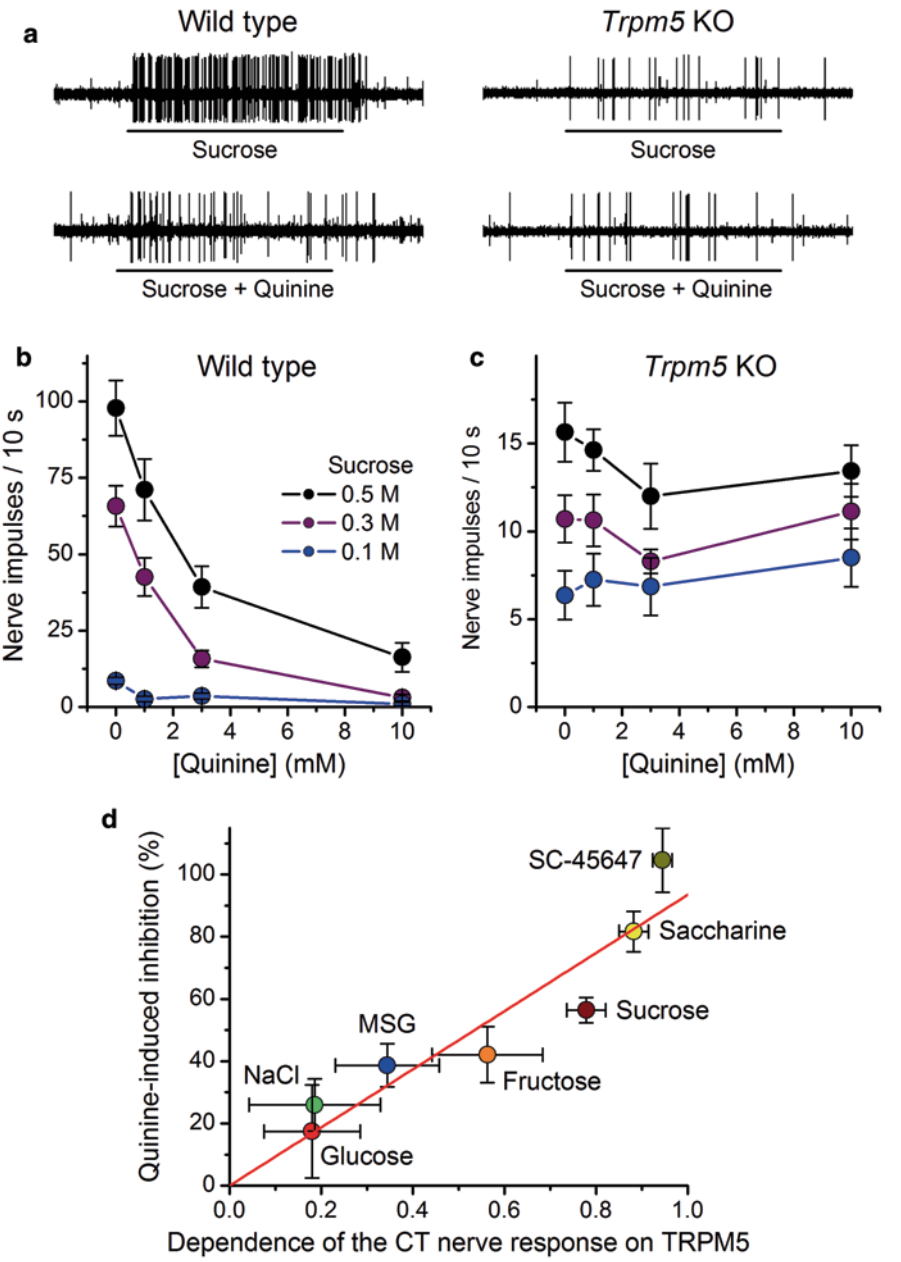

Fig. 6.2 Quinine inhibits the responses of the chorda tympani to sucrose in wild type but not in *Trpm5* knockout mice. **a** Effects of quinine (10 mM) on the responses of single sweet-sensitive fibers from wild type and *Trpm5* knockout mice to sucrose (0.5 M). **b, c** Concentration-dependent effects of quinine on the number of nerve impulses elicited by sucrose in single-sweet sensitive fibers from wild type and *Trpm5* knockout mice. **d** Correlation between the inhibitory effects of quinine of the responses of mouse chorda tympani to different tastants with the degree to which these responses depend on TRPM5. (Figure adapted from (Talavera et al. 2008c), with permission)

The inhibition of TRPM5 by nicotine represents yet another peripheral mechanism of inhibition of gustatory perception. Given the relatively high nicotine concentrations required for TRPM5 inhibition (IC_{50} of 1.3 mM at −50 mV), it is virtually impossible that this effect could be relevant during normal smoking. On the other hand, it could be relevant during tobacco chewing and the use of snuss, in nicotine-replacement therapies, and certainly for the interpretation of experimental studies that used nicotine concentrations of up to 600 mM (Carstens et al. 1998; Boucher et al. 2003; Simons et al. 2006).

Besides shedding light on the mechanisms underlying bitter-sweet taste interactions, the effects of quinine and nicotine on TRPM5 have important implications for our understanding of the mechanisms of bitter taste transduction. The inhibitory effects of these compounds on TRPM5 currents indicate that the perception of their bitter taste cannot be mediated solely by a process requiring TRPM5 activation. Alternative pathways should be recruited to elicit the aversive behavior towards quinine and nicotine at high concentrations. Accordingly, it has been recently shown that nicotine also activates a nAChR-dependent and TRPM5-independent gustatory pathway (Oliveira-Maia et al. 2009). Notably, it is expected that quinine, quinidine and nicotine may also interfere with the perception of umami. However, as for the case of the stimulatory effect of heat on TRPM5 (see above), the effect of TRMP5 inhibitors on the transduction of a certain compound must be related to the extent to which this process depends on TRPM5 activation. This contention was clearly demonstrated for quinine (Talavera et al. 2008c) (Fig. 6.2).

In contrast to quinine, quinidine and nicotine, other bitter compounds known to have an influence in sweet taste perception, including saccharine, caffeine and the related xanthines theophylline and theobromine had no effect on TRPM5 currents, indicating multiple mechanisms of bitter-sweet taste interactions (Gees et al. 2014).

6.3 Concluding Remarks

In recent years we have witnessed evident advances in the understanding of the signaling processes underlying taste transduction, but many issues remain unsolved. For instance, it is particularly challenging that despite the large amount of evidence implicating TRPV1 and TRPP channels, no definitive conclusions have been reached. Regarding TRPM5 we are in a more luxurious position, as some tools, including knockout mice, channel modulators and the inhibitor TPPO, are available to study the roles of this channel. We are particularly expectant about the publication of a full report on the discovery and characterization of a group of selective agonists that may help developing new taste modulators and treatments for type II diabetes (Philippaert 2013). However, it is clear that we still know very little about the details of how this channel is gated in basal conditions and how it is modulated. Furthermore, most findings have been obtained in heterologous expression systems and need to be confirmed in taste cells. It is also clear that studying the pharmaco-

logical properties of individual compounds in detail is of outmost importance for the understanding of their specific chemosensory properties.

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References

Bartel DL, Sullivan SL, Lavoie EG, Sevigny J, Finger TE (2006) Nucleoside triphosphate diphosphohydrolase-2 is the ecto-ATPase of type I cells in taste buds. J Comp Neurol 497:1–12

Boucher Y, Simons CT, Cuellar JM, Jung SW, Carstens MI, Carstens E (2003) Activation of brain stem neurons by irritant chemical stimulation of the throat assessed by c-fos immunohistochemistry. Exp Brain Res 148:211–218

Carstens E, Kuenzler N, Handwerker HO (1998) Activation of neurons in rat trigeminal subnucleus caudalis by different irritant chemicals applied to oral or ocular mucosa. J Neurophysiol 80:465–492

Chaudhari N, Roper SD (2010) The cell biology of taste. J Cell Biol 190:285–296

Cruz A, Green BG (2000) Thermal stimulation of taste. Nature 403:889–892

Damak S, Rong M, Yasumatsu K, Kokrashvili Z, Perez CA, Shigemura N, Yoshida R, Mosinger B Jr, Glendinning JI, Ninomiya Y, Margolskee RF (2006) *Trpm5* null mice respond to bitter, sweet, and umami compounds. Chem Senses 31:253–264

de Araujo IE, Oliveira-Maia AJ, Sotnikova TD, Gainetdinov RR, Caron MG, Nicolelis MA, Simon SA (2008) Food reward in the absence of taste receptor signaling. Neuron 57:930–941

Devantier HR, Long DJ, Brennan FX, Carlucci SA, Hendrix C, Bryant RW, Salemme FR, Palmer RK (2008) Quantitative assessment of TRPM5-dependent oral aversiveness of pharmaceuticals using a mouse brief-access taste aversion assay. Behav Pharmacol 19:673–682

Dotson CD, Roper SD, Spector AC (2005) PLCb2-independent behavioral avoidance of prototypical bitter-tasting ligands. Chem Senses 30:593–600

Dvoryanchikov G, Sinclair MS, Perea-Martinez I, Wang T, Chaudhari N (2009) Inward rectifier channel, ROMK, is localized to the apical tips of glial-like cells in mouse taste buds. J Comp Neurol 517:1–14

Eddy MC, Eschle BK, Peterson D, Lauras N, Margolskee RF, Delay ER (2012) A conditioned aversion study of sucrose and SC45647 taste in TRPM5 knockout mice. Chem Senses 37:391–401

Feng P, Huang L, Wang H (2012) Taste bud homeostasis in health, disease, and aging. Chem Senses 39:3–16

Formaker BK, Frank ME (1996) Responses of the hamster chorda tympani nerve to binary component taste stimuli: evidence for peripheral gustatory mixture interactions. Brain Res 727:79–90

Gao N, Lu M, Echeverri F, Laita B, Kalabat D, Williams ME, Hevezi P, Zlotnik A, Moyer BD (2009) Voltage-gated sodium channels in taste bud cells. BMC Neurosci 10:20

Gees M, Alpizar YA, Luyten T, Parys JB, Nilius B, Bultynck G, Voets T, Talavera K (2014) Differential effects of bitter compounds on the taste transduction channels TRPM5 and IP_3 receptor type 3. Chem Senses 39:295–311

Glendinning JI, Bloom LD, Onishi M, Zheng KH, Damak S, Margolskee RF, Spector AC (2005) Contribution of alpha-gustducin to taste-guided licking responses of mice. Chem Senses 30:299–316

Grunberg NE (1982) The effects of nicotine and cigarette smoking on food consumption and taste preferences. Addict Behav 7:317–331

Grunberg NE (1985) Nicotine, cigarette smoking, and body weight. Br J Addict 80:369–377

Hisatsune C, Yasumatsu K, Takahashi-Iwanaga H, Ogawa N, Kuroda Y, Yoshida R, Ninomiya Y, Mikoshiba K (2007) Abnormal taste perception in mice lacking the type 3 inositol 1,4,5-trisphosphate receptor. J Biol Chem 282:37225–37231

Hofmann T, Chubanov V, Gudermann T, Montell C (2003) TRPM5 is a voltage-modulated and Ca^{2+}-activated monovalent selective cation channel. Curr Biol 13:1153–1158

Huang YA, Roper SD (2010) Intracellular Ca^{2+} and TRPM5-mediated membrane depolarization produce ATP secretion from taste receptor cells. J Physiol 588:2343–2350

Huang YJ, Maruyama Y, Dvoryanchikov G, Pereira E, Chaudhari N, Roper SD (2007) The role of pannexin 1 hemichannels in ATP release and cell-cell communication in mouse taste buds. Proc Natl Acad Sci U S A 104:6436–6441

Ishimaru Y, Matsunami H (2009) Transient receptor potential (TRP) channels and taste sensation. J Dent Res 88:212–218

Ishimaru Y, Inada H, Kubota M, Zhuang H, Tominaga M, Matsunami H (2006) Transient receptor potential family members PKD1L3 and PKD2L1 form a candidate sour taste receptor. Proc Natl Acad Sci U S A 103:12569–12574

Katsumata T, Nakakuki H, Tokunaga C, Fujii N, Egi M, Phan TH, Mummalaneni S, Desimone JA, Lyall V (2008) Effect of Maillard reacted peptides on human salt taste and the amiloride-insensitive salt taste receptor (TRPV1t). Chem Senses 33:665–680

Kinnamon SC (2012) Taste receptor signalling—from tongues to lungs. Acta Physiol (Oxf) 204:158–168

Kinnamon SC, Finger TE (2013) A taste for ATP: neurotransmission in taste buds. Front Cell Neurosci 7:264

Liman ER (2006) Thermal gating of TRP ion channels: food for thought? Sci STKE 2006, pe12

Liman ER (2014) TRPM5. Handb Exp Pharmacol 222:489–502

Liman ER, Zhang YV, Montell C (2014) Peripheral coding of taste. Neuron 81:984–1000

Liu D, Liman ER (2003) Intracellular Ca^{2+} and the phospholipid PIP_2 regulate the taste transduction ion channel TRPM5. Proc Natl Acad Sci U S A 100:15160–15165

Liu L, Zhu W, Zhang ZS, Yang T, Grant A, Oxford G, Simon SA (2004) Nicotine inhibits voltage-dependent sodium channels and sensitizes vanilloid receptors. J Neurophysiol 91:1482–1491

Liu D, Zhang Z, Liman ER (2005) Extracellular acid block and acid-enhanced inactivation of the Ca^{2+}-activated cation channel TRPM5 involve residues in the S3-S4 and S5-S6 extracellular domains. J Biol Chem 280:20691–20699

Liu P, Shah BP, Croasdell S, Gilbertson TA (2011) Transient receptor potential channel type M5 is essential for fat taste. J Neurosci 31:8634–8642

Lopez Jimenez ND, Cavenagh MM, Sainz E, Cruz-Ithier MA, Battey JF, Sullivan SL (2006) Two members of the TRPP family of ion channels, PKD1L3 and PKD2L1, are co-expressed in a subset of taste receptor cells. J Neurochem 98:68–77

Lyall V, Heck GL, Vinnikova AK, Ghosh S, Phan TH, Alam RI, Russell OF, Malik SA, Bigbee JW, Desimone JA (2004) The mammalian amiloride-insensitive non-specific salt taste receptor is a vanilloid receptor-1 variant. J Physiol 558:147–159

Lyall V, Heck GL, Vinnikova AK, Ghosh S, Phan TH, Desimone JA (2005) A novel vanilloid receptor-1 (VR-1) variant mammalian salt taste receptor. Chem Senses 30(Suppl 1):i42–i43

Lyall V, Phan TH, Mummalaneni S, Mansouri M, Heck GL, Kobal G, Desimone JA (2007) Effect of nicotine on chorda tympani responses to salty and sour stimuli. J Neurophysiol 98:1662–1674

Lyall V, Phan TH, Mummalaneni S, Melone P, Mahavadi S, Murthy KS, Desimone JA (2009) Regulation of the benzamil-insensitive salt taste receptor by intracellular Ca^{2+}, protein kinase C, and calcineurin. J Neurophysiol 102:1591–1605

Meseguer VM, Denlinger BL, Talavera K (2011) Methodological considerations to understand the sensory function of TRP channels. Curr Pharm Biotechnol 12:3–11

Mueller KL, Hoon MA, Erlenbach I, Chandrashekar J, Zuker CS, Ryba NJ (2005) The receptors and coding logic for bitter taste. Nature 434:225–229

Nilius B, Appendino G (2013) Spices: the savory and beneficial science of pungency. Rev Physiol Biochem Pharmacol 164:1–76

Nilius B, Talavera K, Owsianik G, Prenen J, Droogmans G, Voets T (2005) Gating of TRP channels: a voltage connection? J Physiol 567:35–44

Öhrwall H (1891) Untersuchungen über den Geschmackssinn. Skand Arch Physiol 2:1–69

Oike H, Wakamori M, Mori Y, Nakanishi H, Taguchi R, Misaka T, Matsumoto I, Abe K (2006) Arachidonic acid can function as a signaling modulator by activating the TRPM5 cation channel in taste receptor cells. Biochim Biophys Acta 1761:1078–1084

Oka Y, Butnaru M, von Buchholtz L, Ryba NJ, Zuker CS (2013) High salt recruits aversive taste pathways. Nature 494:472–475

Oliveira-Maia AJ, Stapleton-Kotloski JR, Lyall V, Phan TH, Mummalaneni S, Melone P, Desimone JA, Nicolelis MA, Simon SA (2009) Nicotine activates TRPM5-dependent and independent taste pathways. Proc Natl Acad Sci U S A 106:1596–1601

Perez CA, Huang L, Rong M, Kozak JA, Preuss AK, Zhang H, Max M, Margolskee RF (2002) A transient receptor potential channel expressed in taste receptor cells. Nat Neurosci 5:1169–1176

Perez CA, Margolskee RF, Kinnamon SC, Ogura T (2003) Making sense with TRP channels: store-operated calcium entry and the ion channel TRPM5 in taste receptor cells. Cell Calcium 33:541–549

Philippaert KP (2013) Screening for and characterization of pharmacological tools to target the Ca^{2+} activated non-selective cation channels TRPM4 and TRPM5. In 22nd Congress of the European-Chemoreception-Research-Organization (ECRO), vol 39. ed. Press, O. U., pp 106–107, Leuven

Prawitt D, Monteilh-Zoller MK, Brixel L, Spangenberg C, Zabel B, Fleig A, Penner R (2003) TRPM5 is a transient Ca^{2+}-activated cation channel responding to rapid changes in $[Ca^{2+}]i$. Proc Natl Acad Sci U S A 100:15166–15171

Pumplin DW, Yu C, Smith DV (1997) Light and dark cells of rat vallate taste buds are morphologically distinct cell types. J Comp Neurol 378:389–410

Ren Z, Rhyu MR, Phan TH, Mummalaneni S, Murthy KS, Grider JR, Desimone JA, Lyall V (2013) TRPM5-dependent amiloride- and benzamil-insensitive NaCl chorda tympani taste nerve response. Am J Physiol Gastrointest Liver Physiol 305:G106–G117

Riera CE, Vogel H, Simon SA, Damak S, Le Coutre J (2009) Sensory attributes of complex tasting divalent salts are mediated by TRPM5 and TRPV1 channels. J Neurosci 29:2654–2662

Romanov RA, Rogachevskaja OA, Bystrova MF, Jiang P, Margolskee RF, Kolesnikov SS (2007) Afferent neurotransmission mediated by hemichannels in mammalian taste cells. EMBO J 26:657–667

Roper SD (2014) TRPs in taste and chemesthesis. Handb Exp Pharmacol 223:827–871

Simons CT, Boucher Y, Carstens MI, Carstens E (2006) Nicotine suppression of gustatory responses of neurons in the nucleus of the solitary tract. J Neurophysiol 96:1877–1886

Talavera K, Yasumatsu K, Voets T, Droogmans G, Shigemura N, Ninomiya Y, Margolskee RF, Nilius B (2005) Heat activation of TRPM5 underlies thermal sensitivity of sweet taste. Nature 438:1022–1025

Talavera K, Ninomiya Y, Winkel C, Voets T, Nilius B (2007) Influence of temperature on taste perception. Cell Mol Life Sci 64:377–381

Talavera K, Nilius B, Voets T (2008a) Neuronal TRP channels: thermometers, pathfinders and lifesavers. Trends Neurosci 31:287–295

Talavera K, Voets T, Nilius B (2008b) Mechanisms of thermosensation in TRP channels. In Martinac B (ed) Sensing with ion channels. Springer, pp 101–120

Talavera K, Yasumatsu K, Yoshida R, Margolskee RF, Voets T, Ninomiya Y, Nilius B (2008c) The taste transduction channel TRPM5 is a locus for bitter-sweet taste interactions. FASEB J 22:1343–1355

Talavera K, Gees M, Karashima Y, Meseguer VM, Vanoirbeek JA, Damann N, Everaerts W, Benoit M, Janssens A, Vennekens R, Viana F, Nemery B, Nilius B, Voets T (2009) Nicotine activates the chemosensory cation channel TRPA1. Nat Neurosci 12:1293–1299

Taruno A, Matsumoto I, Ma Z, Marambaud P, Foskett JK (2013a) How do taste cells lacking synapses mediate neurotransmission? CALHM1, a voltage-gated ATP channel. Bioessays 35:1111–1118

Taruno A, Vingtdeux V, Ohmoto M, Ma Z, Dvoryanchikov G, Li A, Adrien L, Zhao H, Leung S, Abernethy M, Koppel J, Davies P, Civan MM, Chaudhari N, Matsumoto I, Hellekant G, Tordoff MG, Marambaud P, Foskett JK (2013b) CALHM1 ion channel mediates purinergic neurotransmission of sweet, bitter and umami tastes. Nature 495:223–226

Tomassini S, Cuoghi V, Catalani E, Casini G, Bigiani A (2007) Long-term effects of nicotine on rat fungiform taste buds. Neuroscience 147:803–810

Tordoff MG, Shao H, Alarcon LK, Margolskee RF, Mosinger B, Bachmanov AA, Reed DR, Mccaughey S (2008) Involvement of T1R3 in calcium-magnesium taste. Physiol Genomics 34:338–348

Tordoff MG, Alarcon LK, Valmeki S, Jiang P (2012) T1R3: a human calcium taste receptor. Sci Rep 2:496

Treesukosol Y, Lyall V, Heck GL, Desimone JA, Spector AC (2007) A psychophysical and electrophysiological analysis of salt taste in *Trpv1* null mice. Am J Physiol Regul Integr Comp Physiol 292:R1799–R1809

Uchida K, Tominaga M (2013) Extracellular zinc ion regulates transient receptor potential melastatin 5 (TRPM5) channel activation through its interaction with a pore loop domain. J Biol Chem 288:25950–25955

Ullrich ND, Voets T, Prenen J, Vennekens R, Talavera K, Droogmans G, Nilius B (2005) Comparison of functional properties of the Ca^{2+}-activated cation channels TRPM4 and TRPM5 from mice. Cell Calcium 37:267–278

Vandenbeuch A, Kinnamon SC (2009) Why do taste cells generate action potentials? J Biol 8:42

Vandenbeuch A, Clapp TR, Kinnamon SC (2008) Amiloride-sensitive channels in type I fungiform taste cells in mouse. BMC Neurosci 9:1

Vandenbeuch A, Anderson CB, Parnes J, Enjyoji K, Robson SC, Finger TE, Kinnamon SC (2013) Role of the ectonucleotidase NTPDase2 in taste bud function. Proc Natl Acad Sci U S A 110:14789–14794

Voets T, Droogmans G, Wissenbach U, Janssens A, Flockerzi V, Nilius B (2004) The principle of temperature-dependent gating in cold- and heat-sensitive TRP channels. Nature 430:748–754

Voets T, Owsianik G, Janssens A, Talavera K, Nilius B (2007) TRPM8 voltage sensor mutants reveal a mechanism for integrating thermal and chemical stimuli. Nat Chem Biol 3:174–182

Zhang Y, Hoon MA, Chandrashekar J, Mueller KL, Cook B, Wu D, Zuker CS, Ryba NJ (2003) Coding of sweet, bitter, and umami tastes: different receptor cells sharing similar signaling pathways. Cell 112:293–301

Zhang Z, Zhao Z, Margolskee R, Liman E (2007) The transduction channel TRPM5 is gated by intracellular calcium in taste cells. J Neurosci 27:5777–5786

Chapter 7
TRP Channels and Mechanical Transduction

Ana Gomis

Abstract Many physiological processes depend on correctly sensing mechanical forces, including hearing, proprioception and touch. Accordingly, much research has focused on the mechanisms and molecules responsible for mechanotransduction. Studies in the fields of genetics, genomics and electrophysiology have converged to further extend our understanding of mechanosensitive events in invertebrates and vertebrates. Indeed, candidate mechanotransduction genes have been identified in mammalian cells, some of which encode the TRP channels expressed in mechanosensitive neurons. In recent years, functional assays have permitted single or multiple ion channel currents flowing through the membrane to be recorded. Such approaches will help determine the biophysical properties of mechanosensitive currents, a crucial step in the quest to identify transduction channels at the molecular level and probe their activity *in vivo*. Here, the proposed mechanisms to mechanodetection are described, along with the different mechanosensory systems used as models to study mechanotransduction. The TRP channels that represent relevant candidates to be involved in sensing mechanical forces will also be reviewed.

Keywords TRP channels · Mechanotransduction · Sensory systems · Touch · Pain

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7.1 Principles of Mechanotransduction

In many animals, including humans, some of the most important physiological processes occur through mechanical stimulation. These physiological processes depend on the transformation of mechanical energy into ion currents in order to evoke events such as cellular turgor in bacteria, or the sensation of touch, sound and pain in mammals (Hamill and Martinac 2001; Lewin and Moshourab 2004). To detect and decode mechanical stimuli, organisms possess specialised organs like the touch receptor neurons in *Caenorhabditis elegans*, the chordotonal organ and bristles in *Drosophila melanogaster*, and in mammals, the hair cells in the auditory system, the cutaneous tactile receptors, the circumventricular organs that control systemic osmolarity, the baroreceptors that detect blood pressure, and the proprioreceptors that control muscle stretch and the position of the body. In addition, animals also possess free nerve endings of somatosensory and visceral afferents scattered throughout the body (Ernstrom and Chalfie 2002).

As mechanical stimuli are ubiquitous, mechanotransduction may represent one of the oldest sensory processes developed by living organisms. Nevertheless, little is known about the molecular machinery that mediates the sensory detection of force stimuli when compared with our knowledge of other senses.

The mechanisms involved in olfaction, phototransduction and some modalities of taste include molecular mediators that link G protein-coupled receptors to their effector proteins, enzymes or ion channels (Arshavsky et al. 2002; Ronnett and Moon 2002; Hardie and Postma 2008; Kinnamon 2012; Hardie 2014). These mechanisms appear to be evolutionarily conserved and likewise, it could be predicted that there is a general mechanism for mechanical transduction, and a similar sensor that produces different outcomes as a function of its configuration. However, research over the past 10 years indicate that diverse mechanisms and different molecular entities are involved in mechanotransduction, both across species and within single organisms (Sukharev and Corey 2004; Christensen and Corey 2007; Nilius and Honore 2012).

Most mechanosensory cells or mechanoreceptors convert forces into electrical signals, a process that relies on conserved functional principles. The result of this stimulation is the depolarization of the nerve ending and the generation of trains of action potentials (Shepherd 1991; Block 1992; Torre et al. 1995) through the activation of ion channels rather than sensory receptors for odours and most tastes (Garcia-Añoveros and Corey 1997; Sachs and Morris 1998; Hamill and Martinac 2001; Gillespie and Walker 2001) . The impulses propagated travel centrally along sensory nerves, eventually reaching cortical structures in order to evoke a sensation.

The idea of mechanically gated ion channels was proposed by Bernhard Katz in 1950 (Katz 1950) on the basis of studies into the muscle spindle. Years later, experiments carried out on hair cells from the bullfrog sacculus (Corey and Hudspeth 1979) , bristle mechanoreceptors (Walker et al. 2000) , chordotonal hearing receptors from *D. melanogaster* (Albert et al. 2007) and touch receptors in *C. elegans* (O'Hagan et al. 2005) showed that mechanical stimulus produced electrical

responses in the range of microseconds. This rapid response must result from the direct activation of a transduction channel rather than the activation of second messenger systems. In addition to rapid activation kinetics, the channel must also fulfil other requirements (Ernstrom and Chalfie 2002; Christensen and Corey 2007) : first, the channel must be expressed and located in the receptor cell; second, the candidate protein must be necessary for the electrical signal to be produced in response to mechanical stimulation; and third, the mechanical gating and pharmacological properties of the channel must be similar when it is expressed in different cells.

Studies carried out over the past 15 years have also focused on discovering how these ion channels are activated and although this is still to be fully elucidated, several mechanisms have been proposed to explain how mechanosensitive channels (MS) translate mechanical stimuli into channel opening (Fig. 7.1; Voets et al. 2005; Christensen and Corey 2007; Chalfie 2009; Kung 2005). First, MS channels may be directly gated by the force stimulus. A change in the force may lead to changes in tension of the lipid bilayer, causing conformational changes in the channel and the gating of the pore (Fig. 7.1a). An alternative model for the direct activation of the channel proposes that the channels are 'tied' to cytoskeletal elements and/or the extracellular matrix (ECM), and that the tension among these tethered elements controls the gating of the channels (Fig. 7.1b). However, it is not clear whether these links are directly coupled to the channel domains or if they modulate the forces that surround the channel. This model arises from the biophysical studies in hair cells from the auditory and vestibular systems (LeMasurier and Gillespie 2005) . A third model of activation implicates an accessory protein that will transmit the force to the channel, inducing a conformational change of the channel and indirectly gating its activity (Fig. 7.1c). A fourth possibility is that channels may be activated by intracellular signalling cascades that display mechano- or osmosensitivity (Fig. 7.1d). Mechanical stimulation and osmotic stress evoke numerous changes in protein ph osphorylation/dephosphorylation cycles, and it has been shown that some of them play important roles in osmotic and mechanical regulation, acting through protein transport (Pedersen and Nilius 2007).

Multiple mechanisms can converge on a single channel and moreover, the same stimulus may activate different mechanisms. For example, osmotic cell swelling induces tension at the cell membrane that may directly activate channels (mechanism 1) and/or alter the interactions with the cortical cytoskeleton that may in turn affect a tethered channel (mechanism 2). Changes in cell volume may also initiate signalling events that involve the metabolism of membrane components, which may regulate ionic channels by altering the curvature of the membrane (mechanism 4: Pedersen and Nilius 2007) .

How these mechanosensory mechanisms can be studied will be addressed below.

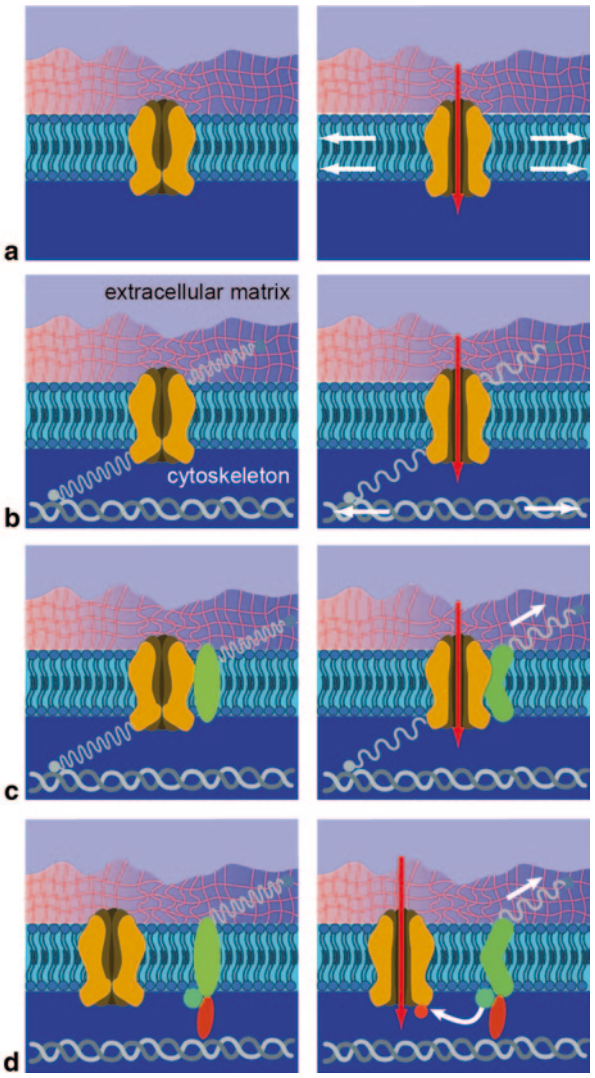

Fig. 7.1 Mechanisms of mechanical channel activation. **a** All membrane channels are exposed to lateral forces or tension produce by the modification of the phospholipid bilayer (indicated by the *arrow*), resulting in pore opening. **b** Channel activation through tethered elements, including the cytoskeleton and elements of the extracellular matrix. This interaction may be direct **b** or through proteins associated to the channel **c**. **d** Channel activation through a secondary signal generated by the activation of other proteins sensitive to mechanical forces. (Adapted with permission from Christensen and Corey 2007)

7.2 Animal Models in Sensory Mechanotransduction: Mechanosensory Systems

Mechanotransduction is not limited to a subset of specialized cells and tissues but rather, it is implicated in a wide range of cellular functions. The molecular bases of touch sensitivity have been investigated extensively in ***C. elegans***. This transparent worm has the particularity of having only 302 neurons, 10% of which are primary mechanosensory neurons, and they have been identified individually through

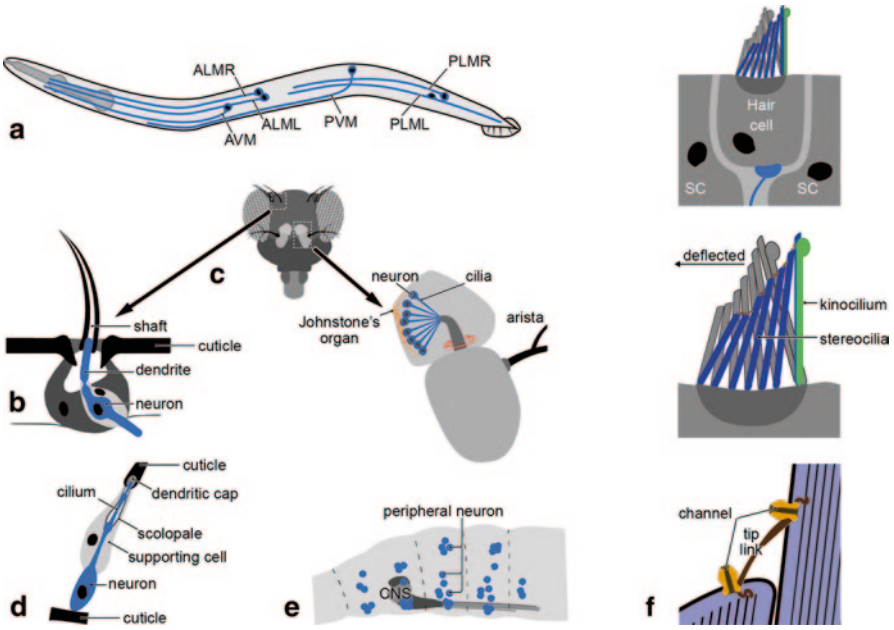

Fig. 7.2 Mechanosensory systems. **a** Schematic drawing of *C elegans* showing the gentle touch neurons. The head of *D. melanogaster* has different mechanosensory organs, bristles (**b**), the Johnston's organ in the antennae (**c**), and chordotonal organs (ch) (**d**). A positive deflection of the bristle stimulates the neurons that innervate it. Sound waves make the arista of the antenna vibrate causing a rotational movement that stretches neurons of Johnston's organ. The characteristic feature of the ch organs is the scolopale cell. Scolopidial receptors are common in tympanal organs and in Johnston's organ, both of which are used in insects to detect external vibration and sound (**d**). Panel (**e**) shows the localization of the multidendritic sensory neurons in *Drosophila* larvae (type II mechanosensory organs). **f** *Hair cell* and supporting cells (SC) in the sensory epithelium (*upper panel*). On top of the HC the stereocilia and kinolicium in the hair bundle can be observed (*middle panel*). Deflection of the stereocilia would increase the tension in the tip links and open the ion channels (*lower panel*). (Adapted with permission from Christensen and Corey 2007 (**a** and **f**) and from Wilson and Corey 2010 (**b–e**))

laser ablation and genetic studies. Specifically, six of these cells are responsible for light touch and proprioception (Fig. 7.2 a: Chalfie and Thomson 1982) . Three cells sense touch in the anterior half of the animal (ALML, ALMR and AVM) and two in the posterior part (PLML and PLMR). Moreover, in addition to touch these cells also appear to regulate other physiological processes (Ernstrom and Chalfie 2002) . Other cell seems not to be essential for touch but they mediate movement in touch-mediated circuits (PVM neurons: Chalfie et al. 1985) . Over the past three decades, genetic screens have generated a large number of *C. elegans* mutants in which light touch is affected. The relevant proteins involved have been named MEC, from mechanosensory, and they have been numbered from 1 to 18. Two of the most interesting MEC proteins, MEC-4 and MEC-10, form ion channels of the degenerin (DEG) family, which are related to the vertebrate epithelial sodium channels (ENaCs). These two proteins form the pore of the channel, probably together

with MEC-6. This channel associates with other MEC proteins, as well as with the extracellular matrix and cytoskeletal proteins, to form a mechanotransduction complex (for extensive revisions see Garcia-Añoveros and Corey 1997; Ernstrom and Chalfie 2002; Geffeney and Goodman 2012). Genetic studies in worms defective for touch sensation have also revealed the involvement of two related TRPV channels, the OSM-9 and OCR-2 proteins, and of the TRPP2 and TRPML channels (Venkatachalam and Montell 2007).

Studies in the fruit fly ***D. melanogaster*** have also contributed to our understanding of mechanosensation. Flies display mechanosensation such a gravity sensing, hearing, proprioception, gentle-touch sensation and mechanical nociception. In *Drosophila* there are two types of touch-sensitive cells, the type I cells that have one sensory dendrite (Figs. 7.2b–d) and the type II cells that are multidendritic (Fig. 7.2e; Keil 1997). The most abundant cells are the type I mechanoreceptors, which are associated with accessory structures and that form complex mechanosensory organs. These organs may be associated with external cuticular structures such as bristles (Fig. 7.2b) or cuticular domes (the campaniformsensilla), or they may be attached to the apparently unspecialized cuticle through support cells (the chordotonal organs). The dendrites of these cells terminate at the base of extracellular bristles or domes (external sensory organs) that can be deflected or deformed by touch, airflow or proprioceptive stimulation, or that are surrounded by a supporting cell called a scolopale (Fig. 7.2d). Scolopidial receptors are spindle-shaped cages enclosing an extracellular cavity, into which the ciliar outer segment extends, and they are common in tympanal organs and in Johnston's organ (Fig. 7.2c), both of which are used to detect external vibration and sound (Kernan and Zuker 1995; Eberl 1999).

Type II mechanoreceptors have multiple non-ciliated dendrites, and they lack accessory cells and innervate the insect epidermis (Grueber et al. 2002).

Genetic screening has helped identify genes that are required for the function of the sensory organs in *Drosophila*, whose mutations cause impaired touch responses. One of these genes, named *nompC* (no mechanoreceptor potential C), encodes a channel protein, located in the ciliated mechanosensory organs responsible for bristle touch sensitivity (NOMPC; Walker et al. 2000). This ion channel belongs to the TRPN branch of the TRP channel family (TRPN1) and NompC mutants display an approximate reduction in 50 % in auditory nerve firing and no amplification of antennal vibration (Eberl et al. 2000), consistent with a hearing deficit. Another gene, *nompA*, was also cloned from Type I cells (Chung et al. 2001), a gene encoding an extracellular linker protein that connects the ciliated dendrites of the auditory mechanosensory neuron to the antennal receiver. Mutation of this gene results in conductive hearing loss. In addition, two more mechanosensory proteins belonging to the TRPV family have been identified, the *nanchung (nan)* and *inactive (iav)*. These proteins are found in the mechanosensory cilia of chordotonal organs, and their mutation results in the loss of auditory responses (Gong et al. 2004; Kim et al. 2003).

Other genetic screens have been performed in *Drosophila* larvae to identify genes responsible for pain responses (pinching and thermal stimuli). In larvae with

deficiencies in the sensation of pain, mutations were identified in a gene (*painless*) that encodes a fly member of the TRPA subfamily channel (Tracey et al. 2003).

More recently, studies on the dendritic arborisation of neurons in *Drosophila* larvae showed that the mechanotransducer channel *piezo* (Dm*piezo*; see below) is essential for sensing noxious mechanical stimulus *in vivo* (Kim et al. 2012), and that NOMPC is a mechanotransducer channel for gentle touch (Yan et al. 2013). However, a separate study indicates that Dm*piezo* is not implicated in the mechanotransduction and/or signal amplification for the detection of sound (Zhang et al. 2013).

Vertebrates possess a variety of external and internal cells that transduce mechanical stimuli. The external stimuli, touch and sound, are detected by receptors located in the skin and hair cells of the inner ear, respectively. The internal mechanoreceptors in the vascular system, muscle, tendon, organs and joints, and in the urinary bladder detect stretch and pressure stimuli. Among them, the most intensely studied mechanoreceptors are the hair cells and those located in the skin.

Hair cells are located in the organ of Corti in the internal ear and they transduce sound and head movements into neuronal signals. The mechanosensory structure of the hair cell consist of a bundle of cilia emerging from the surface of the hear cells (Fig. 7.2f, upper panel), a bundle that contains 30–300 stereocilia and a single kinocilium (Fig. 7.2f middle panel). The stereocilia are connected to each other by a spring protein strand called the tip link, which is thought to be attached to ion channels. The model of mechanotransduction in hair cells proposes that the stimulus produces a deflection of the kinocilia and the associated stereociliary bundle that stretches the tip link. This increase in tension opens ion channels, increasing the inward currents and depolarizing the cells, thereby triggering neurotransmitter release (Fig. 7.2f lower panel) (for extensive reviews see Garcia-Añoveros and Corey 1997; Gillespie and Muller 2009).

Many genes affecting the development and function of hair cells have been identified (http://www.jax.org/), some of which were detected due to spontaneous mutation or systematic mutagenesis causing deafness and/or balance defects (Hrabe de Angelis et al. 2000; Nolan et al. 2000; Shearer and Smith 2012). However, identifying the transduction molecules is proving to be more difficult. Evidence has recently supported a direct role for TMC1 and TMC2, members of the transmembrane channel-like family, as components of the transduction complex (Kawashima et al. 2011; Pan et al. 2013; Kim et al. 2013), although it remains uncertain whether TMC1 and 2 are pore-forming subunits and their specific role has yet to be clarified (Morgan and Barr-Gillespie 2013; Holt et al. 2014). Other channels that are candidates to participate in transduction in vertebrate hair-cells are the members of the TRP superfamily, TRPN1, TRPML3, TRPV4 and TRPA1, as will be discussed later on in this chapter.

Interestingly, novel mechanical activated currents have been recorded in auditory hair cells lacking tip links. These currents have different properties to those recorded when tip links are functional and the channels responsible is not yet clear. Nevertheless, it has been suggested that normal mechanotransducer channels with an altered distribution or configuration may be involved in producing such currents, as well as ion channels other than mechano-electrical transducer channels, or trans-

duction complex elements other than the TMC1 and TMC2 proteins (Marcotti et al. 2014). Further research should shed light on the identity of these new channels.

The somatosensory system in mammals is formed by neurons that innervate the skin, muscle, joints, tendons and internal organs, and that are responsible for the perception and transmission of physical and chemical stimuli originating outside and inside the body. The cell body of these neurons are situated in the dorsal root ganglia (DRG) and trigeminal ganglia (TG), receiving information coming from the body and head, respectively. Transmission of afferent somatosensory information from the periphery to the brain commences with the depolarization of a sensory neuron's terminal, caused by the activation of sensory receptors. These cells include distinct types of mechanoreceptors in function of their anatomy (localization and type of end terminals), their response properties (slow or rapid firing and sensory threshold) or their axonal conduction velocities (myelinated or unmyelinated axons). An important distinction among mechanosensitive neurons is related to the stimulus intensity required to activate their terminals. While some terminals are very sensitive to touch or mechanical displacement, low threshold mechanoreceptors (LTMR), others require stimulation above a high threshold for activation, high threshold mechanonociceptors (HTMRs). Many of the mechanonociceptors are also excited by chemical and thermal stimuli and they are called polymodal nociceptors. Mechanically-evoked nerve impulse discharges are absent in "silent" nociceptor nerve fibres, although they are recorded following local tissue inflammation. The majority of nociceptive fibres are Aδ (myelinated) and C (unmyelinated) type, which terminate with free endings in the tissue they innervate. In terms of the LTMR, the cutaneous receptors are those best known and they can be classified either as Aδ and C fibres whose endings are free nerve terminals, including the D-hair, and as Aβ (myelinated) fibres that terminate in different structures: Pacinian corpuscles, Ruffini endings, Meissner corpuscles (as described in several reviews: Lumpkin and Caterina 2007; Lumpkin et al. 2010; Abraira and Ginty 2013) (Fig. 7.3a). In addition, Merkel's discs have also been shown to function as mechanoreceptors, forming a complex structure with slowly adapting afferents (Maksimovic et al. 2014).

A major issue that is still to be resolved is the identity of the molecules that transduce mechanosensory stimuli, and whether the changes in threshold reflect the activation of different molecular sensors, the differential modulation of the same sensor, or differences in the density or coupling efficiency between the sensor and perireceptor elements. An important reason for the lack of progress regarding these issues is the very small diameter of the mechanosensory terminals and the dispersion of touch receptors in the skin. These factors have prevented the direct recording of mechanogated currents at the site of transduction. In the absence of this type of recordings, different groups have recorded three types of mechanosensory currents from the soma or dendrites of DRG neurons with different kinetics of adaptation: rapidly adapting (RA), intermediately adapting (IA) and slowly adapting (SA: McCarter et al. 1999; Drew et al. 2002; Hu and Lewin 2006; Hao and Delmas 2010) (Fig. 7.3b). Indeed, mechanically activated currents with similar properties have also been recorded in trigeminal neurons (our unpublished results). Some progress

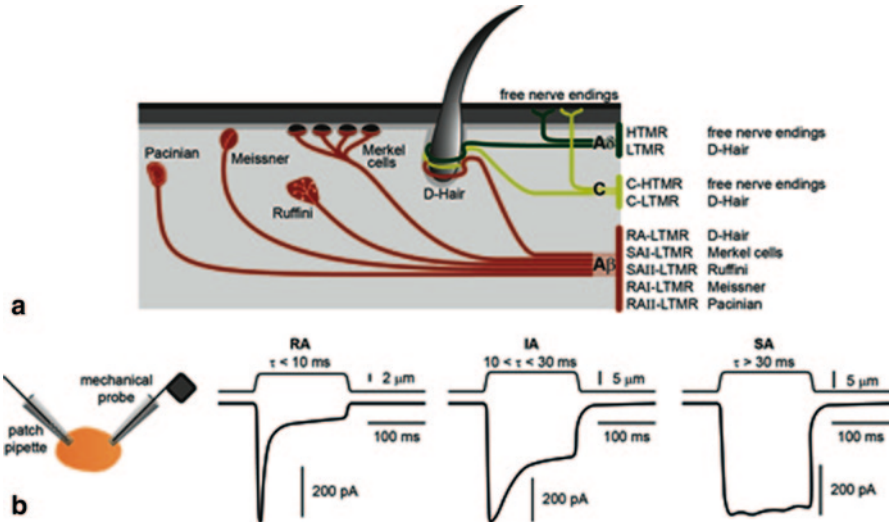

Fig. 7.3 Cutaneous touch receptors and mechanosensitive currents in mammals. **a** Sensory afferents of neurons innervating skin propagate electrical impulses from the body to the central nervous system. Afferents are classified as Aβ (*red*), Aδ (*green*) or C (*yellow*) based on their cell body sizes, axon diameter, degree of myelination and axonal conduction velocities. Most Aβ fibres have low mechanical thresholds and Aδ and *C* fibres respond either to noxious stimuli or light touch, each with different physiological profiles and firing patterns (slowly (SA) and rapidly adapting (RA)). The peripheral afferents terminate in different end organs, Merkel cell-neurite complexes, Ruffini endings, Meissner corpuscles, Pacinian corpuscles and hair follicles, and in free nerve ending. **b** Graphical representation of inward mechanosensitive rapidly-adapting, intermediate-adapting and slowly-adapting currents expressed in mice DRG neurons using the mechano-clamp technique (shown in the *left panel*) at holding potential of −80 mV. Standard mechanical stimuli and time constants of current decay are indicated above the current trace of each panel

has also been made in identifying the mechanotransduction molecules. As such, ASIC 1–3 are expressed in peripheral neurons, although their role in mammalian mechanotransduction is not fully clear (Drew et al. 2004; Roza et al. 2004; Lingueglia 2007). Piezo proteins are pore-forming subunits of a conserved mechanical activated cation family which have recently been considered to be the most promising mechanotransducers candidate (Coste et al. 2010; Coste et al. 2012). In mammals, Piezo1 is necessary for mechanical activated currents in the Neuro2A cell line (Coste et al. 2010) and Piezo2 mediates the mechanical responses in Merkel-cells, evidence of a physiological role in mammalian mechanotransduction (Woo et al. 2014; Maksimovic et al. 2014; Ikeda et el. 2014). Nevertheless, the involvement of TRP channels in mechanotransduction cannot be ruled out, as discussed below.

Mechanotransduction also plays a fundamental role in the **vascular and renal system, and the urinary bladder** among others. These systems are permanently subjected to mechanical forces, such as shear stress and pressure, and impaired handling of these stimuli in these systems can cause severe pathologies. Many studies have focused on the molecular mechanisms of mechanical stretch in visceral

systems and thus, this issue will not be addressed in this chapter (for reviews see Haga et al. 2007; Lehoux et al. 2006; Li and Xu 2007; Orr et al. 2006; Shyu 2009; Hahn and Schwartz 2009; Weinbaum et al. 2010). Mechano-gated ion channels play a key physiological role in the visceral system including Piezo1, that plays a critical role in endothelial cell mechanotransduction (Ranade et al. 2014), and the TRP channels. This family of channels that are expressed in different cell types within the heart, kidney and bladder we will now centre out attention on.

7.3 Mechanosensory TRP Channels

The channel proteins that belong to the TRP family appear to mediate many forms of sensory perception, the reason why they are the focal point of the reviews in this book. There is some controversy as to whether mechanosensitive TRP channels are activated directly or if they are specifically involved in mechanical signalling. Moreover, piezo2 was recently shown to be required for Merkel cell mechanotransduction. Still, there is strong genetic evidence that a variety of TRP channels contribute to mechanosensation (Table 7.1). This includes evidence from loss-of-

Table 7.1 Mechanosensory channels

Molecular identity	Family	Organism	Mechanosensitivity
TRPA1	TRPA	Mammals, worm, fly	Pressure, stretch, osmolarity & touch
TRPC1	TRPC	Mammals/*Xenopus*	Touch, stretch, osmolarity & shear stress
TRPC3	TRPC	Mammals	Gentle touch & audition
TRPC5	TRPC	Mammals	Osmolarity & pressure
TRPC6	TRPC	Mammals	Osmolarity, pressure, touch & audition
TRPV1	TRPV	Mammals	Stretch & osmolarity
TRPV2	TRPV	Mammals	Stretch & osmolarity
TRPV4	TRPV	Mammals	Noxious pressure, shear stress, osmolarity & stretch
TRPM3	TRPM	Mammals	Osmolarity
TRPM4	TRPM	Mammals	Osmolarity
TRPM7	TRPM	Mammals	Osmolarity
TRPN1	TRPN	Fly, fish & amphibian	Touch & audition
OSM-9/OCR-2	TRPV	Worm	Osmolarity, audition & touch
TRPP2	TRPP	Mammals, worm, fly	Pressure, osmolarity & shear force
TRPML3	TRPM	Mammals, worm, fly	Audition

function mutations in organisms ranging from invertebrates to zebra fish, and from mice and humans (Christensen and Corey 2007; Eijkelkamp et al. 2013) .

7.3.1 TRPA1

The ankyrin-repeat channel TRPA1 is strongly conserved in the animal kingdom and it is found in many vertebrates and invertebrates. The *C elegans* TRPA1 is activated by mechanical stimulus, suggesting a role for TRPA1 in mechanosensation (Kindt et al. 2007). Indeed, TRPA1 is required for normal responses to nose-touch, and for a specific foraging behaviour of the worm in the presence of food that consists of high frequency and local exploratory movements of the head. In *Drosophila* larvae that lack *Painless*, the invertebrate orthologue of TRPA1, mechanical nociception is dampened (Tracey et al. 2003). It has been suggested that distinct isoforms of *painless* are responsible for specific sensory modalities that require its function. Moreover, the function of *painless* in noxious mechanosensation is independent of its ankyrin repeats as this phenotype is reverted after the introduction of a short isoform of the *painless* gene in mutant flies (Hwang et al. 2012).

In mammals, TRPA1 is expressed in sensory neurons of the dorsal root, trigeminal and nodose ganglia, and in many organs and tissues, including the brain, inner ear, smooth and skeletal muscle, and pancreas (Nilius et al. 2012). TRPA1 was initially a strong candidate to act as the hair cell transduction channel in vertebrates (Corey et al. 2004) on the basis of three main observations: (i) TRPA1 is expressed in the hair cells of both the cochlea and the vestibular system; (ii) TRPA1 messenger RNA transcripts appear on embryonic day 17, coinciding with the onset of mechanotransduction; and most importantly, (iii) disruption of TRPA1 expression in zebra fish with morpholino oligonucleotides, and in mice with small-interference RNA, strongly inhibits hair cell mechanotransduction. TRPA1 was also found to be expressed in the free nerve endings and in the bundles of stereocilia, leading to the proposal that it fulfils a role in both nociceptive and auditory cell function (Nagata et al. 2005). However, subsequent studies in zebrafish larvae lacking the orthologues of mammalian TRPA1 exhibited normal mechanosensory hair cell function (Prober et al. 2008). In addition, hearing and vestibular function is normal in two different TRPA1$^{-/-}$ mouse lines (Kwan et al. 2006; Bautista et al. 2006), although these two lines have a conflicting nociceptive responses. One of these lines exhibited pain hypersensitivity (Kwan et al. 2006), TRPA1$^{-/-}$ mice displaying a higher mechanosensory pain threshold than their TRPA1$^{+/+}$ littermates as well as a weaker response to supra-threshold stimuli. By contrast, no difference in the mechanical threshold was detected between TRPA1$^{-/-}$ and wild type animals in the other line (Bautista et al. 2006).

More evidence of a role for TRPA1 in sensing mechanical stimuli has been obtained through recordings in the skin-nerve preparation from TRPA1$^{-/-}$ (Kwan et al. 2009). In the absence of TRPA1 both C and AM fibre nociceptors had a lower firing rate at all force intensities and for noxious force, respectively. TRPA1 was

expressed in many medium to large DRG somata and on large-calibre axons associated to Aβ-fibres, and hence it was concluded that TRPA1 reduced the slow adaptation to sustained force in Aβ-fibres but increased the firing of D-hair and RA Aβ-fibres (Kwan et al. 2009). Moreover, pharmacological inhibition of TRPA1 using the selective antagonist HC-030031 and genetic ablation has shown that cutaneous C fibres require TRPA1 to respond to noxious stimulus evoked by formalin application, as well as to high intensity mechanical forces. However, HC-030031 had no effect on mechanical firing in nociceptive Aδ fibres (Kerstein et al. 2009).

The contribution of TRPA1 to mechanical sensitivity was also assessed by studying the mechanically-gated currents in cultured DRG neurons induced by applying an electrically driven mechanical probe to the cell membrane. TRPA1 deletion in knockout mice significantly reduces the maximum IA currents in small-diameter neurons, producing no effect on the RA and SA currents in small and large neurons. Moreover, the inhibitor HC-030031 significantly decreases IA current amplitude in wild type mice activated by the TRPA1 agonist AITC (Brierley et al. 2011). Similarly, SA currents were absent in IB4 negative neurons from TRPA1$^{-/-}$ mice and the amplitude of RA and IA currents in IB4 positive neurons from TRPA1$^{-/-}$ mice was reduced by over the 60 %. Moreover, HC-030031 was only seen to dampen SA currents wild type mice (Vilceanu and Stucky 2010). Together, it seems that TRPA1 mainly participates in SA and IA currents.

So far, direct evidence of TRPA1 activation by mechanical forces has only been obtained in HEK cells transiently expressing TRPA1, and only in response to a hyperosmotic solution but not to a hypoosmotic solution (Zhang et al. 2008). By contrast, TRPA1 does contribute to the Ca^{2+}influx evoked by hypoosmotic stimulation-induced plasma membrane stretching in Merkel cells from hamster buccal mucosa (Soya et al. 2014). TRPA1 also plays a role in the mechanical hyperalgesia and allodynia induced by the gasotransmitter hydrogen sulphide in mice (Okubo et al. 2012) and in the mechanical hyperalgesia caused by a deep-tissue incision in the rat hind paw (Wei et al. 2012). Mechanical hyperalgesia induced by Freund's adjuvant in wild type mice was strongly reduced by blocking TRPA1 with the small molecule, AP18. However, the fact that AP18 does not prevent Freund's adjuvant-induced mechanical hyperalgesia in TRPA1$^{-/-}$ mice may suggest that TRPA1 is involved in the maintenance of mechanical hyperalgesia rather than its generation (Petrus et al. 2007).

Several studies point to a role of TRPA1 in visceral mechanotransduction. TRPA1 is expressed in the rodent and human bladder, and activation of TRPA1 by agonists or changes in its expression is associated with bladder dysfunction (Du et al. 2007, 2008; Streng et al. 2008). A role for TRPA1 in noxious colonic distension was also reported (Brierley et al. 2009).

Together, these results suggest that TRPA1 plays a role in mechanosensation although it remains to be determined whether it acts as a mechanical sensor or if it indirectly participates in mechanosensation by amplifying or modulating the signal from the transduction channel.

7.3.2 TRPC

Examining the mechanosensitivity of TRP channels in heterologous system has provided conflicting data in some instances. This is certainly the case of **TRPC1** which was first considered to be a mechanosensitive non-selective cation channel (MscCa) given that application of positive and negative pressure resulted in a large increase in current that can be attributed to MscCa in *Xenopus* oocytes and in CHO-K1 cells transfected with human TRPC1 (Maroto et al. 2005). However, a study published 3 years later showed the difficulties of testing mechanosensitivity in heterologous systems due to the background mechanical activity observed in cell lines (Gottlieb et al. 2008).

To date, the only proven role of TRPC1 in mechanosensation was described by studying the recordings of the afferent terminals that innervate the hairy skin of TRPC1$^{-/-}$ mice (Garrison et al. 2012). In these animals there was an ~40% reduction in action potential firing of SA-Aβ fibres in response to forces at intensities lower than 4–200 mN yet not in the RA-Aβ fibres. A reduction of 50% in the discharges was observed in the D-hair Aδ fibres, whereas C-fibres and nociceptive AM fibres innervating hairy skin responded normally to noxious mechanical stimulation in TRPC1$^{-/-}$ mice, meaning that TRPC1 does not play significant role in cutaneous mechanical nociception. However, in a behavioural light-touch test a significant decrease in mechanical sensitivity was evident in TRPC1 deficient mice compared to wild type mice (Garrison et al. 2012). More recent findings indicate a reduced static firing rate of SA-Aδ fibres and a moderate decrease in the behavioural response to light touch in an epidermal piezo2 knockout mice (Maksimovic et al. 2014). These latter data might suggest that TRPC1 and piezo2 mediate mechanotransduction in Merkel cells.

Within the cardiovascular system, pressure-induced constriction of cerebral arteries in smooth muscle cells is unaffected in TRPC1 knock-out mice, indicating that TRPC1 is not an obligatory component of stretch-activated ion channel complexes in vascular smooth muscle (Dietrich et al. 2007).

TRPC3 has been implicated in the kinetics of mechanical activated currents, given that the proportion of sensory neurons that display IA currents increased in TRPC3$^{-/-}$ mice while those responding with RA currents decreased (Quick et al. 2012). Interestingly, TRPC3 was also attributed a role in the detection of light touch when it forms a complex with TRPC6. Indeed, there were deficits in innocuous mechanosensation in the TRPC3 and TRPC6double knockout mice but not in the single KO mice. There was also a significant decrease in the proportion of neurons displaying RA currents in these double KO animals, and elimination of TRPC3 and TRPC6 also produced hearing deficits (Quick et al. 2012).

Mechanical activation of **TRPC6** has been addressed, and it was reported that osmotic and pressure-induced membrane stretch directly activates the TRPC6 channel expressed in heterologous systems. The fact that TRPC6 is strongly expressed in vascular smooth muscle cells made it a candidate mechanotransducer in smooth muscle (Spassova et al. 2006). However, later studies demonstrated the activation

of TRPC6 through a Gq/11-coupled protein receptor (Schnitzler et al. 2008; Inoue et al. 2009; Sharif-Naeini et al. 2010). TRPC6 has also been involved in kidney regulation via podocin modulation, a protein involved in the function of the filtration barrier in the mammalian kidney. However, the implication of podocin in mechanosensation is in any case speculative, such that the participation of TRPC6 and other members of the TRPC subfamily is probably indirect (Huber et al. 2006).

TRPC5 has been proposed to act as a direct sensor of lysophospholipids (Flemming et al. 2006), suggesting that this channel is sensitive to the structure of the lipid bilayer.We characterized the activation of **TRPC5** in response to hypoosmotic stimulation and positive pressure in a heterologous system expressing TRPC5. This activation is blocked by the tarantula peptide GsMTx-4, a specific inhibitor of stretch activated channels, and it is independent of PLC activation (Gomis et al. 2008). Interestingly TRPC5 is also activated by applying force to the cell's surface via a glass pipette controlled by a piezo-electric system, provoking currents with RA kinetics (unpublished data). TRPC5 channels are expressed in trigeminal and DRG neurons, suggesting a possible role in mechanotransduction, although their physiological role in mechanotransduction and osmotic regulation remains to be clarified.

7.3.3 TRPV

Several TRPV channels have been described to be sensitive to mechanical stimulus or volume/osmotic change. **TRPV1** is required for osmosensory transduction in the organumvasculosum lamina terminalis, a primary osmosensor nucleus in the brain (Ciura and Bourque 2006; Ciura et al. 2011). Although controversial, the TRPV1 channel has been implicated in mechanical hypersensitivity as a consequence of inflammation, heat injury or nerve ligation (Honore et al. 2005; Bolcskei et al. 2005). The reason for the discrepancies observed is not clear but it is possible that TRPV1 may be involved in cutanous mechanical hyperalgesia through an indirect mechanism rather than as a direct noxoius mechanical sensor. The involvement of TRPV1 in mechanical hyperalgesia is more obvious in deeper tissues than in cutaneous pain.

TRPV2 is expressed in mesensteric and basilar arterial myocytes and it is activated by membrane stretch in TRPV2-CHO cells. Therefore, it would seem that TRPV2 can function as a stretch-activated channel in vascular smooth muscle (Muraki et al. 2003), although studies in intact arteries are still needed to confirm the physiological role of TRPV2. Studies in somatosensory systems indicated that TRPV2 is not essential in mechanical nociception (Park et al. 2011).

TRPV4 was first reported as an osmotically activated channel over a decade ago, when it was shown that the osmotic stimulus activates the TRPV4 (previously known as VR-OAC or OTRPC4) expressed in HEK and in CHO cells (Liedtke et al. 2000; Strotmann et al. 2000; Wissenbach et al. 2000). However, a subsequent study failed to find significant activation of TRPV4 expressed in CHO cells by hy-

poosmotic stimulation (Suzuki et al. 2003), while the activation of TRPV4 by cell swelling was seen to occur through the PLA2-dependent formation of arachidonic acid and its subsequent metabolism to 5',6'-epoxyeicosatrienoic acid (Vriens et al. 2004). The contribution of TRPV4 *in vivo* was studied by expressing the mammalian TRPV4 in *C elegans* sensory neurons and using TRPV4$^{-/-}$ mice. TRPV4 is required for nose touch avoidance in *C elegans* (Liedtke et al. 2003) and its loss markedly reduced the sensitivity of the mouse tail to pressure (Suzuki et al. 2003). Hypotonic solutions activate 54% of C-fibres in rats and the effect of hypoosmotic stimulation is more effective in inflammation states, suggesting that TRPV4 plays an important role in mechanical hyperalgesia (Alessandri-Haber et al. 2003; Alessandri-Haber et al. 2006). Moreover, proteases generated during inflammation cause mechanical hyperalgesia through TRPV4 sensitisation (Grant et al. 2007). Interestingly, interactions between different TRP channels, such as TRPC1, TRPC6 and TRPV4 contribute to the development of mechanical hyperalgesia, although they may fulfil different roles (Alessandri-Haber et al. 2009).

TRPV4 is expressed in hair cells and thus, the role of TRPV4 in the transduction of acoustic stimuli has also been studied. The cochlea of TRPV4$^{-/-}$ mice has a normal morphology but these animals develop late-onset hearing loss, which is similar to the autosomal dominant non-syndromic hearing loss in humans, and loss of TRPV4 makes the cochlea vulnerable to acoustic injury (Tabuchi et al. 2005). There is also evidence of a possible role for TRPV4 in skeletal muscle fibres. Recordings of single channel activity showed that hypoosmotic solutions activate mechanosensory channels and the absence of mechanosensory activity in the muscle of TRPV4$^{-/-}$ mice (Ho et al. 2012). However, the implication of other TRP channels forming heteromeric proteins with TRPV4 cannot be ruled out.

7.3.4 TRPM

Three members of the TRPM subfamily have been implicated in osmosensation: the **TRPM3** channel that is expressed in the odontoblast (Son et al. 2009) and kidney (Grimm et al. 2003); **TRPM4** in blood vessels (Morita et al. 2007) and that has been implicated in cerebral blood flow regulation (Reading and Brayden 2007); and **TRPM7** that is expressed in human epithelial HeLa cells and is thought to be involved in regulation associated with a volume decrease (Numata et al. 2007). Although these data imply an inherent stretch or mechanosensitivity of these channels, further studies are necessary to define the functional role of these channels in mechanotransduction.

7.3.5 TRPN1

The *nompC* gene encodes the **TRPN1** channel in *Drosophila melanogaster* and it is involved in touch, hearing and proprioception. Mutations, in this gene result in de-

ficiencies in bristle mechanotransduction (Kernan et al. 1994; Walker et al. 2000). However, while TRPN1 is an excellent candidate mechanotransduction channel in flies and fish, *nompC* homologues have not been found in the mammalian genome (Sidi et al. 2003; Shin et al. 2005; Li et al. 2006).

7.4 Concluding Remarks

Since their discovery three decades ago, TRP channels have been shown to participate in a number of different sensory processes. Still, our understanding of the functional roles of this ion channel superfamily is limited in comparison to what we know about those of ligand- and voltage-activated channels. Progress included the discovery of the involvement of some of the TRP channels in the detection of mechanical forces, although as discussed previously in this chapter, the activation mechanisms and functional significance of TRP channel's contribution to mechanotransduction is still under discussion. This is not surprising, considering that, as occurs in other cell types, diverse members of the TRP superfamily responding to mechanical energy are expressed by sensory neurons. Moreover, TRP proteins may interact forming heteromultimeric complexes. The expected functional redundancy of TRP channels participating in mechanosensation complicates the definition of the individual functional role of each channel in mechanical stimulus detection.

The identification of the molecules and/or transduction mechanisms that allow sensory neurons to diferentiate stimuli of low and high intensity *in vivo* is still an important unanswered question in mechanotransduction. It is possible that non-nociceptive and nociceptive neurons express different ion channels, or the same channels but with different density.

Other factors aside from neuron sensitivity may also determine its ability to detect stimuli of different intensity, as for example a filtering of the stimulus by perireceptor structures, which could distort the efficiency of final transmission of mechanical force to the channel.

This chapter has offered a brief overview of the potential contribution of various members of the TRP channel superfamily to mechanical transduction in the somatosensory system, which leads ultimately to mechanosensation. It is evident that further work is required to fully understand the molecular and cellular processes underlying the different modalities of mechanosensation evoked by activation of the various functional classes of mechanosensitive cells, and the contribution to these processes of TRP channels.

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References

Abraira VE, Ginty DD (2013) The sensory neurons of touch. Neuron 79:618–639

Albert JT, Nadrowski B, Gopfert MC (2007) Mechanical signatures of transducer gating in the *Drosophila* ear. Curr Biol 17:1000–1006

Alessandri-Haber N, Yeh JJ, Boyd AE, Parada CA, Chen X, Reichling DB, Levine JD (2003) Hypotonicity induces TRPV4-mediated nociception in rat. Neuron 39:497–511

Alessandri-Haber N, Dina OA, Joseph EK, Reichling D, Levine JD (2006) A transient receptor potential vanilloid 4-dependent mechanism of hyperalgesia is engaged by concerted action of inflammatory mediators. J Neurosci 26:3864–3874

Alessandri-Haber N, Dina OA, Chen X, Levine JD (2009) TRPC1 and TRPC6 channels cooperate with TRPV4 to mediate mechanical hyperalgesia and nociceptor sensitization. J Neurosci 29:6217–6228

Arshavsky VY, Lamb TD, Pugh EN Jr (2002) G proteins and phototransduction. Annu Rev Physiol 64:153–187

Bautista DM, Jordt SE, Nikai T, Tsuruda PR, Read AJ, Poblete J, Yamoah EN, Basbaum AI, Julius D (2006) TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. Cell 124:1269–1282

Block SM (1992) Biophysical principles of sensory transduction. Soc Gen Physiol Ser 47:1–17

Bolcskei K, Helyes Z, Szabo A, Sandor K, Elekes K, Nemeth J, Almasi R, Pinter E, Petho G, Szolcsanyi J (2005) Investigation of the role of TRPV1 receptors in acute and chronic nociceptive processes using gene-deficient mice. Pain 117:368–376

Brierley SM, Hughes PA, Page AJ, Kwan KY, Martin CM, O'Donnell TA, Cooper NJ, Harrington AM, Adam B, Liebregts T, Holtmann G, Corey DP, Rychkov GY, Blackshaw LA (2009) The ion channel TRPA1 is required for normal mechanosensation and is modulated by algesic stimuli. Gastroenterology 137:2084–2095

Brierley SM, Castro J, Harrington AM, Hughes PA, Page AJ, Rychkov GY, Blackshaw LA (2011) TRPA1 contributes to specific mechanically activated currents and sensory neuron mechanical hypersensitivity. J Physiol 589:3575–3593

Chalfie M (2009) Neurosensory mechanotransduction. Nat Rev Mol Cell Biol 10:44–52

Chalfie M, Thomson JN (1982) Structural and functional diversity in the neuronal microtubules of *Caenorhabditis elegans*. J Cell Biol 93:15–23

Chalfie M, Sulston JE, White JG, Southgate E, Thomson JN, Brenner S (1985) The neural circuit for touch sensitivity in *Caenorhabditis elegans*. J Neurosci 5:956–964

Christensen AP, Corey DP (2007) TRP channels in mechanosensation: direct or indirect activation? Nat Rev Neurosci 8:510–521

Chung YD, Zhu J, Han Y, Kernan MJ (2001) nompA encodes a PNS-specific, ZP domain protein required to connect mechanosensory dendrites to sensory structures. Neuron 29:415–428

Ciura S, Bourque CW (2006) Transient receptor potential vanilloid 1 is required for intrinsic osmoreception in organum vasculosum lamina terminalis neurons and for normal thirst responses to systemic hyperosmolality. J Neurosci 26:9069–9075

Ciura S, Liedtke W, Bourque CW (2011) Hypertonicity sensing in organumvasculosum lamina terminalis neurons: a mechanical process involving TRPV1 but not TRPV4. J Neurosci 31:14669–14676

Corey DP, Hudspeth AJ (1979) Response latency of vertebrate hair cells. Biophys J 26:499–506

Corey DP, Garcia-Anoveros J, Holt JR, Kwan KY, Lin SY, Vollrath MA, Amalfitano A, Cheung EL, Derfler BH, Duggan A, Geleoc GS, Gray PA, Hoffman MP, Rehm HL, Tamasauskas D, Zhang DS (2004) TRPA1 is a candidate for the mechanosensitive transduction channel of vertebrate hair cells. Nature 432:723–730

Coste B, Mathur J, Schmidt M, Earley TJ, Ranade S, Petrus MJ, Dubin AE, Patapoutian A (2010) Piezo1 and Piezo2 are essential components of distinct mechanically activated cation channels. Science 330:55–60

Coste B, Xiao B, Santos JS, Syeda R, Grandl J, Spencer KS, Kim SE, Schmidt M, Mathur J, Dubin AE, Montal M, Patapoutian A (2012) Piezo proteins are pore-forming subunits of mechanically activated channels. Nature 483:176–181

Dietrich A, Kalwa H, Storch U, Schnitzler M, Salanova B, Pinkenburg O, Dubrovska G, Essin K, Gollasch M, Birnbaumer L, Gudermann T (2007) Pressure-induced and store-operated cation influx in vascular smooth muscle cells is independent of TRPC1. Pflugers Arch 455:465–477

Drew LJ, Wood JN, Cesare P (2002) Distinct mechanosensitive properties of capsaicin-sensitive and -insensitive sensory neurons. J Neurosci 22:RC228

Drew LJ, Rohrer DK, Price MP, Blaver KE, Cockayne DA, Cesare P, Wood JN (2004) Acid-sensing ion channels ASIC2 and ASIC3 do not contribute to mechanically activated currents in mammalian sensory neurones. J Physiol 556:691–710

Du S, Araki I, Yoshiyama M, Nomura T, Takeda M (2007) Transient receptor potential channel A1 involved in sensory transduction of rat urinary bladder through C-fiber pathway. Urology 70:826–831

Du S, Araki I, Kobayashi H, Zakoji H, Sawada N, Takeda M (2008) Differential expression profile of cold (TRPA1) and cool (TRPM8) receptors in human urogenital organs. Urology 72:450–455

Eberl DF (1999) Feeling the vibes: chordotonal mechanisms in insect hearing. Curr Opin Neurobiol 9:389–393

Eberl DF, Hardy RW, Kernan MJ (2000) Genetically similar transduction mechanisms for touch and hearing in *Drosophila*. J Neurosci 20:5981–5988

Eijkelkamp N, Quick K, Wood JN (2013) Transient receptor potential channels and mechanosensation. Annu Rev Neurosci 36:519–546

Ernstrom GG, Chalfie M (2002) Genetics of sensory mechanotransduction. Annu Rev Genet 36:411–453

Flemming PK, Dedman AM, Xu SZ, Li J, Zeng F, Naylor J, Benham CD, Bateson AN, Muraki K, Beech DJ (2006) Sensing of lysophospholipids by TRPC5 calcium channel. J Biol Chem 281:4977–4982

Garcia-Añoveros J, Corey DP (1997) The molecules of mechanosensation. Annu Rev Neurosci 20:567–594

Garrison SR, Dietrich A, Stucky CL (2012) TRPC1 contributes to light-touch sensation and mechanical responses in low-threshold cutaneous sensory neurons. J Neurophysiol 107:913–922

Geffeney SL, Goodman MB (2012) How we feel: ion channel partnerships that detect mechanical inputs and give rise to touch and pain perception. Neuron 74:609–619

Gillespie PG, Muller U (2009) Mechanotransduction by hair cells: models, molecules, and mechanisms. Cell 139:33–44

Gillespie PG, Walker RG (2001) Molecular basis of mechanosensory transduction. Nature 413:194–202

Gomis A, Soriano S, Belmonte C, Viana F (2008) Hypoosmotic- and pressure-induced membrane stretch activate TRPC5 channels. J Physiol 586:5633–5649

Gong Z, Son W, Chung YD, Kim J, Shin DW, McClung CA, Lee Y, Lee HW, Chang DJ, Kaang BK, Cho H, Oh U, Hirsh J, Kernan MJ, Kim C (2004) Two interdependent TRPV channel subunits, inactive and Nanchung, mediate hearing in *Drosophila*. J Neurosci 24:9059–9066

Gottlieb P, Folgering J, Maroto R, Raso A, Wood TG, Kurosky A, Bowman C, Bichet D, Patel A, Sachs F, Martinac B, Hamill OP, Honore E (2008) Revisiting TRPC1 and TRPC6 mechanosensitivity. Pflugers Arch 455:1097–1103

Grant AD, Cottrell GS, Amadesi S, Trevisani M, Nicoletti P, Materazzi S, Altier C, Cenac N, Zamponi GW, Bautista-Cruz F, Lopez CB, Joseph EK, Levine JD, Liedtke W, Vanner S, Vergnolle N, Geppetti P, Bunnett NW (2007) Protease-activated receptor 2 sensitizes the transient receptor potential vanilloid 4 ion channel to cause mechanical hyperalgesia in mice. J Physiol 578:715–733

Grimm C, Kraft R, Sauerbruch S, Schultz G, Harteneck C (2003) Molecular and functional characterization of the melastatin-related cation channel TRPM3. J Biol Chem 278:21493–21501

Grueber WB, Jan LY, Jan YN (2002) Tiling of the Drosophila epidermis by multidendritic sensory neurons. Development 129:2867–2878
Haga JH, Li YS, Chien S (2007) Molecular basis of the effects of mechanical stretch on vascular smooth muscle cells. J Biomech 40:947–960
Hahn C, Schwartz MA (2009) Mechanotransduction in vascular physiology and atherogenesis. Nat Rev Mol Cell Biol 10:53–62
Hamill OP, Martinac B (2001) Molecular basis of mechanotransduction in living cells. Physiol Rev 81:685–740
Hao J, Delmas P (2010) Multiple desensitization mechanisms of mechanotransducer channels shape firing of mechanosensory neurons. J Neurosci 30:13384–13395
Hardie RC (2014) Photosensitive TRPs. Handb Exp Pharmacol. 223:795–826
Hardie RC, Postma M (2008) Phototransduction in microvillar photoreceptors of *Drosophila* and other invertebrates. In Masland R., Albright TD (eds) The senses: a comprehensive reference, Vol 1. Academic Press, Oxford, pp 77–130
Ho TC, Horn NA, Huynh T, Kelava L, Lansman JB (2012) Evidence TRPV4 contributes to mechanosensitive ion channels in mouse skeletal muscle fibers. Channels (Austin) 6:246–254
Holt JR, Pan B, Koussa MA, Asai Y (2014) TMC function in hair cell transduction. Hear Res 311:17-24
Honore P, Wismer CT, Mikusa J, Zhu CZ, Zhong C, Gauvin DM, Gomtsyan A, El KR, Lee CH, Marsh K, Sullivan JP, Faltynek CR, Jarvis MF (2005) A-425619 [1-isoquinolin-5-yl-3-(4-trifluoromethyl-benzyl)-urea], a novel transient receptor potential type V1 receptor antagonist, relieves pathophysiological pain associated with inflammation and tissue injury in rats. J Pharmacol Exp Ther 314:410–421
Hrabe de Angelis MH et al (2000) Genome-wide, large-scale production of mutant mice by ENU mutagenesis. Nat Genet 25:444–447
Hu J, Lewin GR (2006) Mechanosensitive currents in the neurites of cultured mouse sensory neurones. J Physiol 577:815–828
Huber TB, Schermer B, Muller RU, Hohne M, Bartram M, Calixto A, Hagmann H, Reinhardt C, Koos F, Kunzelmann K, Shirokova E, Krautwurst D, Harteneck C, Simons M, Pavenstadt H, Kerjaschki D, Thiele C, Walz G, Chalfie M, Benzing T (2006) Podocin and MEC-2 bind cholesterol to regulate the activity of associated ion channels. Proc Natl Acad Sci U S A 103:17079–17086
Hwang RY, Stearns NA, Tracey WD (2012) Theankyrin repeat domain of the TRPA protein painless is important for thermal nociception but not mechanical nociception. PLoS One 7:e30090
Ikeda R, Cha M, Ling J, Jia Z, Coyle D, Gu JG (2014) Merkel cells transduce and encode tactile stimuli to drive aβ-afferent impulses. Cell 157:664–675
Inoue R, Jensen LJ, Jian Z, Shi J, Hai L, Lurie AI, Henriksen FH, Salomonsson M, Morita H, Kawarabayashi Y, Mori M, Mori Y, Ito Y (2009) Synergistic activation of vascular TRPC6 channel by receptor and mechanical stimulation via phospholipase C/diacylglycerol and phospholipase A2/omega-hydroxylase/20-HETE pathways. Circ Res 104:1399–1409
Katz B (1950) Depolarization of sensory terminals and the initiation of impulses in the muscle spindle. J Physiol 111:261–282
Kawashima Y, Geleoc GS, Kurima K, Labay V, Lelli A, Asai Y, Makishima T, Wu DK, la Santina CC, Holt JR, Griffith AJ (2011) Mechanotransduction in mouse inner ear hair cells requires transmembrane channel-like genes. J Clin Invest 121:4796–4809
Keil TA (1997) Functional morphology of insect mechanoreceptors. Microsc Res Tech 39:506–531
Kernan M, Zuker C (1995) Genetic approaches to mechanosensory transduction. Curr Opin Neurobiol 5:443–448
Kernan M, Cowan D, Zuker C (1994) Genetic dissection of mechanosensory transduction: mechanoreception-defective mutations of Drosophila. Neuron 12:1195–1206
Kerstein PC, del CD, Moran MM, Stucky CL (2009) Pharmacological blockade of TRPA1 inhibits mechanical firing in nociceptors. Mol Pain 5:19

Kim J, Chung YD, Park DY, Choi S, Shin DW, Soh H, Lee HW, Son W, Yim J, Park CS, Kernan MJ, Kim C (2003) A TRPV family ion channel required for hearing in *Drosophila*. Nature 424:81–84

Kim SE, Coste B, Chadha A, Cook B, Patapoutian A (2012) The role of Drosophila Piezo in mechanical nociception. Nature 483:209–212

Kim KX, Beurg M, Hackney CM, Furness DN, Mahendrasingam S, Fettiplace R (2013) The role of transmembrane channel-like proteins in the operation of hair cell mechanotransducer channels. J Gen Physiol 142:493–505

Kindt KS, Viswanath V, Macpherson L, Quast K, Hu H, Patapoutian A, Schafer WR (2007) *Caenorhabditis elegans* TRPA-1 functions in mechanosensation. Nat Neurosci 10:568–577

Kinnamon SC (2012) Taste receptor signalling—from tongues to lungs. Acta Physiol (Oxf) 204:158–168

Kung C (2005) A possible unifying principle for mechanosensation. Nature 436:647–654

Kwan KY, Allchorne AJ, Vollrath MA, Christensen AP, Zhang DS, Woolf CJ, Corey DP (2006) TRPA1 contributes to cold, mechanical, and chemical nociception but is not essential for hair-cell transduction. Neuron 50:277–289

Kwan KY, Glazer JM, Corey DP, Rice FL, Stucky CL (2009) TRPA1 modulates mechanotransduction in cutaneous sensory neurons. J Neurosci 29:4808–4819

Lehoux S, Castier Y, Tedgui A (2006) Molecular mechanisms of the vascular responses to haemodynamic forces. J Intern Med 259:381–392

LeMasurier M, Gillespie PG (2005) Hair-cell mechanotransduction and cochlear amplification. Neuron 48:403–415

Lewin GR, Moshourab R (2004) Mechanosensation and pain. J Neurobiol 61:30–44

Li C, Xu Q (2007) Mechanical stress-initiated signal transduction in vascular smooth muscle cells in vitro and in vivo. Cell Signal 19:881–891

Li W, Feng Z, Sternberg PW, Xu XZ (2006) A *C. elegans* stretch receptor neuron revealed by a mechanosensitive TRP channel homologue. Nature 440:684–687

Liedtke W, Choe Y, Marti-Renom MA, Bell AM, Denis CS, Sali A, Hudspeth AJ, Friedman JM, Heller S (2000) Vanilloid receptor-related osmotically activated channel (VR-OAC), a candidate vertebrate osmoreceptor. Cell 103:525–535

Liedtke W, Tobin DM, Bargmann CI, Friedman JM (2003) Mammalian TRPV4 (VR-OAC) directs behavioral responses to osmotic and mechanical stimuli in *Caenorhabditis elegans*. Proc Natl Acad Sci U S A 100(Suppl. 2):14531–14536

Lingueglia E (2007) Acid-sensing ion channels in sensory perception. J Biol Chem 282:17325–17329

Lumpkin EA, Caterina MJ (2007) Mechanisms of sensory transduction in the skin. Nature 445:858–865

Lumpkin EA, Marshall KL, Nelson AM (2010) The cell biology of touch. J Cell Biol 191:237–248

Maksimovic S, Nakatani M, Baba Y, Nelson AM, Marshall KL, Wellnitz SA, Firozi P, Woo SH, Ranade S, Patapoutian A, Lumpkin EA (2014) Epidermal Merkel cells are mechanosensory cells that tune mammalian touch receptors. Nature 509:617–621

Marcotti W, Corns LF, Desmonds T, Kirkwood NK, Richardson GP, Kros CJ (2014) Transduction without tip links in Cochlear hair cells is mediated by ion channels with permeation properties distinct from those of the mechano-electrical transducer channel. J Neurosci 34:5505–5514

Maroto R, Raso A, Wood TG, Kurosky A, Martinac B, Hamill OP (2005) TRPC1 forms the stretch-activated cation channel in vertebrate cells. Nat Cell Biol 7:179–185

McCarter GC, Reichling DB, Levine JD (1999) Mechanical transduction by rat dorsal root ganglion neurons in vitro. Neurosci Lett 273:179–182

Morgan CP, Barr-Gillespie PG (2013) Mechanotransduction: the elusive hair cell transduction channel revealed? Curr Biol 23:R887–R890

Morita H, Honda A, Inoue R, Ito Y, Abe K, Nelson MT, Brayden JE (2007) Membrane stretch-induced activation of a TRPM4-like nonselective cation channel in cerebral artery myocytes. J Pharmacol Sci 103:417–426

Muraki K, Iwata Y, Katanosaka Y, Ito T, Ohya S, Shigekawa M, Imaizumi Y (2003) TRPV2 is a component of osmotically sensitive cation channels in murine aortic myocytes. Circ Res 93:829–838

Nagata K, Duggan A, Kumar G, Garcia-Anoveros J (2005) Nociceptor and hair cell transducer properties of TRPA1, a channel for pain and hearing. J Neurosci 25:4052–4061

Nilius B, Honore E (2012) Sensing pressure with ion channels. Trends Neurosci 35:477–486

Nilius B, Appendino G, Owsianik G (2012) The transient receptor potential channel TRPA1: from gene to pathophysiology. Pflugers Arch 464:425–458

Nolan PM et al (2000) A systematic, genome-wide, phenotype-driven mutagenesis programme for gene function studies in the mouse. Nat Genet 25:440–443

Numata T, Shimizu T, Okada Y (2007) TRPM7 is a stretch- and swelling-activated cation channel involved in volume regulation in human epithelial cells. Am J Physiol Cell Physiol 292:C460–C467

O'Hagan R, Chalfie M, Goodman MB (2005) The MEC-4 DEG/ENaC channel of *Caenorhabditis elegans* touch receptor neurons transduces mechanical signals. Nat Neurosci 8:43–50

Okubo K, Matsumura M, Kawaishi Y, Aoki Y, Matsunami M, Okawa Y, Sekiguchi F, Kawabata A (2012) Hydrogen sulfide-induced mechanical hyperalgesia and allodynia require activation of both Cav3.2 and TRPA1 channels in mice. Br J Pharmacol 166:1738–1743

Orr AW, Helmke BP, Blackman BR, Schwartz MA (2006) Mechanisms of mechanotransduction. Dev Cell 10:11–20

Pan B, Geleoc GS, Asai Y, Horwitz GC, Kurima K, Ishikawa K, Kawashima Y, Griffith AJ, Holt JR (2013) TMC1 and TMC2 are components of the mechanotransduction channel in hair cells of the mammalian inner ear. Neuron 79:504–515

Park U, Vastani N, Guan Y, Raja SN, Koltzenburg M, Caterina MJ (2011) TRP vanilloid 2 knock-out mice are susceptible to perinatal lethality but display normal thermal and mechanical nociception. J Neurosci 31:11425–11436

Pedersen SF, Nilius B (2007) Transient receptor potential channels in mechanosensing and cell volume regulation. Methods Enzymol 428:183–207

Petrus M, Peier AM, Bandell M, Hwang SW, Huynh T, Olney N, Jegla T, Patapoutian A (2007) A role of TRPA1 in mechanical hyperalgesia is revealed by pharmacological inhibition. Mol Pain 3:40

Prober DA, Zimmerman S, Myers BR, McDermott BM Jr, Kim SH, Caron S, Rihel J, Solnica-Krezel L, Julius D, Hudspeth AJ, Schier AF (2008) Zebrafish TRPA1 channels are required for chemosensation but not for thermosensation or mechanosensory hair cell function. J Neurosci 28:10102–10110

Quick K, Zhao J, Eijkelkamp N, Linley JE, Rugiero F, Cox JJ, Raouf R, Gringhuis M, Sexton JE, Abramowitz J, Taylor R, Forge A, Ashmore J, Kirkwood N, Kros CJ, Richardson GP, Freichel M, Flockerzi V, Birnbaumer L, Wood JN (2012) TRPC3 and TRPC6 are essential for normal mechanotransduction in subsets of sensory neurons and cochlear hair cells. Open Biol 2:120068

Ranade SS, Qiu Z, Woo SH, Hur SS, Murthy SE, Cahalan SM, Xu J, Mathur J, Bandell M, Coste B, Li YS, Chien S, Patapoutian A (2014) Piezo1, a mechanically activated ion channel, is required for vascular development in mice. Proc Natl Acad Sci U S A 28:10347–10352

Reading SA, Brayden JE (2007) Central role of TRPM4 channels in cerebral blood flow regulation. Stroke 38:2322–2328

Ronnett GV, Moon C (2002) G proteins and olfactory signal transduction. Annu Rev Physiol 64:189–222

Roza C, Puel JL, Kress M, Baron A, Diochot S, Lazdunski M, Waldmann R (2004) Knockout of the ASIC2 channel in mice does not impair cutaneous mechanosensation, visceral mechanonociception and hearing. J Physiol 558:659–669

Sachs F, Morris CE (1998) Mechanosensitive ion channels in nonspecialized cells. Rev Physiol Biochem Pharmacol 132:1–77

Schnitzler M, Storch U, Meibers S, Nurwakagari P, Breit A, Essin K, Gollasch M, Gudermann T (2008) Gq-coupled receptors as mechanosensors mediating myogenic vasoconstriction. EMBO J 27:3092–3103

Sharif-Naeini R, Folgering JH, Bichet D, Duprat F, Delmas P, Patel A, Honore E (2010) Sensing pressure in the cardiovascular system: Gq-coupled mechanoreceptors and TRP channels. J Mol Cell Cardiol 48:83–89

Shearer AE, Smith RJ (2012) Genetics: advances in genetic testing for deafness. Curr Opin Pediatr 24:679–686

Shepherd GM (1991) Sensory transduction: entering the mainstream of membrane signaling. Cell 67:845–851

Shin JB, Adams D, Paukert M, Siba M, Sidi S, Levin M, Gillespie PG, Grunder S (2005) *Xenopus* TRPN1 (NOMPC) localizes to microtubule-based cilia in epithelial cells, including inner-ear hair cells. Proc Natl Acad Sci U S A 102:12572–12577

Shyu KG (2009) Cellular and molecular effects of mechanical stretch on vascular cells and cardiac myocytes. Clin Sci (Lond) 116:377–389

Sidi S, Friedrich RW, Nicolson T (2003) NompC TRP channel required for vertebrate sensory hair cell mechanotransduction. Science 301:96–99

Son AR, Yang YM, Hong JH, Lee SI, Shibukawa Y, Shin DM (2009) Odontoblast TRP channels and thermo/mechanical transmission. J Dent Res 88:1014–1019

Soya M, Sato M, Sobhan U, Tsumura M, Ichinohe T, Tazaki M, Shibukawa Y (2014) Plasma membrane stretch activates transient receptor potential vanilloid and ankyrin channels in Merkel cells from hamster buccal mucosa. Cell Calcium 55:208-218

Spassova MA, Hewavitharana T, Xu W, Soboloff J, Gill DL (2006) A common mechanism underlies stretch activation and receptor activation of TRPC6 channels. Proc Natl Acad Sci U S A 103:16586–16591

Streng T, Axelsson HE, Hedlund P, Andersson DA, Jordt SE, Bevan S, Andersson KE, Hogestatt ED, Zygmunt PM (2008) Distribution and function of the hydrogen sulfide-sensitive TRPA1 ion channel in rat urinary bladder. Eur Urol 53:391–399

Strotmann R, Harteneck C, Nunnenmacher K, Schultz G, Plant TD (2000) OTRPC4, a nonselective cation channel that confers sensitivity to extracellular osmolarity. Nat Cell Biol 2:695–702

Sukharev S, Corey DP (2004) Mechanosensitive channels: multiplicity of families and gating paradigms. Sci STKE 2004:re4

Suzuki M, Mizuno A, Kodaira K, Imai M (2003) Impaired pressure sensation in mice lacking TRPV4. J Biol Chem 278:22664–22668

Tabuchi K, Suzuki M, Mizuno A, Hara A (2005) Hearing impairment in TRPV4 knockout mice. Neurosci Lett 382:304–308

Torre V, Ashmore JF, Lamb TD, Menini A (1995) Transduction and adaptation in sensory receptor cells. J Neurosci 15:7757–7768

Tracey WD Jr, Wilson RI, Laurent G, Benzer S (2003) painless, a *Drosophila* gene essential for nociception. Cell 113:261–273

Venkatachalam K, Montell C (2007) TRP channels. Annu Rev Biochem 76:387–417

Vilceanu D, Stucky CL (2010) TRPA1 mediates mechanical currents in the plasma membrane of mouse sensory neurons. PLoS One 5:e12177

Voets T, Talavera K, Owsianik G, Nilius B (2005) Sensing with TRP channels. Nat ChemBiol 1:85–92

Vriens J, Watanabe H, Janssens A, Droogmans G, Voets T, Nilius B (2004) Cell swelling, heat, and chemical agonists use distinct pathways for the activation of the cation channel TRPV4. Proc Natl Acad Sci U S A 101:396–401

Walker RG, Willingham AT, Zuker CS (2000) A Drosophila mechanosensory transduction channel. Science 287:2229–2234

Wei H, Karimaa M, Korjamo T, Koivisto A, Pertovaara A (2012) Transient receptor potential ankyrin 1 ion channel contributes to guarding pain and mechanical hypersensitivity in a rat model of postoperative pain. Anesthesiology 117:137–148

Weinbaum S, Duan Y, Satlin LM, Wang T, Weinstein AM (2010) Mechanotransduction in the renal tubule. Am J Physiol Renal Physiol 299:F1220–F1236

Wilson RI, Corey DP (2010) The force be with you: a mechanoreceptor channel in proprioception and touch. Neuron 67:349–351

Wissenbach U, Bodding M, Freichel M, Flockerzi V (2000) Trp12, a novel Trp related protein from kidney. FEBS Lett 485:127–134

Woo SH, Ranade S, Weyer AD, Dubin AE, Baba Y, Qiu Z, Petrus M, Miyamoto T, Reddy K, Lumpkin EA, Stucky CL, Patapoutian A (2014) Piezo2 is required for Merkel-cell mechanotransduction. Nature 509:622–626

Yan Z, Zhang W, He Y, Gorczyca D, Xiang Y, Cheng LE, Meltzer S, Jan LY, Jan YN (2013) Drosophila NOMPC is a mechanotransduction channel subunit for gentle-touch sensation. Nature 493:221–225

Zhang XF, Chen J, Faltynek CR, Moreland RB, Neelands TR (2008) Transient receptor potential A1 mediates an osmotically activated ion channel. Eur J Neurosci 27:605–611

Zhang W, Yan Z, Jan LY, Jan YN (2013) Sound response mediated by the TRP channels NOMPC, NANCHUNG, and INACTIVE in chordotonal organs of *Drosophila* larvae. Proc Natl Acad Sci U S A 110:13612–13617

Chapter 8
TRP Channels in the Sensation of Heat

Chun-Hsiang Tan and Peter A. McNaughton

Abstract Animals must sense temperature in the external environment in order to find ambient temperatures appropriate for different activities, such as hunting or sleeping, and to avoid even brief exposure to damaging extremes of temperature. They must also sense their internal bodily temperature in order to regulate it. Some members of the TRP channel family are activated by thermal stimuli, and are consequently named the thermoTRPs; thermally sensitive ion channels are also found in the potassium and chloride channel families. When thermoTRP channels are expressed in a sensory neuron, channel opening in response to heat leads to depolarization and generation of action potentials. It is still an open question which channels are important for sensation of thermal stimuli in the external environment, and channels involved in the sensation of internal bodily temperature for the purposes of thermoregulation are even less understood. In this chapter we review the properties of thermosensitive ion channels and their roles in thermosensation and thermoregulation, with an emphasis on TRP channels.

Keywords TRP channels · Heat transduction · Thermosensation · Thermoregulation

8.1 Introduction

Thermosensation and thermoregulation are crucial for the survival of animals, which must be able both to maintain their bodily temperature within a certain range in order to carry out their physiological activities normally, and to detect and

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avoid external noxious temperatures which could cause tissue injury. Cold-blooded animals (ectotherms or poikilotherms) rely mainly on the external temperature to maintain an appropriate internal temperature, while warm-blooded animals (endotherms or hometherms) create most of their bodily heat via internal metabolic processes, and must therefore possess thermal detection mechanisms of exquisite sensitivity in order to avoid damaging hyperthermia caused by overproduction of internal heat. Animals use various strategies to achieve the goal of thermoregulation. Both ectotherms and endotherms use behavioural thermoregulation, in which sensation of the ambient temperature initiates movement towards an appropriate thermal environment. In addition to behavioural thermoregulation, mammals have a more complex thermoregulatory system under the control of the autonomic nervous system, which includes thermogenesis from brown adipose tissue (BAT) and muscle, vasoconstriction or vasodilatation of the peripheral vasculature, and evaporative heat loss via sweating. While these output mechanisms are relatively well-understood, possible mechanisms by which external and internal temperatures are sensed were mysterious until the thermally sensitive ion channels were identified and cloned. In this chapter, a brief review of the roles of heat-sensitive ion channels in mammalian thermosensation and thermoregulation will be presented, with an emphasis on TRP channels.

8.2 Thermosensation and Thermoregulation in Mammals

8.2.1 Thermosensation

Thermosensory neurons in the dorsal root ganglia (DRG) and trigeminal ganglia (TG) are pseudounipolar neurons, with a peripheral thinly myelinated or unmyelinated neurite that ends as bare dendritic terminals extending into skin, muscle, or mucosa, and a central branch that enters the spinal cord or brain stem. Thermosensitive ion channels, which depolarize the neuron when activated and thus convert thermal stimuli into firing of action potentials, are expressed in the neuronal membrane. Action potentials are transmitted to the central branch of the neuron, and from there to the spinal cord or brainstem, where thermal information is processed by excitatory and inhibitory interneurons and then transmitted onward to the thalamus and then to the primary somatosensory cortex and insular cortex to facilitate perception and discrimination of cutaneous thermosensation (Craig 2002; Todd 2010; Martinez et al. 2013). Figure 8.1a illustrates the main pathway for conscious thermosensation.

8.2.2 Thermoregulation

The current concept holds that thermoregulation in mammals consists of three major components: a sensory afferent input component, a central integration/sensory

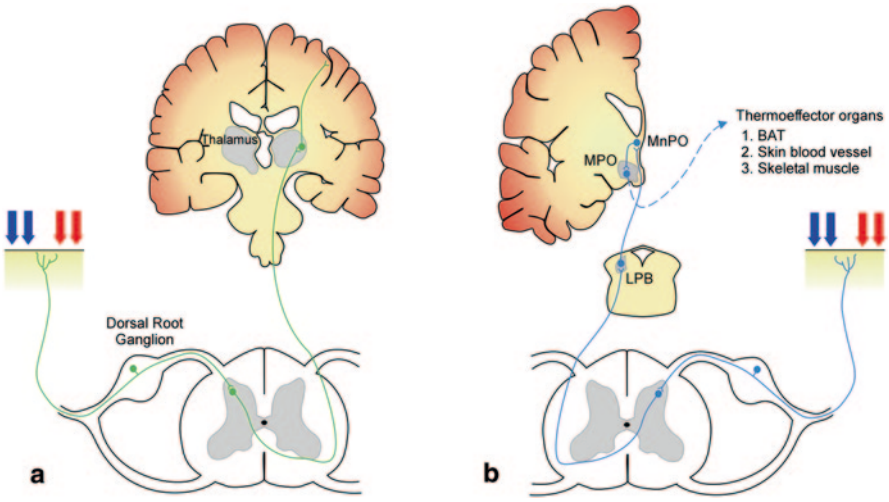

Fig. 8.1 Pathways for thermosensation and thermoregulation. **a** Nerve terminals of somatosensory neurons in the dorsal root ganglion receive external thermal stimuli, which result in generation of action potentials. The action potentials are transmitted by the somatosensory neuron and to dorsal horn neurons in the spinal cord. Through the spinothalamic tract, the information is transmitted to the thalamus, and further relayed to the somatosensory cortex for the perception of temperature, which can lead to behavioral thermoregulation. **b** Action potentials generated in somatosensory neurons are also transmitted via dorsal horn neurons to neurons in the lateral parabrachial nucleus (LPB). LPB neurons send information to interneurons in the median preoptic (MnPO) subnucleus, which is further relayed to warm-sensitive neurons in the medial preoptic (MPO) subnucleus for integration. Preoptic warm-sensitive neurons then control thermoeffector organs, which include brown adipose tissue (BAT), skin blood vessels, and skeletal muscles

component, and a motor efferent component. Figure 8.1b illustrates the proposed three components of thermoregulation (Morrison 2011). We discuss here the first two of these components, which are thought to contain the major sensory elements.

8.2.2.1 Sensory Afferent Input to Thermoregulation

There are two sensory afferent pathways important for thermoregulation. The first pathway is that used for thermosensation: DRG or TG neurons sense external warm or cold stimuli, transfer the temperature signal to secondary neurons in the spinothalamic or trigeminothalamic tract respectively, and the signal is then relayed to the cortex. Activation of the primary somatosensory cortex or insular cortex then triggers various voluntary thermoregulatory behaviors, as seen (for instance) when mice choose a preferred temperature in a thermal preference test or when humans turn on air conditioners (Flouris 2011). A good example is the initiation of warmth-seeking behavior in mice when TRPM8 is activated by menthol or cold; this behavior is reduced in TRPM8 knockout mice or by the co-application of a TRPM8 blocker (Tajino et al. 2011; Almeida et al. 2012).

A second sensory afferent pathway is initiated by the same peripheral thermosensory neurons but relays temperature signals to the pre-optic area of the hypothalamus (POAH). This pathway is crucial for controlling involuntary thermoregulation (including cold-activated vasoconstriction, sympathetic thermogenesis in BAT, and shivering thermogenesis in skeletal muscle; and heat-activated vasodilation and sweating). Cold and hot signals detected by DRG and TG neurons are transmitted through dorsal horn neurons to the external lateral subnucleus of the lateral parabrachial nucleus (LPBel) and the dorsal subnucleus of the lateral parabrachial nucleus (LPBd), respectively (Nakamura et al. 2008b; Nakamura et al. 2010). LPB neurons responsible for relaying cutaneous thermal signals then synapse on neurons in the median preoptic nucleus (MnPO), a subregion of the POAH (Nakamura et al. 2008a, b). Afferent pathways mediating cold and hot signals from the body surface to the MnPO of the POAH are essential for eliciting involuntary responses to external thermal challenges.

8.2.2.2 Thermoregulatory Sensorimotor Integration in the POAH

Information from the MnPO is relayed to the medial preoptic area (MPO), another subregion of the POAH (Nakamura et al. 2011). The MPO plays an important role in controlling thermoregulation by driving a potent inhibition of cold defense effector activation. The inhibitory role of the MPO on cold-defensive responses is evident from several results. First, transections of the neuraxis immediately caudal to the POAH induced a rapid and large rise in brown adipose tissue activity and increased rectal temperatures (Chen et al. 1998), and also increased cutaneous vasoconstrictor sympathetic outflow (Rathner et al. 2008). Second, reducing the activity of neurons in the MPO, either by pharmacological tools such as local injection of GABA or muscimol, a $GABA_A$ agonist, or by causing electrolytic lesions, induced cold defensive responses. Thirdly, glutamate injection, as well as electrical stimulation, suppresses cold-defensive responses and elicits vasodilatation of the paw skin and the tail (Zhang et al. 1995).

Interestingly, in the POAH, about one neuron out of five increases its rate of spontaneous discharge with an increase in POAH temperature from 32–41 °C (Nakayama et al. 1961, 1963). Moreover, local warming has the same effect as glutamate injection and electrical stimulation in suppressing cold-defensive responses and evoking cutaneous vasodilation (Kanosue et al. 1994; Zhang et al. 1995). These results indicate that the POAH neurons are intrinsically warm-sensitive and modulate their activities both according both to the local temperature of the POAH and to the afferent thermal information received from the body surface, in order to cause tonic inhibition of cold defense effector responses (Zhang et al. 1995).

However, the mechanism of how POAH neurons sense warmth remains controversial. There are two types of thermosensitive neurons in the preoptic area, primary thermosensitive neurons, which retain thermosensitivity after synaptic block, and secondary thermosensitive neurons, in which thermosensitivity disappears after synaptic block (Hori et al. 1999; Kobayashi et al. 2006). In primary thermosensitive

neurons warming increased the firing frequency by increasing the rate of depolarization between action potentials, but one group attributes this to the activation of an inward current (Hori et al. 1999; Kobayashi et al. 2006), while a second finds evidence for warmth-inhibition of an A-type potassium current (Griffin et al. 1996).

Several ion channels have been suggested to be the origin of the central thermoregulatory mechanism, such as hyperpolarization-activated cyclic nucleotide-gated channels, two-pore domain potassium channels, and the thermoTRP channels TRPV1 and TRPV4 (Wechselberger et al. 2006; Caterina 2007; Romanovsky et al. 2009). However, no thermoregulatory deficits have been reported from knockout mouse models of these channels, or from application of agonists or antagonists of hyperpolarization-activated cyclic nucleotide-gated channels or two-pore domain potassium channels. Detailed studies with TRPV1 and TRPV4 knockout mice and with TRPV1 antagonists also failed to reveal an involvement of TRPV1 and TRPV4 in warmth sensitivity in the hypothalamus (see sections below on TRPV1 and TRPV4). Several attempts with different approaches have been made to identify the thermosensitive mechanism, including a single cell transcriptome analysis of GABAergic warmth-sensitive neurons (Eberwine et al. 2011), but the mechanism remains elusive.

These studies on heat-sensitivity in the central nervous system (CNS) indicate the presence in the hypothalamus of unidentified thermosensitive ion channels which act as the thermostat of the body.

8.3 Heat-Sensitive Ion Channels

The use of ion channels to directly transduce information from the external environments into nerve impulses can be seen in many different modes of sensation, including thermal, acid and mechano-sensation (Waldmann et al. 1997; Coste et al. 2010). For heat-sensitivity, several families of cation channels have been reported to be heat-sensitive, including members of the TRP channel family (Vay et al. 2012; Voets 2012), the two-pore domain potassium channel family (Maingret et al. 2000; Kang et al. 2005) and the voltage-gated calcium channel family (Iftinca et al. 2006). In addition, anoctamin 1, a Ca^{2+}-activated chloride channel, has recently been reported to be heat-sensitive (Cho et al. 2012). Store-operated calcium channels of the ORAI family located in the surface membrane, while not intrinsically heat-sensitive, are modulated by the calcium-sensitive protein STIM1, in the membrane of the endoplasmic reticulum, which has also recently been reported to be heat-sensitive (Xiao et al. 2011).

Heat-sensitive ion channels expressed in the membrane of peripheral nerve terminals are responsible for transducing elevated environmental temperatures into nerve action potentials. When thermal stimuli reach the temperature threshold of the heat-sensitive ion channels, the heat-sensitive ion channels are activated, in the case of ion channels carrying an inward current, or suppressed, in the case of channels carrying an outward current, resulting in the depolarization of the neuron and the

firing of action potentials. The properties of the heat-sensitive ion channels, with emphasis on the heat-sensitive TRP channels, will be presented briefly here.

8.3.1 Transient Receptor Potential Channels

The TRP channel family shares the common features of six transmembrane segments, varying degrees of sequence homology, and permeability to cations. There are 28 TRP channels in mice, while there are 27 TRP channels in humans, with TRPC2 being a pseudogene. These TRP channels can be further divided into six subfamilies, namely TRPC 1–7 (Canonical), TRPV 1–6 (Vanilloid), TRPM 1–8 (Melastatin), TRPA 1 (Ankyrin) and the more remotely related TRPP2, TRPP3, and TRPP5 (Polycystin), and TRPML 1–3 (Mucolipin) (Venkatachalam et al. 2007). TRP channels, unlike other known families of ion channels, display an enormous diversity of cation selectivities and gating mechanisms. Many TRP channels function as signal integrators, and several of them have been shown to be thermosensitive. Since all chemical and biochemical reactions are to some extent temperature-dependent, and therefore all ion channels could fall within the definition of temperature-sensitive, we define thermosensitivity from the Q_{10} value, which is calculated as the relative increase in current amplitude at a specific voltage when the temperature increases by 10 °C. Here, a brief overview of the heat-sensitive TRP channels which have Q_{10} values higher than 5 will be presented. These are TRPV1, TRPV2, TRPV3, TRPV4, TRPM2, TRPM3, TRPM4 and TRPM5 (Voets 2012).

8.3.1.1 Transient Receptor Potential Vanilloid 1 (TRPV1)

The activation of ion channels by noxious heat in somatosensory neurons was first characterized in 1996 (Cesare et al. 1996). The ion channel responsible for heat sensitivity in at least some primary afferent neurons was cloned in the following year (Caterina et al. 1997), and was later named TRPV1 to emphasize its membership of the TRP ion channel family. TRPV1 is a nonselective cation channel displaying outward rectification that can be activated by temperature > 42ºC with a Q_{10} value of 25 (Vlachova et al. 2003) and P_{Ca}/P_{Na} value of around 10 (Owsianik et al. 2006). TRPV1 can also be activated by a wide range of agonists, including capsaicin, resiniferatoxin and extracellular protons, and is inhibited by capsazepine, N-(4-tertiarybutylphenyl)-4-(3-chloropyridin-2-yl)tetrahydropyrazine-1(2 H)-carboxamide (BCTC) and ruthenium red, amongst others. TRPV1 was found to be mainly expressed in C-fibers and Aδ nociceptive fibers of somatosensory neurons that express the neuropeptides substance P, neurokinin A, and CGRP, indicating its role as a sensor of noxious heat stimuli (Tominaga et al. 1998; Kobayashi et al. 2005; Julius 2013).

TRPV1 KO mice show a partial deficit in sensing noxious heat, but they still withdraw their tails from hot water (Caterina et al. 2000), and TRPV1 KO mice

also show no difference from wild type mice in two-plate temperature preference tests (Pogorzala et al. 2013). In addition, no differences in heat responses could be observed in polymodal nociceptors between WT and TRPV1 KO mice, and the role of TRPV1 seems to be restricted to the C-heat fiber class (Woodbury et al. 2004; Lawson et al. 2008). These findings demonstrate the existence of additional TRPV1-independent mechanisms of heat sensation.

Several studies have sought to determine whether TRPV1 is involved in thermoregulation, in pursuit of the long-sought central mechanism responsible for regulation of body temperature. A possible role of TRPV1 in thermoregulation was supported by evidence showing that TRPV1 blockade with a highly selective TRPV1 antagonist, AMG 517, elicits a marked and plasma concentration-dependent hyperthermia in rodents and humans (Gavva et al. 2007, 2008). However, a peripherally restricted TRPV1 antagonist causes similar increases in body core temperature, demonstrating that the site of action for TRPV1 blockade is outside the blood-brain barrier, and that TRPV1 is therefore not directly involved in central thermoregulation in the hypothalamus (Tamayo et al. 2008). In addition, several studies have failed to reveal any difference between TRPV1 KO and WT mice in basal deep body temperature or in the change in the deep body temperature in responses to thermal challenges (Szelenyi et al. 2004; Iida et al. 2005; Garami et al. 2011). All these results suggest that TRPV1 is unlikely to be a thermosensitive mechanism playing an important role in central thermoregulation.

8.3.1.2 Transient Receptor Potential Vanilloid 2 (TRPV2)

Two years after the cloning of TRPV1, a TRPV1 homologue with a high threshold for noxious heat was discovered, and was subsequently named TRPV2 (Caterina et al. 1999). TRPV2 carries an outwardly rectifying nonselective cation current with P_{Ca}/P_{Na} of around 3 (Clapham et al. 2005; Owsianik et al. 2006). TRPV2 was once thought to be a candidate for the TRPV1-independent mechanism for heat sensation, because the channel has an even higher temperature threshold than TRPV1 (~52°C) with a Q_{10} value higher than 100 (Leffler et al. 2007), and is expressed in medium- to large- diameter myelinated sensory neurons (Caterina et al. 1999). TRPV2 is also activated by hypo-osmolarity, membrane stretch and cannabinoids in addition to heat (Muraki et al. 2003; Qin et al. 2008), and is inhibited by ruthenium red, lanthanum, and SKF96365 (Muraki et al. 2003; Clapham et al. 2005; Qin et al. 2008; Zhang et al. 2012a). The activation of TRPV2 by 2-APB is controversial, with some reports showing that TRPV2 can be activated by 2-APB (Hu et al. 2004; Neeper et al. 2007), and others not (Chung et al. 2004; Bender et al. 2005). In addition to expression in somatosensory neurons, TRPV2 is also expressed in the epithelium of several different tissues (including pancreatic duct, mammary gland, parotid gland, submandibular gland, renal tubule and tracheal gland) and in immune cells (Kowase et al. 2002; Link et al. 2010).

The wide distribution of expression in various tissues suggests a possible range of physiological functions mediated by TRPV2 but the physiological or pathologi-

cal functions mediated by TRPV2 still remain unclear, because behavioral assays of TRPV1/TRPV2 double knockout mice, or TRPV2 knockout mice treated with resiniferatoxin to desensitize TRPV1-expressing afferents, reveal no thermosensory deficits as a consequence of absence of TRPV2 (Park et al. 2011). Whether TRPV2 could be involved in thermoregulation remains unclear. However, the finding showing that the overall pattern and amplitude of the oscillation in core body temperature was not different in TRPV2 knockout mice from WT mice, along with the high activation threshold by heat, makes the possibility less likely (Park 2008).

8.3.1.3 Transient Receptor Potential Vanilloid 3 (TRPV3)

TRPV3, also a homologue of TRPV1, was identified by searching for sequences with a high degree of similarity to TRPV1 in the human genome (Peier et al. 2002; Smith et al. 2002) or by searching for TRP-related expressed sequence tags (ESTs) in a human testis library (Xu et al. 2002). TRPV3 carries an outwardly rectifying nonselective cation current with a P_{Ca}/P_{Na} value of around 12 (Xu et al. 2002; Clapham et al. 2005; Owsianik et al. 2006). TRPV3 can be activated by warmth with an activation threshold at a temperature around 31 °C and has a Q_{10} value higher than 6 (Peier et al. 2002; Xu et al. 2002). In addition to temperature, TRPV3 can also be activated by carvacrol, eugenol and thymol, which are found in plants such as oregano, savory, clove and thyme (Xu et al. 2006b). TRPV3 is also activated by 2-APB and is inhibited by ruthenium red (Hu et al. 2004; Clapham et al. 2005). In addition, TRPV3 exhibits a unique property known as sensitization, which causes progressive increases in its response with repeated application of a stimulus (Peier et al. 2002).

TRPV3 is mainly expressed in skin keratinocytes and has been proposed to act as a thermosensor, the excitation of which could be transferred to primary afferent terminals of sensory neurons by an unknown mediator (Peier et al. 2002). TRPV3 null mice on an intercrossed C57BL6/129 J background were initially reported to have some relatively minor deficits in responses to innocuous and noxious heat but not in other sensory modalities (Moqrich et al. 2005). However, TRPV3 knockout mice on a homogeneous C57BL6 background exhibited no obvious alterations in thermal preference behaviour, while the absence of TRPV3 on a 129S6 background resulted in a more restricted range of occupancy centered around cooler floor temperatures (Huang et al. 2011). In addition, TRPV3 knockout mice showed no deficits in acute heat nociception on either a C57BL6 or a 129S6 background (Huang et al. 2011). These results suggest that TRPV3 is not a major contributor for thermosensation, and the previously reported deficits in responses to innocuous and noxious heat in the TRPV3 null mice on the intercrossed C57BL6/129J background are possibly due to inhomogeneous inheritance of non-TRPV3 determinants. The warmth-sensitive TRPV3 could plausibly be involved in thermoregulation, but the finding that mice in which TRPV3 has been genetically inactivated appeared morphologically normal and have a normal internal temperature does not favour this idea (Moqrich et al. 2005).

8.3.1.4 Transient Receptor Potential Vanilloid 4 (TRPV4)

TRPV4 was cloned with an initial motivation to find osmotically-sensitive channels (Liedtke et al. 2000; Strotmann et al. 2000). It was almost 2 years after the cloning of TRPV4 that it was found to be thermo-sensitive (Guler et al. 2002). TRPV4 carries an outwardly rectifying nonselective cation current that can be activated by non-noxious heat at a temperature higher than 25 °C (Clapham 2003) with a Q_{10} value of around 10 (Guler et al. 2002) and P_{Ca}/P_{Na} value of around 9.3 (Strotmann et al. 2003). In addition to warmth and hypotonic stress, TRPV4 can also be activated by the compound bisandrographolide from the Chinese herbal plant *Andrographis paniculata* and by a specific TRPV4 agonist, GSK1016790A (Smith et al. 2006; Willette et al. 2008). TRPV4 is also inhibited by ruthenium red and by a specific TRPV4 antagonist, HC-06704 (Clapham et al. 2005; Everaerts et al. 2010). The expression of TRPV4 has been reported in a variety of tissues, including kidney, corneal epithelial cells (Pan et al. 2008), cerebral microvascular endothelial cells (Ma et al. 2008), cortical astrocytes (Benfenati et al. 2007), tracheal epithelial cells (Lorenzo et al. 2008), and keratinocytes (Guler et al. 2002).

Like TRPV3, the fact that TRPV4 is expressed in keratinocytes led to the proposal that activation of TRPV4 in keratinocytes could be transferred to sensory neurons via an unidentified extracellular mediator. However, as noted above, mice deficient in both TRPV3 and TRPV4 show thermal preference behavior similar to wild type mice on a thermal gradient and little or no change in acute heat perception (Huang et al. 2011). The findings suggest that TRPV4 probably also makes a very limited contribution to heat sensation.

TRPV4 also seems not to be involved in thermoregulation. Application of a TRPV4 antagonist did not affect core body temperature (Everaerts et al. 2010). Second, no difference in basal body temperature recordings and no difference in core body temperature after cold stress or heat stress was found between TRPV4 KO and WT mice (Liedtke et al. 2003; Lee et al. 2005).

8.3.1.5 Transient Receptor Potential Melastatin 2 (TRPM2)

TRPM2 was cloned in 1998 with an initial goal of finding the genes responsible for several diseases, such as autoimmune polyglandular disease type I, bipolar affective disorder, and nonsyndromic hereditary deafness, and was confirmed to be located at 21q22.3 (Nagamine et al. 1998). Of these diseases, bipolar disorder was found to be associated with TRPM2 (Yoon et al. 2001; Xu et al. 2006a). TRPM2 carries a voltage-insensitive nonselective cation current that was found later to be activated by heat at a temperature higher than 35 °C with Q_{10} value at around 15.6 (Togashi et al. 2006) and P_{Ca}/P_{Na} value of 0.5–1.6 (Perraud et al. 2001). In addition to heat, the activity of TRPM2 can also be synergistically modulated by intracellular ADP-ribose (ADPr) and Ca^{2+} (Heiner et al. 2006). TRPM2 can also be activated by reactive oxygen species (ROS), such as H_2O_2 (Hara et al. 2002; Wehage et al. 2002), and enhanced by additional factors, such as cyclic ADP-ribose and nicotinic

acid adenine dinucleotide phosphate (NAADP) (Kolisek et al. 2005; Beck et al. 2006). TRPM2 is inhibited by permeating protons (both intracellular and extracellular acidic pH) (Du et al. 2009; Starkus et al. 2010) and intracellular adenosine monophosphate (AMP) (Kolisek et al. 2005; Beck et al. 2006).

TRPM2 is highly expressed in brain (with the vast majority in non-neuronal microglial cells) and in immune cells such as macrophages and neutrophils (Naziroglu 2011), and has also been reported to be expressed in DRG neurons (Naziroglu et al. 2014). However, TRPM2 KO mice showed no deficits in noxious heat sensitivity when tested with the Hargreaves test and hot plate test at 52 and 55°C (Haraguchi et al. 2012). Thermal hyperalgesia was, however, found to be attenuated in carrageenan-induced inflammatory pain and sciatic nerve injury-induced neuropathic pain models (Haraguchi et al. 2012). It was proposed that rather than being a direct effect mediated via TRPM2 expressed in sensory nerve terminals, the effect of TRPM2 deletion on pain was due to TRPM2 expressed in macrophages and microglia aggravating peripheral and spinal pronociceptive inflammatory responses and hence contributing to the pathogenesis of inflammatory and neuropathic pain (Haraguchi et al. 2012).

TRPM2 KO mice have a similar body temperature to WT mice at ambient temperatures (Uchida et al. 2011). However, the activation threshold of TRPM2 at around body temperature (Togashi et al. 2006) makes TRPM2 a possible candidate as the long-sought thermosensitive mechanism for thermoregulation. In addition, TRPM2 KO mice have been reported to have higher energy expenditure (Zhang et al. 2012b), which in general is correlated with thermoregulation. More detailed studies into the thermoregulatory phenotype of TRPM2 KO mice are needed to determine whether TRPM2 may be involved in thermoregulation.

8.3.1.6 Transient Receptor Potential Melastatin 3 (TRPM3)

TRPM3 has the largest number of splice variants within the TRP channel family (11 splice variants have been reported) (Oberwinkler et al. 2007; Thiel et al. 2013) and was first identified by sequencing projects. TRPM3 carries an outwardly rectifying non-selective cation current that can be activated by heat (>40 °C) with a Q_{10} value of 7.2 (Vriens et al. 2011) and P_{Ca}/P_{Na} at 0.1–10 (depending on splice variant) (Owsianik et al. 2006). TRPM3 exhibits constitutive activity when overexpressed in HEK293 cells. Agonists of TRPM3 include pregnenolone sulphate, nifedipine, and β-cyclodextrin (Wagner et al. 2008; Naylor et al. 2010). Secondary metabolites from citrus fruit flavanones, such as naringenin (the predominant flavanone in grapefruits), hesperetin (the predominant flavanone in oranges), and fabacea (from the pea and bean family) selectively inhibit TRPM3 channel activation (Felgines et al. 2000; Erlund et al. 2001; Straub et al. 2013a, b). TRPM3 channels are prominently expressed in various tissues, including kidney, liver, ovary, brain, spinal cord, pituitary, vascular smooth muscle, and testis (Grimm et al. 2003; Lee et al. 2003; Naylor et al. 2010) and are also found to be expressed in small diameter DRG neurons (Vriens et al. 2011).

TRPM3 knockout mice exhibited reduced but not absent sensitivity to noxious heat, similar to what was seen with TRPV1 knockout mice. This result is corroborated by the effect of block or deletion of TRPV1 and TRPM3 in sensory neurons; up to 50% of DRG neurons from TRPM3 knockout mice are still heat-responsive after treatment with AMG9810, a selective TRPV1 antagonist (Vriens et al. 2011). These results demonstrate that both TRPV1 and TRPM3 contribute to the sensation of noxious heat, but that there are additional novel heat-sensitive mechanisms independent of TRPV1 and TRPM3.

Whether TRPM3 is involved in thermoregulation in TRPM3 KO mice is undetermined, because no detailed studies have been reported. The only result available is that the body temperatures of TRPM3 KO mice are not statistically different from WT mice (Vriens et al. 2011).

8.3.1.7 Transient Receptor Potential Melastatin 4 (TRPM4)

Within the TRPM channel family most channels are non-selective cationic channels, but TRPM4 and TRPM5 are not permeable to divalent cations (Guinamard et al. 2010). TRPM4 carries an outwardly rectifying current that has a Q_{10} value of around 8.5 between 15 and 25 °C (Talavera et al. 2005). In addition to heat, TRPM4 can be activated directly by intracellular calcium with an EC_{50} of 20 μM (Ullrich et al. 2005). Decavanadate and BTP2 are also activators of TRPM4 (Nilius et al. 2004a; Takezawa et al. 2006). Intracellular ATP can inhibit TRPM4 with an IC_{50} value of 1.7 μM (Nilius et al. 2004b). Other reported inhibitors of TRPM4 include flufenamic acid, 9-phenanthrol, MPB-104, glibenclamide, and clotrimazole (Abriel et al. 2012). TRPM4 expression is widespread and, in humans, it has been detected at high levels in the heart, pancreas, placenta, and prostate and at lower levels in the kidney, skeletal muscle, liver, intestines, thymus, and spleen (Abriel et al. 2012).

TRPM4 mRNA has also been shown to be expressed in trigeminal ganglion and in DRG at all the anatomical segments of the spinal cord tested (Jang et al. 2012; Vandewauw et al. 2013). However, no studies focusing on a possible thermosensory or thermoregulatory phenotype of TRPM4 KO mice have been reported. A possible role for TRPM4 in thermosensation and thermoregulation needs further investigation.

8.3.1.8 Transient Receptor Potential Melastatin 5 (TRPM5)

TRPM5, like TRPM4, is impermeable to divalent cations (Vandewauw et al. 2013). TRPM5 carries an outwardly rectifying current that has a Q_{10} value of around 10.3 between 15 and 25 °C (Talavera et al. 2005). In addition to warmth, TRPM5 can also be activated directly by intracellular calcium at an even lower concentration than TRPM4, with EC_{50} at 0.7 μM, whereas higher concentrations are inhibitory (Prawitt et al. 2003; Ullrich et al. 2005). TRPM5 can be inhibited by triphenylphosphine oxide (Palmer et al. 2010). External acidification can also cause a rapid and

reversible block of the current mediated by TRPM5, with IC_{50} pH of 6.2 and a slower irreversible enhancement of current inactivation (Liu et al. 2005). TRPM5 was first cloned from mammalian taste buds (Perez et al. 2002) and has been shown to confer thermal sensitivity to sweet taste (Damak et al. 2006; Liman 2007) (See Chap. 6 by Talavera in this book). A temperature increase from 15 to 35 °C significantly enhances the response of the gustatory nerve to sweet compounds in WT but not in TRPM5 KO mice (Talavera et al. 2005). TRPM5 expression was later found to be widespread and includes chemosensory organs (the main olfactory epithelium and the vomeronasal organ) (See Chap. 5 by Restrepo et al. in this book) and epithelial cells of the respiratory and gastrointestinal tract (Kaske et al. 2007).

TRPM5 mRNA has also been reported to be detectable in DRG at all anatomical segments of the spinal cord tested (Vandewauw et al. 2013). However, no studies of the role of TRPM5 in either thermosensation or thermoregulation have been reported, and a possible role of TRPM5 in these physiological functions is undetermined.

8.3.2 Two-Pore Domain Potassium Channels

Mammalian two-pore domain potassium channels (K2P) are encoded by 15 known genes (KCNK family). The two-pore domain potassium channels share the common features of four transmembrane segments, two pore domains, an extended M1P1 extracellular loop and intracellular amino (N) and carboxyl (C) termini. However, they have low sequence identity outside the pore regions. The K2P channels can be distributed into six subfamilies [two-pore domain weak inwardly rectifying K^+ channel (TWIK), TWIK-related K^+ channel (TREK), TWIK-related acid-sensitive K^+ channel (TASK), TWIK-related alkaline-activated K^+ channel (TALK), TWIK-related halothane-inhibited K^+ channel (THIK), and TWIK-related spinal cord K^+ channel (TRESK)]. These two-pore domain potassium channels give rise to leak K^+currents, and have also been shown to be important in determining neuronal excitability and cell firing (Enyedi et al. 2010). Among the two-pore potassium channels, TREK-1, TREK-2, and TRAAK have been shown to be thermo-sensitive (Maingret et al. 2000; Kang et al. 2005).

TREK-1 is activated by heat with a Q_{10} from 22 to 32 °C of ~7-fold, while a temperature decrease from 22 to 16 °C inhibits TREK-1 basal activity. Noteworthy is that cell integrity is required for temperature sensitivity, because TREK-1 in the inside-out state is not sensitive to heat (Maingret et al. 2000). In addition to the sensitivity to temperature, TREK-1 can be activated by arachidonic acid, unsaturated fatty acids, lysophospholipids, volatile anesthetics, mechanical stress, and internal acidification, and is inhibited by barium and quinidine (Goldstein et al. 2005; Ma et al. 2011).

TREK-2 can also be activated by heat, with a ~20-fold increase in current amplitude from 24 to 42 °C. Cell integrity is also required for temperature sensitivity, suggesting the participation of an intracellular second messenger in temperature transduction (Kang et al. 2005). TREK2 is activated by arachidonic acid, docosa-

hexaenoic acid, chloroform, halothane, and isoflurane, mechanical stress, intracellular acidification and riluzole, and is inhibited by quinidine (Lesage et al. 2000; Goldstein et al. 2005).

TRAAK has a similar sensitivity to heat to TREK-2, with a ~20-fold increase in current amplitude with a temperature increase from 24 to 42 °C, and like TREK-1 and TREK-2, cell integrity is required for thermosensitivity (Kang et al. 2005). Arachidonic acid, mechanical stress, unsaturated fatty acids, lysopholipids and riluzole can activate TRAAK in addition to heat, and gadolinium can inhibit TRAAK (Goldstein et al. 2005).

TREK-1, TREK-2 and TRAAK have been shown to be expressed in both somatosensory neurons and the CNS (Maingret et al. 2000; Medhurst et al. 2001; Pollema-Mays et al. 2013) and therefore could be involved in thermosensation and/or thermoregulation. TREK-1 and TRAAK KO mice were reported to be more sensitive to heat stimuli, but not to cold (Alloui et al. 2006)(Noel et al. 2009). Interestingly, in TREK-1/TRAAK double knockout mice, heat hypersensitivity persists and cold hypersensitivity develops. Recently, TREK-2 KO mice were shown to have increased excitability to thermal stimuli above 40 °C and TREK-1/TREK-2/TRAAK triple knockout mice also express hypersensitivity to heat (Pereira et al. 2014). These results suggest that inhibition of these K2P channels by cold could promote firing in cold-sensitive neurons, while in response to heat, activation of these channels could act to dampen the effect of activation of TRP channels and so to reduce heat sensitivity.

It remains unknown whether two-pore domain potassium channels are involved in thermoregulation. None of the two-pore domain potassium channel KO animals have been found to have any obvious deficits in thermoregulation, but there is a lack of definitive studies and participation in thermoregulation remains a possibility.

8.3.3 Calcium-Activated Chloride Channel Anoctamin 1 (ANO1)

The calcium-activated chloride channel ANO1 was recently reported to be heat-sensitive. ANO1 is activated by heat (>44 °C) with a Q_{10} value of 19.4 (Cho et al. 2012) and by intracellular calcium. ANO1 can also be activated by other divalent cations, including Ba^{2+}, Sr^{2+}, and Ni^{2+}. Niflumic acid, often considered a specific blocker for calcium-activated chloride channels, blocks ANO1. Other blockers of ANO1 have been investigated and include T16inh-A01, CaCCinh-A01, tannic acid, eugenol, and benzbromarone (Pedemonte et al. 2014).

ANO1 has been shown to be expressed in epithelial cells of pulmonary bronchioles, pancreatic acinar cells, retinal cell layers, proximal renal tubules, submandibular glands, Leydig cells, and sensory neurons of dorsal root ganglia (Yang et al. 2008). ANO1 knockdown mice and ANO1 conditional knockout mice were both shown to have deficits in sensing noxious heat, showing that ANO1 is involved in heat sensation (Cho et al. 2012). However, there are no reports showing whether ANO1 may be involved in thermoregulation.

8.4 Concluding Remarks

In the last two decades several TRP channels have been shown to be activated by heat, and both TRPV1 and TRPM3 have been demonstrated to play a role in heat sensation *in vivo*. Thermal sensitivity is not confined to the TRP channel family, however, as members of the two-pore domain potassium channel family and the calcium-activated chloride channel anoctamin 1 are also involved in the detection of heat from the external environment. The evidence for an involvement of other heat-sensitive TRP channels in thermosensation *in vivo* is not currently persuasive. However, it is still undetermined whether the combination of these known heat-sensitive mechanisms is enough to fully explain the exquisite sensitivity of animals to thermal stimuli, or whether there are other novel thermosensitive mechanisms remaining to be discovered. More importantly, none of the existing knockout animals show significant deficits in thermoregulation, and an understanding of the molecular mechanisms underlying the regulation of the core temperature of mammals remains elusive.

References

Abriel H et al (2012) TRPM4 channels in the cardiovascular system: physiology, pathophysiology, and pharmacology. Biochem Pharmacol 84(7):873–881

Alloui A et al (2006) TREK-1, a K+ channel involved in polymodal pain perception. EMBO J 25(11):2368–2376

Almeida MC et al (2012) Pharmacological blockade of the cold receptor TRPM8 attenuates autonomic and behavioral cold defenses and decreases deep body temperature. J Neurosci 32(6):2086–2099

Beck A et al (2006) Nicotinic acid adenine dinucleotide phosphate and cyclic ADP-ribose regulate TRPM2 channels in T lymphocytes. FASEB J 20(7):962–964

Bender FL et al (2005) The temperature-sensitive ion channel TRPV2 is endogenously expressed and functional in the primary sensory cell line F-11. Cell Physiol Biochem 15(1–4):183–194

Benfenati V et al (2007) Expression and functional characterization of transient receptor potential vanilloid-related channel 4 (TRPV4) in rat cortical astrocytes. Neuroscience 148(4):876–892

Caterina MJ (2007) Transient receptor potential ion channels as participants in thermosensation and thermoregulation. Am J Physiol Regul Integr Comp Physiol 292(1):R64–R76

Caterina MJ et al (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389(6653):816–824

Caterina MJ et al (1999) A capsaicin-receptor homologue with a high threshold for noxious heat. Nature 398(6726):436–441

Caterina MJ et al (2000) Impaired nociception and pain sensation in mice lacking the capsaicin receptor. Science 288(5464):306–313

Cesare P, McNaughton P (1996) A novel heat-activated current in nociceptive neurons and its sensitization by bradykinin. Proc Natl Acad Sci U S A 93(26):15435–15439

Chen XM et al (1998) Efferent projection from the preoptic area for the control of non-shivering thermogenesis in rats. J Physiol 512(Pt 3):883–892

Cho H et al (2012) The calcium-activated chloride channel anoctamin 1 acts as a heat sensor in nociceptive neurons. Nat Neurosci 15(7):1015–1021

Chung MK et al (2004) 2-aminoethoxydiphenyl borate activates and sensitizes the heat-gated ion channel TRPV3. J Neurosci 24(22):5177–5182
Clapham DE (2003) TRP channels as cellular sensors. Nature 426(6966):517–524
Clapham DE et al (2005) International Union of Pharmacology. XLIX. Nomenclature and Structure-function relationships of transient receptor potential channels. Pharmacol Rev 57(4):427–450
Coste B et al (2010) Piezo1 and Piezo2 are essential components of distinct mechanically activated cation channels. Science 330(6000):55–60
Craig AD (2002) How do you feel? Interoception: the sense of the physiological condition of the body. Nat Rev Neurosci 3(8):655–666
Damak S et al (2006) Trpm5 null mice respond to bitter, sweet, and umami compounds. Chem Senses 31(3):253–264
Du J et al (2009) Modulation of TRPM2 by acidic pH and the underlying mechanisms for pH sensitivity. J Gen Physiol 134(6):471–488
Eberwine J, Bartfai T (2011) Single cell transcriptomics of hypothalamic warm sensitive neurons that control core body temperature and fever response signaling asymmetry and an extension of chemical neuroanatomy. Pharmacol Ther 129(3):241–259
Enyedi P, Czirjak G (2010) Molecular background of leak K+ currents: two-pore domain potassium channels. Physiol Rev 90(2):559–605
Erlund I et al (2001) Plasma kinetics and urinary excretion of the flavanones naringenin and hesperetin in humans after ingestion of orange juice and grapefruit juice. J Nutr 131(2):235–241
Everaerts W et al (2010) Inhibition of the cation channel TRPV4 improves bladder function in mice and rats with cyclophosphamide-induced cystitis. Proc Natl Acad Sci U S A 107(44):19084–19089
Felgines C et al (2000) Bioavailability of the flavanone naringenin and its glycosides in rats. Am J Physiol Gastrointest Liver Physiol 279(6):G1148–G1154
Flouris AD (2011) Functional architecture of behavioural thermoregulation. Eur J Appl Physiol 111(1):1–8
Garami A et al (2011) Thermoregulatory phenotype of the Trpv1 knockout mouse: thermoeffector dysbalance with hyperkinesis. J Neurosci 31(5):1721–1733
Gavva NR et al (2007) Repeated administration of vanilloid receptor TRPV1 antagonists attenuates hyperthermia elicited by TRPV1 blockade. J Pharmacol Exp Ther 323(1):128–137
Gavva NR et al (2008) Pharmacological blockade of the vanilloid receptor TRPV1 elicits marked hyperthermia in humans. Pain 136(1–2):202–210
Goldstein SA et al (2005) International Union of Pharmacology. LV. Nomenclature and molecular relationships of two-P potassium channels. Pharmacol Rev 57(4):527–540
Griffin JD et al (1996) Cellular mechanisms for neuronal thermosensitivity in the rat hypothalamus. J Physiol 492(Pt 1):231–242
Grimm C et al (2003) Molecular and functional characterization of the melastatin-related cation channel TRPM3. J Biol Chem 278(24):21493–21501
Guinamard R et al (2010) Physiological roles of the TRPM4 channel extracted from background currents. Physiology (Bethesda) 25(3):155–164
Guler AD et al (2002) Heat-evoked activation of the ion channel, TRPV4. J Neurosci 22(15):6408–6414
Hara Y et al (2002) LTRPC2 Ca2+ -permeable channel activated by changes in redox status confers susceptibility to cell death. Mol Cell 9:163–173
Haraguchi K et al (2012) TRPM2 contributes to inflammatory and neuropathic pain through the aggravation of pronociceptive inflammatory responses in mice. J Neurosci 32(11):3931–3941
Heiner I et al (2006) Endogenous ADP-ribose enables calcium-regulated cation currents through TRPM2 channels in neutrophil granulocytes. Biochem J 398(2):225–232
Hori A et al (1999) Warming-activated channels of warm-sensitive neurons in rat hypothalamic slices. Neurosci Lett 275(2):93–96
Hu HZ et al (2004) 2-aminoethoxydiphenyl borate is a common activator of TRPV1, TRPV2, and TRPV3. J Biol Chem 279(34):35741–35748

Huang SM et al (2011) TRPV3 and TRPV4 ion channels are not major contributors to mouse heat sensation. Mol Pain 7:37
Iftinca M et al (2006) Temperature dependence of T-type calcium channel gating. Neuroscience 142(4):1031–1042
Iida T et al (2005) Attenuated fever response in mice lacking TRPV1. Neurosci Lett 378(1):28–33
Jang Y et al (2012) Quantitative analysis of TRP channel genes in mouse organs. Arch Pharm Res 35(10):1823–1830
Julius D (2013) TRP channels and pain. Annu Rev Cell Dev Biol 29:355–384
Kang D et al (2005) Thermosensitivity of the two-pore domain K+ channels TREK-2 and TRAAK. J Physiol 564(Pt 1):103–116
Kanosue K et al (1994) Hypothalamic network for thermoregulatory vasomotor control. Am J Physiol 267(1 Pt 2):R283–R288
Kaske S et al (2007) TRPM5, a taste-signaling transient receptor potential ion-channel, is a ubiquitous signaling component in chemosensory cells. BMC Neurosci 8:49
Kobayashi K et al (2005) Distinct expression of TRPM8, TRPA1, and TRPV1 mRNAs in rat primary afferent neurons with adelta/c-fibers and colocalization with trk receptors. J Comp Neurol 493(4):596–606
Kobayashi S et al (2006) Point: heat-induced membrane depolarization of hypothalamic neurons: a putative mechanism of central thermosensitivity. Am J Physiol Regul Integr Comp Physiol 290(5):R1479–R1480 discussion R1484
Kolisek M et al (2005) Cyclic ADP-ribose and hydrogen peroxide synergize with ADP-ribose in the activation of TRPM2 channels. Mol Cell 18(1):61–69
Kowase T et al (2002) Immunohistochemical localization of growth factor-regulated channel (GRC) in human tissues. Endocr J 49(3):349–355
Lawson JJ et al (2008) TRPV1 unlike TRPV2 is restricted to a subset of mechanically insensitive cutaneous nociceptors responding to heat. J Pain 9(4):298–308
Lee N et al (2003) Expression and characterization of human transient receptor potential melastatin 3 (hTRPM3). J Biol Chem 278(23):20890–20897
Lee H et al (2005) Altered thermal selection behavior in mice lacking transient receptor potential vanilloid 4. J Neurosci 25(5):1304–1310
Leffler A et al (2007) A high-threshold heat-activated channel in cultured rat dorsal root ganglion neurons resembles TRPV2 and is blocked by gadolinium. Eur J Neurosci 26(1):12–22
Lesage F et al (2000) Human TREK2, a 2P domain mechano-sensitive K + channel with multiple regulations by polyunsaturated fatty acids, lysophospholipids, and Gs, Gi, and Gq protein-coupled receptors. J Biol Chem 275(37):28398–28405
Liedtke W, Friedman JM (2003) Abnormal osmotic regulation in trpv4-/- mice. Proc Natl Acad Sci U S A 100(23):13698–13703
Liedtke W et al (2000) Vanilloid receptor-related osmotically activated channel (VR-OAC), a candidate vertebrate osmoreceptor. Cell 103(3):525–535
Liman ER (2007) TRPM5 and taste transduction. Handb Exp Pharmacol (179): 287–298
Link TM et al (2010) TRPV2 has a pivotal role in macrophage particle binding and phagocytosis. Nat Immunol 11(3):232–239
Liu D et al (2005) Extracellular acid block and acid-enhanced inactivation of the Ca2+ -activated cation channel TRPM5 involve residues in the S3-S4 and S5-S6 extracellular domains. J Biol Chem 280(21):20691–20699
Lorenzo IM et al (2008) TRPV4 channel participates in receptor-operated calcium entry and ciliary beat frequency regulation in mouse airway epithelial cells. Proc Natl Acad Sci U S A 105(34):12611–12616
Ma YY et al (2008) Effects of cinnamaldehyde on PGE2 release and TRPV4 expression in mouse cerebral microvascular endothelial cells induced by interleukin-1beta. Biol Pharm Bull 31(3):426–430
Ma XY et al (2011) External Ba2+ block of the two-pore domain potassium channel TREK-1 defines conformational transition in its selectivity filter. J Biol Chem 286(46):39813–39822

Maingret F et al (2000) TREK-1 is a heat-activated background K(+) channel. EMBO J 19(11):2483–2491
Martinez C, Peña García E (2013) In Galizia CG, Lledo P-M (eds) Thermosensation. Neurosciences—from molecule to behavior: a university textbook. Springer, Berlin, pp 303–319
Medhurst AD et al (2001) Distribution analysis of human two pore domain potassium channels in tissues of the central nervous system and periphery. Brain Res Mol Brain Res 86(1–2):101–114
Moqrich A et al (2005) Impaired thermosensation in mice lacking TRPV3, a heat and camphor sensor in the skin. Science 307(5714):1468–1472
Morrison SF (2011) 2010 Carl Ludwig distinguished lectureship of the APS neural control and autonomic regulation section: central neural pathways for thermoregulatory cold defense. J Appl Physiol 110(5):1137–1149
Muraki K et al (2003) TRPV2 is a component of osmotically sensitive cation channels in murine aortic myocytes. Circ Res 93(9):829–838
Nagamine K et al (1998) Molecular cloning of a novel putative Ca2+ channel protein (TRPC7) highly expressed in brain. Genomics 54(1):124–131
Nakamura K, Morrison SF (2008a) Preoptic mechanism for cold-defensive responses to skin cooling. J Physiol 586(10):2611–2620
Nakamura K, Morrison SF (2008b) A thermosensory pathway that controls body temperature. Nat Neurosci 11(1):62–71
Nakamura K, Morrison SF (2010) A thermosensory pathway mediating heat-defense responses. Proc Natl Acad Sci U S A 107(19):8848–8853
Nakamura K, Morrison SF (2011) Central efferent pathways for cold-defensive and febrile shivering. J Physiol 589(Pt 14):3641–3658
Nakayama T et al (1961) Single unit activity of anterior hypothalamus during local heating. Science 134(3478):560–561
Nakayama T et al (1963) Thermal stimulation of electrical activity of single units of the preoptic region. Am J Physiol Leg Content 204(6):1122–1126
Naylor J et al (2010) Pregnenolone sulphate- and cholesterol-regulated TRPM3 channels coupled to vascular smooth muscle secretion and contraction. Circ Res 106(9):1507–1515
Naziroglu M (2011) TRPM2 cation channels, oxidative stress and neurological diseases: where are we now? Neurochem Res 36(3):355–366
Naziroglu M et al (2014) Modulation of oxidative stress and Ca(2+) mobilization through TRPM2 channels in rat dorsal root ganglion neuron by hypericum perforatum. Neuroscience 263:27–35
Neeper MP et al (2007) Activation properties of heterologously expressed mammalian TRPV2: evidence for species dependence. J Biol Chem 282(21):15894–15902
Nilius B et al (2004a) Decavanadate modulates gating of TRPM4 cation channels. J Physiol 560(Pt 3):753–765
Nilius B et al (2004b) Intracellular nucleotides and polyamines inhibit the Ca2+ -activated cation channel TRPM4b. Pflugers Arch 448(1):70–75
Noel J et al (2009) The mechano-activated K+ channels TRAAK and TREK-1 control both warm and cold perception. EMBO J 28(9):1308–1318
Oberwinkler J, Phillipp SE (2007) TRPM3. Handb Exp Pharmacol (179): 253–267
Owsianik G et al (2006) Permeation and selectivity of TRP channels. Annu Rev Physiol 68:685–717
Palmer RK et al (2010) Triphenylphosphine oxide is a potent and selective inhibitor of the transient receptor potential melastatin-5 ion channel. Assay Drug Dev Technol 8(6):703–713
Pan Z et al (2008) Dependence of regulatory volume decrease on transient receptor potential vanilloid 4 (TRPV4) expression in human corneal epithelial cells. Cell Calcium 44(4):374–385
Park U (2008) In vivo function of transient receptor potential vanilloid 2 (TRPV2). Ann Arbor, The Johns Hopkins University, 3309822:128
Park U et al (2011) TRP vanilloid 2 knock-out mice are susceptible to perinatal lethality but display normal thermal and mechanical nociception. J Neurosci 31(32):11425–11436
Pedemonte N, Galietta LJ (2014) Structure and function of TMEM16 proteins (Anoctamins). Physiol Rev 94(2):419–459

Peier AM et al (2002) A heat-sensitive TRP channel expressed in keratinocytes. Science 296(5575):2046–2049
Pereira V et al (2014) Role of the TREK2 potassium channel in cold and warm thermosensation and in pain perception. Pain 155(12):2534–2544
Perez CA et al (2002) A transient receptor potential channel expressed in taste receptor cells. Nat Neurosci 5(11):1169–1176
Perraud AL et al (2001) ADP-ribose gating of the calcium-permeable LTRPC2 channel revealed by Nudix motif homology. Nature 411(6837):595–599
Pogorzala LA et al (2013) The cellular code for mammalian thermosensation. J Neurosci 33(13):5533–5541
Pollema-Mays SL et al (2013) Expression of background potassium channels in rat DRG is cell-specific and down-regulated in a neuropathic pain model. Mol Cell Neurosci 57:1–9
Prawitt D et al (2003) TRPM5 is a transient Ca2+ -activated cation channel responding to rapid changes in [Ca2+]i. Proc Natl Acad Sci U S A 100(25):15166–15171
Qin N et al (2008) TRPV2 is activated by cannabidiol and mediates CGRP release in cultured rat dorsal root ganglion neurons. J Neurosci 28(24):6231–6238
Rathner JA et al (2008) Central pathway for spontaneous and prostaglandin E2-evoked cutaneous vasoconstriction. Am J Physiol Regul Integr Comp Physiol 295(1):R343–R354
Romanovsky A et al (2009) The transient receptor potential vanilloid-1 channel in thermoregulation: a thermosensor it is not. Pharmacol Rev 61:228–261
Smith GD et al (2002) TRPV3 is a temperature-sensitive vanilloid receptor-like protein. Nature 418(6894):186–190
Smith PL et al (2006) Bisandrographolide from Andrographis paniculata activates TRPV4 channels. J Biol Chem 281(40):29897–29904
Starkus JG et al (2010) The calcium-permeable non-selective cation channel TRPM2 is modulated by cellular acidification. J Physiol 588(Pt 8):1227–1240
Straub I et al (2013a) Flavanones that selectively inhibit TRPM3 attenuate thermal nociception in vivo. Mol Pharmacol 84(5):736–750
Straub I et al (2013b) Citrus fruit and fabacea secondary metabolites potently and selectively block TRPM3. Br J Pharmacol 168(8):1835–1850
Strotmann R et al (2000) OTRPC4, a nonselective cation channel that confers sensitivity to extracellular osmolarity. Nat Cell Biol 2(10):695–702
Strotmann R et al (2003) Ca2+ -dependent potentiation of the nonselective cation channel TRPV4 is mediated by a C-terminal calmodulin binding site. J Biol Chem 278(29):26541–26549
Szelenyi Z et al (2004) Daily body temperature rhythm and heat tolerance in TRPV1 knockout and capsaicin pretreated mice. Eur J Neurosci 19(5):1421–1424
Tajino K et al (2011) Cooling-sensitive TRPM8 is thermostat of skin temperature against cooling. PLoS One 6(3):e17504
Takezawa R et al (2006) A pyrazole derivative potently inhibits lymphocyte Ca2+ influx and cytokine production by facilitating transient receptor potential melastatin 4 channel activity. Mol Pharmacol 69(4):1413–1420
Talavera K et al (2005) Heat activation of TRPM5 underlies thermal sensitivity of sweet taste. Nature 438(7070):1022–1025
Tamayo N et al (2008) Design and synthesis of peripherally restricted transient receptor potential vanilloid 1 (TRPV1) antagonists. J Med Chem 51(9):2744–2757
Thiel G et al (2013) Signal transduction via TRPM3 channels in pancreatic beta-cells. J Mol Endocrinol 50(3):R75–R83
Todd AJ (2010) Neuronal circuitry for pain processing in the dorsal horn. Nat Rev Neurosci 11(12):823–836
Togashi K et al (2006) TRPM2 activation by cyclic ADP-ribose at body temperature is involved in insulin secretion. EMBO J 25(9):1804–1815
Tominaga M et al (1998) The cloned capsaicin receptor integrates multiple pain-producing stimuli. Neuron 21(3):531–543

Uchida K et al (2011) Lack of TRPM2 impaired insulin secretion and glucose metabolisms in mice. Diabetes 60(1):119–126
Ullrich ND et al (2005) Comparison of functional properties of the Ca2+ -activated cation channels TRPM4 and TRPM5 from mice. Cell Calcium 37(3):267–278
Vandewauw I et al (2013) Systematic and quantitative mRNA expression analysis of TRP channel genes at the single trigeminal and dorsal root ganglion level in mouse. BMC Neurosci 14:21
Vay L et al (2012) The thermoTRP ion channel family: properties and therapeutic implications. Br J Pharmacol 165(4):787–801
Venkatachalam K, Montell C (2007) TRP channels. Annu Rev Biochem 76:387–417
Vlachova V et al (2003) Functional role of C-terminal cytoplasmic tail of rat vanilloid receptor 1. J Neurosci 23(4):1340–1350
Voets T (2012) Quantifying and modeling the temperature-dependent gating of TRP channels. Rev Physiol Biochem Pharmacol 162:91–119
Vriens J et al (2011) TRPM3 is a nociceptor channel involved in the detection of noxious heat. Neuron 70(3):482–494
Wagner TF et al (2008) Transient receptor potential M3 channels are ionotropic steroid receptors in pancreatic beta cells. Nat Cell Biol 10(12):1421–1430
Waldmann R et al (1997) A proton-gated cation channel involved in acid-sensing. Nature 386(6621):173–177
Wechselberger M et al (2006) Ionic channels and conductance-based models for hypothalamic neuronal thermosensitivity. Am J Physiol Regul Integr Comp Physiol 291(3):R518–R529
Wehage E et al (2002) Activation of the cation channel long transient receptor potential channel 2 (LTRPC2) by hydrogen peroxide. A splice variant reveals a mode of activation independent of ADP-ribose. J Biol Chem 277(26):23150–23156
Willette RN et al (2008) Systemic activation of the transient receptor potential vanilloid subtype 4 channel causes endothelial failure and circulatory collapse: Part 2. J Pharmacol Exp Ther 326(2):443–452
Woodbury CJ et al (2004) Nociceptors lacking TRPV1 and TRPV2 have normal heat responses. J Neurosci 24(28):6410–6415
Xiao B et al (2011) Temperature-dependent STIM1 activation induces Ca(2) + influx and modulates gene expression. Nat Chem Biol 7(6):351–358
Xu H et al (2002) TRPV3 is a calcium-permeable temperature-sensitive cation channel. Nature 418(6894):181–186
Xu C et al (2006a) Association of the putative susceptibility gene, transient receptor potential protein melastatin type 2, with bipolar disorder. Am J Med Genet B Neuropsychiatr Genet 141B(1):36–43
Xu H et al (2006b) Oregano, thyme and clove-derived flavors and skin sensitizers activate specific TRP channels. Nat Neurosci 9(5):628–635
Yang YD et al (2008) TMEM16A confers receptor-activated calcium-dependent chloride conductance. Nature 455(7217):1210–1215
Yoon IS et al (2001) Altered TRPC7 gene expression in bipolar-I disorder. Biol Psychiatry 50(8):620–626
Zhang YH et al (1995) Warm and cold signals from the preoptic area: which contribute more to the control of shivering in rats? J Physiol 485(Pt 1):195–202
Zhang D et al (2012a) Mast-cell degranulation induced by physical stimuli involves the activation of transient-receptor-potential channel TRPV2. Physiol Res 61(1):113–124
Zhang Z et al (2012b) TRPM2 Ca2+ channel regulates energy balance and glucose metabolism. Am J Physiol Endocrinol Metab 302(7):E807–E816

Chapter 9
TRP Channels in Cold Transduction

Alejandro González, Gonzalo Ugarte, Ricardo Piña, María Pertusa and Rodolfo Madrid

Abstract In the somatosensory system, cold thermoreceptor neurons and cold nociceptors are responsible for the detection of environmental low temperatures. The underlying machinery is far from simple; it is a result of the participation of several classes of transduction and voltage-gated ion channels that functionally coexist to give shape to the cold-induced receptor potential and subsequent action potential firing in response to cold stimulation. The cold-induced electrical responses begin in the free nerve endings of these sensory neurons, where a subgroup of thermosensitive **T**ransient **R**eceptor **P**otential channels (thermo**TRP**s) plays a critical role. These channels have evolved as molecular thermal sensors activated by a wide range of cold temperatures, and they have been proposed as key elements of the transduction machinery responsible for detection of environmental cold in primary somatosensory neurons. In this chapter, we summarize the most important functional properties of the primary sensory neurons involved in cutaneous cold detection, and the corresponding role of the thermoTRP channels TRPM8, TRPA1 and TRPC5 in cold transduction.

Keywords Cold transduction · TRPM8 · TRPA1 · TRPC5 · Primary sensory neurons · TRP channels

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9.1 Introduction

Primary sensory neurons of the peripheral somatosensory system are responsible for converting a wide range of environmental physical and chemical stimuli into electrical signals. Sensory signals begin at the nerve endings of these pseudounipolar neurons that innervate the skin and exposed mucosae, which home the molecular machinery necessary for the detection and transduction of these stimuli into a receptor potential (Fig. 9.1). The soma of primary somatosensory neurons is located in the dorsal root ganglia (DRG) and trigeminal ganglia (TG). These neurons project one axonal branch peripherally innervating the skin and mucosae, and the other branch centrally to dorsal horn neurons at the spinal cord and brain stem nuclei,

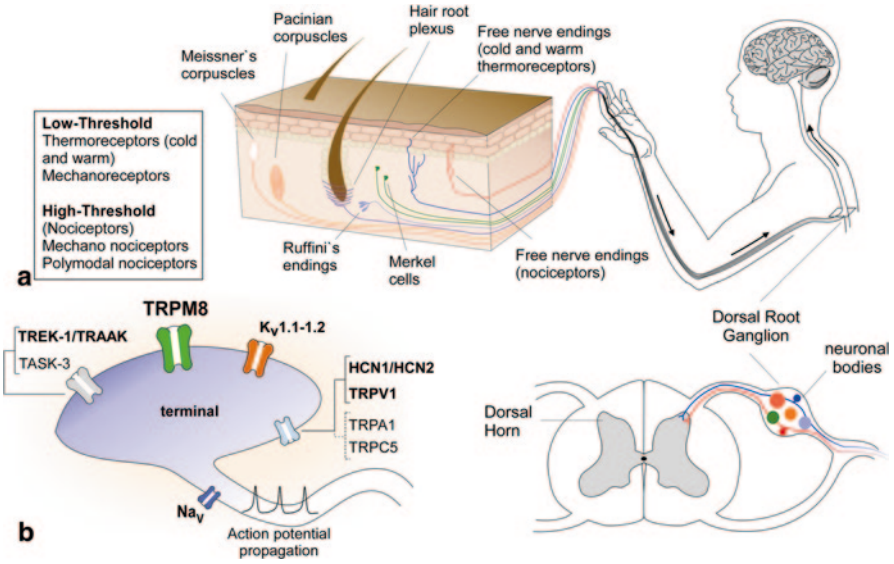

Fig. 9.1 Sensory innervation of the skin. **a**. Simplified schematic representation of the different primary sensory neurons innervating the skin. **b.** In the dorsal root ganglion at right, the somas of the different subclasses of primary sensory neurons that project to dorsal horn are represented in different colors. The nerve ending of a cold thermoreceptor neuron (CTN) is shown in the *left panel*. The site of cold transduction (terminal) is depicted as separated from the site of action potential generation and propagation. In this scheme, TRPM8 is the most prominent channel, representing its contribution to cold sensing. Kv1.1-1.2 channels (the molecular counterpart of the break potassium current I_{KD}), TREK-1/TRAAK and TASK-3 background potassium channels are also important components of the molecular machinery underlying cold temperature transduction. HCN1 and, to a lower extent, HCN2 channels (the molecular counterpart of I_h current) contribute to give shape to the net electrical response to temperature reductions in primary somatosensory neurons. The heat- and capsaicin-activated TRPV1 channel is also expressed in a large subpopulation of CTNs (~25%); TRPA1 is only expressed in a small subpopulation of CTNs (~5% of the TRPM8-expressing neurons). Although depicted, further studies are necessary to determine the role of TRPC5 in cold transduction. Among Na_v channels, $Na_{v1.8}$ channels are critical to allow impulse generation in primary sensory neurons at very low temperatures

where the information is transmitted towards the central integrative areas of the brain.

TG and DRG comprise a variety of neurons responding to a wide range of mechanical stimuli, chemical agents of different nature, and temperature, and the activation of functional subpopulations of the primary sensory neurons evoke diverse sensations of touch, itch, irritation, cold, heat and pain. Thus, primary somatosensory receptors can be divided into cold and warm thermoreceptors, mechanoreceptors and nociceptors. Based on the intensity of the stimuli necessary to initiate a sensory signal, primary somatosensory neurons can be separated into low-threshold and high-threshold receptors. Under this general classification, low-threshold receptor neurons correspond to thermoreceptors (cold and warm) and low-threshold mechanoreceptors, whereas high-threshold receptors correspond to nociceptors, which include polymodal nociceptors and mechanonociceptors (Fig. 9.1a).

The wide diversity of cold sensations that arise from the exposure of the skin and mucosae to environmental temperature reductions varies from comfortable cold to intense pain, depending on the intensity of the thermal stimulus. The perception of cold as an innocuous temperature drop takes place when the surface of the skin is cooled by as little as 1 °C, or even less, and cold normally feels painful or noxious at temperatures below 15 °C. The detection of innocuous and noxious cold is mediated by cold thermoreceptors and nociceptors respectively, expressing a large variety of transduction and voltage-gated channels. These channels work concertedly in generating the cold-induced receptor potential and subsequent action potential firing in response to an environmental temperature decrease. In the last years, the identification of ion channels with a strong thermal sensitivity, including several cold- and heat-activated members of the superfamily of **T**ransient **R**eceptor **P**otential (**TRP**) (also called thermoTRP channels), has shed light on the molecular mechanisms underlying the diverse sensory modalities that emerge from the activation of distinct sets of peripheral receptors, including cold-sensitive neurons (Vriens et al. 2014).

9.2 Cold Thermoreceptor and Cold Nociceptor Neurons

In mammals, cold thermoreceptor neurons (CTNs) correspond to subpopulations of small diameter unmyelinated primary afferent C-fibers and medium diameter thinly myelinated Aδ-fibers, in a proportion that varies among different species and somatic territories (Hensel and Zotterman 1951b; Iriuchijima and Zotterman 1960; Hensel 1981; Heppelmann et al. 1990). CTNs show a wide range of temperature thresholds, and can be separated into low-threshold and high-threshold cold-sensitive neurons, the latter having a role in the detection of cold discomfort under physiological conditions (Belmonte et al. 2009). At a normal skin temperature of 33 °C, cold thermoreceptors detecting and encoding innocuous or moderate cold typically exhibit spontaneous electrical activity, mainly in a beating (regular) pattern of action potential firing. The firing of these exquisitely temperature-sensitive neurons increases in response to temperature reductions of less than 1 °C, and is reduced

by heating. These cold afferents are sensitized by micromolar concentrations of the natural cold-mimetic compound menthol (Hensel and Zotterman 1951a; Brock et al. 2001). Cold thermoreceptor neurons comprise about 8–15 % of the neuronal population in DRG and TG.

The functional properties of primary somatosensory neurons that respond to innocuous and moderate cold have been extensively characterized by Ca^{2+}-imaging, patch-clamping and extracellular recordings in several somatic territories (Fig. 9.2). In culture, these neurons respond to cooling with a depolarizing inward current. The depolarization induces an increase in the action potential firing followed by a rise in intracellular Ca^{2+} concentration that depends on external Ca^{2+}. In CTNs,

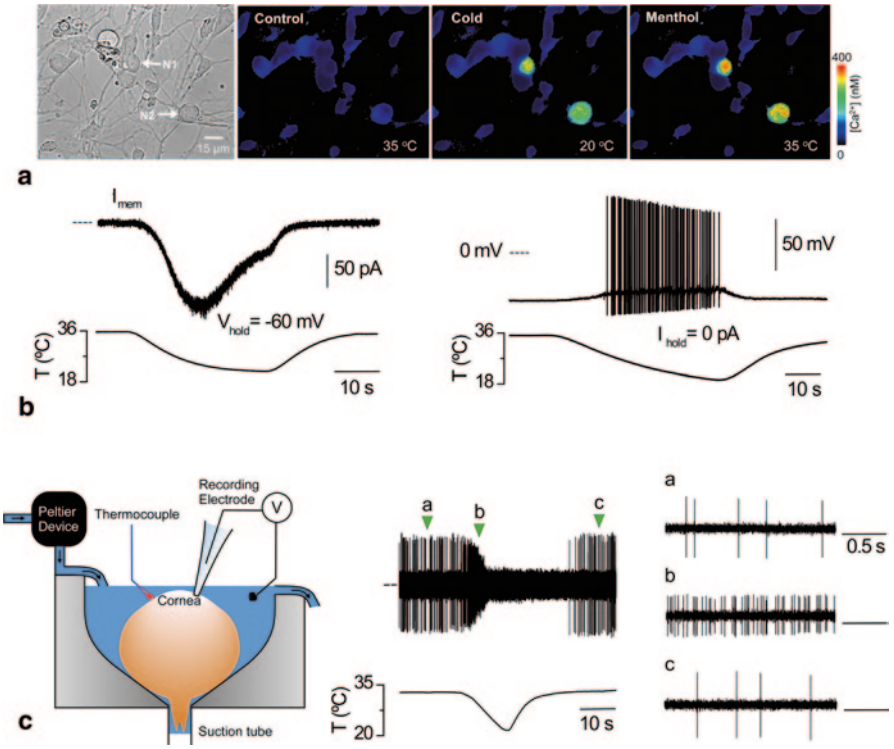

Fig. 9.2 Calcium imaging and electrophysiological tools in the study of thermal responses in cold thermoreceptor neurons. **a**. Transmitted and pseudocolor ratiometric $[Ca^{2+}]_i$ images cultured trigeminal neurons showing the effects of cold and menthol on its intracellular calcium concentration. Note that the same neurons are sensitive to both stimuli (Madrid and Viana, unpublished). Scale bar in *left* panel, 15 µm. **b**. *Left*, simultaneous recording of membrane current using patch clamp technique (*top* trace) and bath temperature (*bottom* trace) during a cooling ramp from 34 to 22 °C in a cold-sensitive trigeminal neuron (V_{hold}=−60 mV). *Right*, simultaneous recording of membrane potential (*top* trace) and bath temperature during a cooling ramp in a cold-sensitive neuron recorded in current-clamp mode (I_{hold}=0 pA) (González and Madrid, unpublished). **c**. Extracellular recording of nerve terminal impulses (NTI) activity in a corneal cold-sensitive neuron in response to cooling (González and Madrid, unpublished; Scheme at *left* modified from Parra et al. 2010)

intracellular Ca^{2+} increases in response to cold stimulation are mainly due to the activation of voltage-gated Ca^{2+} channels during action potential firing. As a consequence, there is a tight correlation between temperature threshold for firing of action potentials and for $[Ca^{2+}]_i$ elevations in each individual neuron (Viana et al. 2002; Madrid et al. 2006) (Fig. 9.3a). Canonical CTNs fire short duration action potentials (~ 1 ms at the half-amplitude of the depolarizing phase) (Fig. 9.3b) and are characterized by a low rheobase current and by the presence of a prominent hyperpolarization-activated current (I_h), mediated by HCN1 and, to a lower extent, HCN2 channels (Reid et al. 2002; Viana et al. 2002; Madrid et al. 2006, 2009; Orio et al. 2009, 2012).

As mentioned before, the vast majority of cold thermoreceptor neurons are sensitive to menthol, a natural cyclic terpene alcohol that induces cold sensations

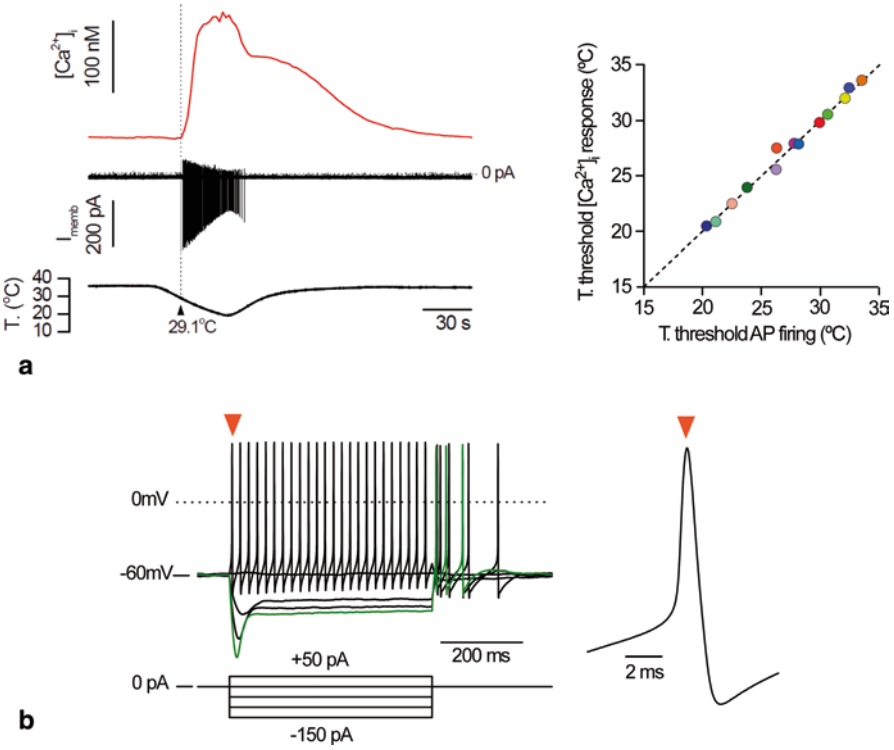

Fig. 9.3 The intracellular calcium increase in response to cold in cultured cold-sensitive neurons depends on the action potential firing. **a**. *Left*, simultaneous recording of $[Ca^{2+}]_i$ signals (*top* trace), action currents (*middle*) and bath temperature (*bottom*) during a cooling ramp in a cold-sensitive neuron in culture, recorded in cell-attached condition. *Right*, scatter plot of thresholds for action potential firing and $[Ca^{2+}]_i$ signals in response to cooling in 14 cold-sensitive neurons (each neuron has been color coded). The dotted line represents the unity line (González and Madrid, unpublished). **b**. *Left*, typical voltage responses (*upper* traces) to 500 ms hyperpolarizing and depolarizing current pulses (*lower* traces) of a trigeminal CTN. Note the fast repetitive discharge, the strong sag and the presence of rebound firing (*in green*). The first action potential of the train (*orange arrow*) is shown at *right* (González and Madrid, unpublished)

(Hensel and Zotterman 1951a). Menthol sensitivity in cold-sensitive fibers (and cold-sensitive cultured neurons) is manifested as an increase in the action potential firing frequency in response to this so-called cooling compound at 33 °C. Expressed differently, menthol shifts the temperature threshold of the cold-induced response to higher temperatures. This is not a trivial point, since high-threshold cold-sensitive neurons that do not respond to menthol at 33 °C could be erroneously classified as menthol-insensitive cells (Fig. 9.4).

CTNs maintain an ongoing firing activity at normal skin temperature (33 °C), suggesting a tone of excitatory input of CTNs to the central cells where they synapse. This basal activity has been attributed to a temperature-dependent rhythmic oscillation of the membrane potential (Braun et al. 1980) (See also Chap. 10 by *Orio and Olivares* in this book). After the action potential firing frequency in response to a temperature drop reaches the peak (dynamic response), it slowly decreases to a lower sustained rate at the new temperature (static response) (Hensel and Iahn 1973) (see Fig. 10.3 in Chap. 10 of this book). The amplitude and rate of change of the cold-induced response is proportional to the rate and magnitude of the temperature decrease. The firing changes gradually from a beating to a bursting pattern at lower temperatures, and when the temperature is raised back, the electrical activity transiently silences with the rewarming process (Hensel and Iahn 1973; Darian-Smith et al. 1973).

In contrast to cold thermoreceptor neurons, C- and Aδ-nociceptors responding to intense cold are silent at normal temperature of the skin, and fire only in response to temperatures below 15 °C. These neurons fire wide action potentials (>2 ms at the half-amplitude) with an inflection (or hump) in the falling phase, and also respond to other modalities of noxious stimuli (Bessou and Perl 1969; Croze et al. 1976;

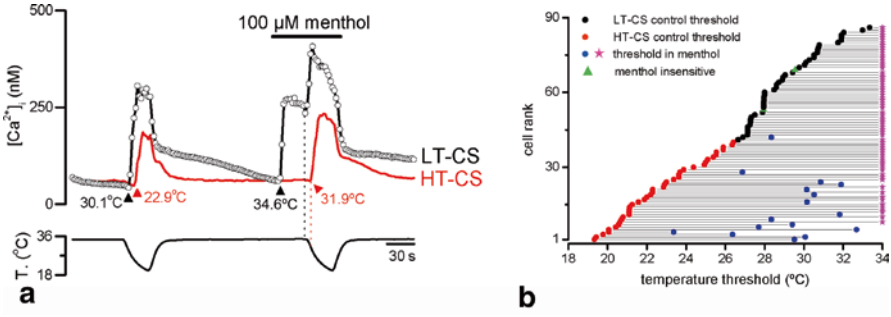

Fig. 9.4 Differential menthol sensitivity in low- and high-threshold cold thermoreceptor neurons. **a**. Simultaneous recordings of cold-induced $[Ca^{2+}]_i$ and bath temperature in two cold-sensitive neurons with different temperature thresholds (low-threshold (LT-CS) and high-threshold (HT-CS)) during two consecutive cooling ramps, in control condition and in the presence of 100 µM menthol. Note that in the low-threshold CSN menthol evoked a robust $[Ca^{2+}]_i$ rise at 34 °C, while in the HT-CS neurons menthol only shifted the threshold of cold induced response to higher temperatures. **b**. Dot plot summarizing the effect of 100 µM menthol on cold threshold in 86 trigeminal cold-sensitive neurons in culture. The neurons activated by 100 µM menthol at 34 °C are marked by a magenta star. The menthol-insensitive neurons are marked by a *green triangle*. (Modified from Madrid et al. 2009)

LaMotte and Thalhammer 1982; Campero et al. 1996; Simone and Kajander 1996, 1997; Craig et al. 2001). Interestingly, additional nociceptors are excited with larger temperature reductions. Moreover, almost all primary somatosensory neurons respond to cold temperatures below 0 °C, complicating the determination of the exact percentage of cold nociceptors in the peripheral somatosensory system (Simone and Kajander 1996, 1997; Campero et al. 2001).

In primary somatosensory neurons, TTx-insensitive $Na_v1.8$ sodium channels are critical for noxious cold response. Unlike the TTx-sensitive Na^+ channels, whose slow inactivation is potentiated by cold, the inactivation of $Na_v1.8$ is largely cold-insensitive. Thus, $Na_v1.8$ channels allow the impulse generation of nociceptive neurons at very low temperatures (Zimmermann et al. 2007). In addition, the increase in membrane resistance and the decrease in the activation threshold of the Na^+ currents induced by cold augment the membrane potential change in response to a depolarizing stimulus.

9.3 TRPM8 Channels in Cold Transduction

9.3.1 Molecular Cloning of TRPM8 and Expression in Primary Sensory Neurons

TRPM8 is the **8th** member of the **M**elastatin-related family of **TRP** channels. The encoding gene was originally identified by its expression in normal prostate epithelial cells and prostate carcinomas, and the full-length cDNA of human TRPM8 was formerly called trp-p8 (Tsavaler et al. 2001). The transcript corresponds to a 1104 amino acid protein with a marked homology to some members of the TRP channels superfamily. The expression of this protein in primary somatosensory neurons was not reported until 2002, when TRPM8 was cloned by two groups independently, and characterized as a cold- and menthol-activated ion channel (McKemy et al. 2002; Peier et al. 2002). In order to identify the putative cold and menthol receptor protein(s) responsible for cold and menthol sensitivity of CTNs, McKemy and coworkers constructed a cDNA expression library of trigeminal ganglia. They carried out a functional screening with this material, transfecting HEK293 cells with discrete cDNA pools obtained from this library. Using calcium imaging in isolated cells that exhibited intracellular calcium rises in response to menthol, they were able to identify a single cDNA sequence that conferred both menthol and cold sensitivity to these normally cold-insensitive cells, and classified it as a TRP channel (McKemy et al. 2002). Peier and colleagues, on the other hand, used a different strategy. Reasoning that the cold- and menthol-sensitive receptor could be a protein related to the recently cloned heat- and capsaicin-activated channel TRPV1 (Caterina et al. 1997), they searched directly for TRP-like proteins in genomic DNA databases looking for putative exons with similarity to the S4 and S6 transmembrane domains of TRPV1. One of these sequences was used to design primers to amplify a fragment of a puta-

tive TRP channel from a DRG cDNA library, and by using a **r**apid **a**mplification of **c**DNA **e**nds-PCR (RACE-PCR) in combination with an exon-prediction software, they obtained the full length sequence of the protein (Peier et al. 2002).

The TRPM8 channel is robustly expressed in trigeminal and dorsal root ganglia. TRPM8-positive neurons, corresponding to ~8–15 % of the neurons of these sensory ganglia, exhibit a relatively diverse neural and biochemical phenotype. These neurons show immunoreactivity to peripherin, a marker of C-fibers, and intermediate filament NF200, a marker of Aδ fibers. Furthermore, TRPM8 is co-expressed to a variable extent with nociceptive markers such as calcitonin gene-related peptide (CGRP), substance P, artemin receptor GFRα3 and the nociceptive channel TRPV1 (Viana et al. 2002; Babes et al. 2004; Okazawa et al. 2004; Xing et al. 2006; Takashima et al. 2007; Dhaka et al. 2008; Axelsson et al. 2009; Parra et al. 2010; Zimmermann et al. 2011; Lippoldt et al. 2013). The NGF receptor TrkA is also expressed in a large subpopulation of TRPM8-positive neurons, and mediates the increase in cold-sensitivity of CTNs induced by this growth factor (Babes et al. 2004). A fraction of cold- and menthol-sensitive neurons also responds to proinflammatory mediators such as bradykinin, histamine and prostaglandin E2, with a reduction of its sensitivity to agonists (Linte et al. 2007; Zhang et al. 2012). On the other hand, non-peptidergic primary sensory neurons that bind isolectin B4 do not show significant expression levels of TRPM8 (Takashima et al. 2007).This neurochemical phenotype is in line with a sensory role of TRPM8 channels beyond innocuous cold thermosensation.

9.3.2 Functional Properties and Modulation of TRPM8 Channels

As mentioned before, TRPM8 is a nonselective cationic channel activated by cold, by natural and artificial cooling compounds such as menthol and icilin, and by voltage (for recent reviews, see (Babes et al. 2011; Latorre et al. 2011; McCoy et al. 2011; Yudin and Rohacs 2012; Almaraz et al. 2014; Madrid and Pertusa 2014). Ion substitution experiments show a low discrimination of this channel among monovalent cations, but a significantly higher permeability for calcium ions ($P_{Ca}/P_{Na}=3.2$; $P_K/P_{Na}=1.1$; $P_{Cs}/P_K=1.2$) (McKemy et al. 2002). Both cold and menthol increase the open probability of the channel. Activation of TRPM8 by cooling-mimetic compounds such as menthol explains the refreshing sensation evoked by natural and artificial activators of this channel, widely used in many toiletries. Interestingly, higher concentrations of menthol can evoke sensations of irritation and burning pain, probably due to the activation of TRPA1 channels in nociceptors (Karashima et al. 2007). TRPM8 shows a strong outward rectification at depolarized membrane potentials and an estimated single channel conductance of 60–90 pS depending on temperature. It is directly activated by cold, with a Q_{10} temperature coefficient as high as 25. Activation of the TRPM8 channel by low temperatures and by most of its natural and artificial agonists is related to a shift in its voltage-activation curve

to more negative voltages. This shift results in an increase in the open probability of the channel at physiologically relevant membrane potentials in response to thermal and chemical stimulation (Brauchi et al. 2004; Voets et al. 2004, 2007b).

TRPM8 is a homotetramer, where each subunit consists of six transmembrane domains with the N- and C- termini facing the cytosolic side of the plasma membrane (Fig. 9.5a). As in other members of the TRP channels family, the C-terminal domain of TRPM8 contains a TRP domain (Clapham 2003; Montell 2005). This domain plays an important role in its modulation by phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) (Rohacs et al. 2005) (see below). The coiled-coil motif located in the distal C-terminal domain plays an important role in the tetramerization and function of the channel (Erler et al. 2006; Tsuruda et al. 2006; Phelps and Gaudet 2007). Several regions have recently been described in the N-terminal domain as relevant for both cold and menthol sensitivity and proper folding and assembly of the channel protein (Pedretti et al. 2009; Pertusa et al. 2014). The pore module consists of the S5 and S6 domains and the interconnecting loop, where the selectivity filter is located. A unique N-glycosylation, at asparagine 934 facilitates the segregation of the channel to lipid rafts (Morenilla-Palao et al. 2009). This N-glycosylation site is flanked by two cysteine residues that form a conserved double cysteine motif (C929 and C940), which is essential for channel function (Dragoni et al. 2006). N-glycosylation of TRPM8 occurs in both native and recombinant channels, and it has a direct effect on their biophysical properties. The membrane potential for half-maximal activation ($V_{1/2}$) of the channel carrying the single point mutation N934Q is 60 mV more positive than the wild type channel, shifting the mean temperature threshold of the cold response to lower temperatures (Pertusa et al. 2012) (see Chap. 3 by *Pertusa and Madrid* in this book).

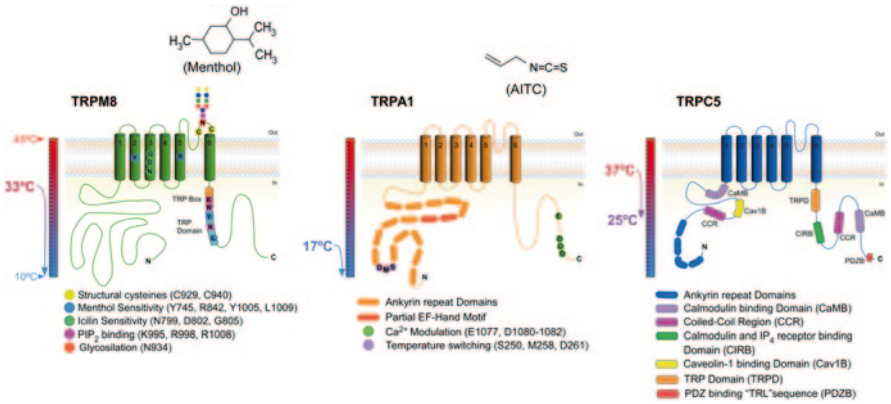

Fig. 9.5 Schematic representation of a TRPM8, TRPA1 and TRPC5 channel subunit. Left, TRPM8. Middle, TRPA1. Right, TRPC5. Each subunit presents six transmembrane domains, and the N- and C-terminal tails are located in the intracellular side of the plasma membrane. The most conspicuous functional domains and residues are indicated. Temperature-activation ranges and typical chemical activators are also depicted

Mean cold thresholds over 30 °C can be found in corneal cold-sensitive nerve endings and cultured CTNs, as opposed to a mean value of 25 °C observed in transfected hippocampal neurons or HEK293 cells heterologously expressing TRPM8 (Reid et al. 2002; Viana et al. 2002; de la Peña et al. 2005; Madrid et al. 2006, 2009; Malkia et al. 2007). At the same temperature, the $V_{1/2}$ by equivalent cold stimulation is ~ 140 mV more negative in native TRPM8 than in recombinant channels. This results in a significantly lower thermal excitation threshold of CTNs compared to the heterologous expression systems such as HEK293 cells (Malkia et al. 2007). Thus, the activation of TRPM8 at physiological membrane potentials results in larger inward currents in cold-sensitive neurons than in recombinant systems. Interestingly, it has been reported different temperature activation threshold and menthol sensitivity among TRPM8 orthologs, which is coincident with the mean body temperature in endothermic animals and tuned to the environmental temperature of its ecological niche in ectotherms (Myers et al. 2009). Recently, Fujita and coworkers have also reported that warm ambient temperatures shift the cold threshold of TRPM8 to higher temperatures (Fujita et al. 2013).

Charge-neutralizing mutations of positively charged residues in the S4 transmembrane segment and the S4-S5 linker of TRPM8 cause a decrease in its voltage dependence, suggesting that this region may be part of the voltage sensor (Voets et al. 2007a). Nevertheless, despite the advances in the study of the voltage and temperature dependence of TRPM8, and in contrast to its activation by cooling compounds, both the precise location of the voltage sensor and the molecular determinants of the temperature sensitivity remain elusive.

Functional and biophysical properties of TRPM8 can be finely tuned by diverse mechanisms, including lipid-protein interactions, protein-protein interactions, activation of intracellular cascades of second messengers and post-translational modifications such as phosphorylation and N-glycosylation. These mechanisms can influence its contribution to cold sensing under physiological and physiopathological conditions (see (Almaraz et al. 2014; Madrid and Pertusa 2014) for recent reviews; see also Chap. 3 by *Pertusa and Madrid* in this Book). In fact, both cold and menthol responses of TRPM8 are reduced by its basal association with lipid rafts at the plasma membrane (Morenilla-Palao et al. 2009). Although still unexplored, these results suggest that the association of TRPM8 with specific membrane microdomains may affect the targeting and trafficking dynamics of this channel in the nerve endings of cold-sensitive neurons. On the other hand, $PI(4,5)P_2$ is a critical cofactor for TRPM8 activation, and the positions K995, R998 and R1008 within the TRP domain appear to mediate the interaction of this lipid with the channel (Rohacs et al. 2005). $PI(4,5)P_2$ is able to activate TRPM8 in the absence of other physical and chemical stimuli (Liu and Qin 2005; Rohacs et al. 2005). Similarly to cold and cooling compounds, positive modulation of TRPM8 by $PI(4,5)P_2$ seems to be related to a shift in the $V_{1/2}$ value of voltage activation towards more negative membrane potentials (Daniels et al. 2009).

Desensitization of TRPM8 to sustained cold and menthol stimulation strongly depends on extracellular Ca^{2+} (McKemy et al. 2002). It has been suggested that this property is related to a depletion of $PI(4,5)P_2$ of the plasma membrane. Cal-

cium entry through TRPM8 activates Ca^{2+}-dependent PLC, reducing the sensitivity of the channel to thermal and chemical stimulation by diminishing $PI(4,5)P_2$ levels (Daniels et al. 2009). Sarria and coworkers on the other hand have proposed that tachyphylaxis in response to repetitive stimulation of TRPM8 would be mediated by PLC-dependent hydrolysis of $PI(4,5)P_2$ and the activation of a PKC/Protein Phosphatase 1 cascade, and that the desensitization by single sustained stimulation of the channel would depend on calmodulin activation and $PI(4,5)P_2$ availability (Sarria et al. 2011).

TRPM8 channel function can be also modulated by different protein kinases (see Chap. 3 by *Pertusa and Madrid* in this Book). These intracellular modulatory mechanisms could explain the desensitization of the channel induced by inflammatory mediators. Thus, it has been suggested that bradykinin reduces the cold and menthol responses of TRPM8 though the activation of Gq protein and PKC-dependent mechanisms, respectively (Premkumar et al. 2005; Linte et al. 2007). Nevertheless, recently Zhang and coworkers proposed an alternative explanation, suggesting that the inhibition of TRPM8 activity is due to a direct interaction of the Gαq subunit with the channel, independent of the signaling pathway downstream of Gαq-coupled receptors (Zhang et al. 2012).

9.3.3 TRPM8 as the Main Molecular Entity in Cold Transduction

In CTNs, the depolarizing inward cold-induced current (I_{cold}) is the main responsible for the excitatory receptor potential in response to temperature reductions. I_{cold} displays a biophysical and pharmacological profile consistent with a depolarizing current depending on the TRPM8 channel. This current exhibits outward rectification and can be fully suppressed by BCTC, a potent blocker of TRPM8 (Madrid et al. 2006).

CTNs display a wide range of temperature thresholds. This differential thermal sensitivity is to a large extent determined by the functional counterbalance of two conductances with opposite effects on temperature-dependent excitability. Differential functional expression of TRPM8 (the main responsible for the excitatory cold-activated current) and Kv1.1-1.2 Shaker -like potassium channels (responsible for the excitability break current I_{KD}) is intimately linked to the thermosensitive phenotype of individual CTNs. The fast-activating slow-inactivating outward I_{KD} current dampens the effect of the cold-induced depolarizing TRPM8-dependent current, shifting the temperature threshold of the neuron to higher values, reducing the net response of the CTN to temperature reductions. Interestingly, pharmacological suppression of I_{KD} induces cold-sensitivity in cold-insensitive neurons (Viana et al. 2002). It is important to mention here that a fraction of cold-sensitive neurons does not express TRPM8, suggesting the presence of other mechanisms. Closure of thermosensitive background K^+ channels TREK-1 and TRAAK by temperature drops also contributes to increase the excitability of CTNs neurons under cold

stimulation (Reid and Flonta 2001; Viana et al. 2002; Noel et al. 2009). Recently, TASK-3 and Kv7.2-7.3 potassium channels have also been proposed as modulators of cold-sensitivity in TRPM8-expressing neurons (Vetter et al. 2013; Morenilla-Palao et al. 2014).

The use of genetically modified mice has been critical to unveil the role of TRPM8 in sensing innocuous and noxious cold. Mice lacking functional expression of TRPM8 were developed by three groups independently (Bautista et al. 2007; Colburn et al. 2007; Dhaka et al. 2007), and all three TRPM8 knockout strains presented a strongly impaired cold sensitivity. TRPM8 knockout mice failed to discriminate between cold and warm environments. These mice exhibited a clearly reduced avoidance to cold in two-temperature tests and in thermotaxis assays of temperature gradients, when compared to wild type animals (Bautista et al. 2007; Colburn et al. 2007; Dhaka et al. 2007). Molecular ablation of the TRPM8 channel also reduced the behavioral responses to cooling agents and abolished their response to systemic stimulation with icilin, one of the strongest chemical agonists of TRPM8 (Dhaka et al. 2007). Using calcium imaging techniques in cultured primary sensory neurons, Bautista and coworkers additionally found that TRPM8 knockout animals showed a strong decrease in the incidence and magnitude of cold-induced responses (Bautista et al. 2007). Extracellular recordings of single sensory fibers revealed that both low- and high-threshold cold-sensitive primary afferents have impaired responses to temperature reductions. Cold-sensitive C-fibers from TRPM8 knockout mice also display lower basal action potential firing, but no effect on their general excitability (Bautista et al. 2007). Accordingly, basal firing of corneal CTNs and their responses to cold and menthol are virtually absent in TRPM8 knockout mice and proportionally reduced in heterozygous animals (Parra et al. 2010). Thus, not only the static and dynamic responses to temperature drops, but also the ongoing spontaneous activity of CTNs is largely dependent of the functional expression level of the cold- and menthol-activated channel TRPM8.

More recently, Knowlton and coworkers demonstrated that selective ablation of TRPM8-expressing neurons yielded animals with an even higher cold insensitivity in both innocuous and noxious range than observed in TRPM8 knockout animals (Knowlton et al. 2013). This result not only supports a critical role of TRPM8-expressing neurons in cold sensing, but also suggests the participation of other molecular mechanisms in cold-detection. Animals that lack TRPM8-expressing neurons have normal mechanical and heat sensitivity, suggesting that these neurons could be largely dispensable for other somatosensory modalities (Knowlton et al. 2013). Using a similar ablation strategy to eliminate TRPM8-expressing neurons but in adult mice, Pogorzala and coworkers demonstrated that this approach strongly reduced the responses of these animals to cold in a wide range of low temperatures (Pogorzala et al. 2013).

Summarizing, molecular, cellular, biophysical and behavioral studies support a key role of TRPM8 in the molecular and neural machinery responsible for cold sensing in a wide range of low temperatures.

9.4 TRPA1 Channels in Cold Transduction

9.4.1 Molecular Cloning of TRPA1 and Expression in Primary Sensory Neurons

Transient **Re**ceptor **P**otential **A**nkiryn **1** channel (**TRPA1**) , first identified in human fibroblasts and liposarcoma cells (Jaquemar et al. 1999), is the only member of the subfamily of TRP**A** channels found in mammals. A similar bioinformatic approach used in the molecular cloning of TRPM8 (Peier et al. 2002) was used by Patapoutian and his group to design primers for obtaining the full-length TRPA1 from mouse TG cDNA, encoding a 1115 amino acid protein (Story et al. 2003). TRPA1 was originally described as an ion channel activated by intense cold (< 17 °C) (Story et al. 2003). TRPA1 works as a sensor of cell threat, responding to a plethora of structurally diverse pungent and noxious compounds, and nowadays it is seen as the main molecular entity in the somatosensory system responsible for the detection of irritant and potentially harmful substances. The TRPA1 channel also plays an important role as regulator of neuropeptide release and neurogenic inflammation, and is considered a promising molecular target for new analgesic and anti-inflammatory drugs (for reviews see (Bautista et al. 2006; Baraldi et al. 2010; Nilius et al. 2012; Zygmunt and Hogestatt 2014)). Moreover, TRPA1 functions as a detector of potentially toxic compounds throughout the animal kingdom, implying that it emerged early in evolution (Kang et al. 2010; Macpherson and Patapoutian 2010).

The proportion of sensory neurons expressing TRPA1 in sensory ganglia varies from one report to another, with observations ranging from 3.6 to 56.7% (Story et al. 2003; Jordt et al. 2004; Kobayashi et al. 2005; Nagata et al. 2005). TRPA1 can be found in both peptidergic (substance P- and CGRP-positive) and non-peptidergic (IB4-positive) primary sensory neurons (Hjerling-Leffler et al. 2007; Barabas et al. 2012). The channel colocalizes with the heat-activated polymodal channel TRPV1 in a subpopulation of nociceptors, while its co-expression with TRPM8 channels in cold thermoreceptor neurons is very low, comprising about 5% of TRPM8-expressing cells (Story et al. 2003; Jordt et al. 2004; Parra et al. 2010).

The particularly high threshold for the activation of TRPA1 by cold temperatures, along with its expression in nociceptive neurons, makes it a strong candidate as the molecular entity responsible for transducing painful cold.

9.4.2 Functional Properties and Modulation of TRPA1 Channels

TRPA1 is most likely a homotetramer, with each subunit having a topology of six transmembrane segments (S1-S6), a putative pore loop and selectivity filter between S5 and S6, and large cytoplasmic N- and C- terminal domains (Fig. 9.5b). TRPA1 is highly permeable to Ca^{2+}, with a PCa/PNa of 5.8 and 17% of the inward current carried by Ca^{2+} ions measured under physiological ionic conditions

(Karashima et al. 2010). TRPA1 is also activated by voltage, showing a shift in its voltage dependence in response to agonists toward more negative membrane potentials (Karashima et al. 2007; Zurborg et al. 2007). In cell-attached patches, TRPA1 shows conductances of ~ 65 pS and ~ 110 pS in the inward and outward directions respectively under physiological conditions. In the absence of divalent cations, TRPA1 shows a linear I-V relationship with a slope conductance of ~ 120 pS (see (Nilius et al. 2011)). In heterologously expressed TRPA1 channels, cold produces an exponential increase in the open probability, which is accompanied by an important decrease in single channel conductance and an increase in the mean open time (Sawada et al. 2007; Karashima et al. 2009).

The molecular nature of TRPA1 agonists is remarkably diverse. The TRPA1 agonists include active ingredients in many natural compounds commonly used as spices in cooking: cinnamaldehyde present in cinnamon (Bandell et al. 2004), isothiocyanates in mustard oil, wasabi, and horseradish (Bandell et al. 2004; Jordt et al. 2004), allicin and diallyldisulfide in garlic (Bautista et al. 2005; Macpherson et al. 2005) and methyl salicylate in winter green oil (Bandell et al. 2004) are some examples. Noxious compounds like acrolein (in smoke and tear gas) (Bautista et al. 2006), Δ9-tetrahydrocannabinol (in marijuana) (Jordt et al. 2004), as well as irritant compounds generated endogenously during inflammatory, oxidative and nitrative stress responses, such as 4-hydroxynonenal, 15-deoxy-Δ12,14-prostaglandin J2 and nitrooleic acid, have also been reported as activators of TRPA1 (Trevisani et al. 2007; Macpherson et al. 2007b; Taylor-Clark et al. 2008, 2009), just to name a few. Many agonists of TRPA1 are electrophilic compounds that activate the channel through covalent modification of cysteine or lysine residues (Hinman et al. 2006; Macpherson et al. 2007a; Takahashi et al. 2008; Bang and Hwang 2009). In contrast, non-electrophilic compounds that activate TRPA1 bind to the channel in a non-covalent manner (Karashima et al. 2007; Fajardo et al. 2008; Talavera et al. 2009; Nilius et al. 2012).

Ca^{2+} ions modulate TRPA1 function. The N-terminal domain of this channel contains an EF-hand Ca^{2+} binding motif between **A**nkyrin **R**epeat **D**omain (ARD) 11 and 12, which has been related with Ca^{2+}-dependent modulation (Doerner et al. 2007; Zurborg et al. 2007). Ca^{2+} ions in the micromolar range induce a concentration-dependent leftward shift in the $V_{1/2}$ toward physiologically relevant membrane potentials, enhancing the channels' chemical responses (Doerner et al. 2007; Zurborg et al. 2007). However, later studies suggest that apparently EF-hand motif is not required for this phenomenon (Wang et al. 2008).

Furthermore, it has been shown that activation and desensitization of TRPA1 channels in response to cold and chemical agonists are modulated by Ca^{2+} ions (Wang et al. 2008; Karashima et al. 2009). Thus, when Ca^{2+} is present, the current elicited by addition of the potent TRPA1 agonist mustard-oil shows a rapid activation and further desensitization. In the absence of Ca^{2+}, both processes are still present but with higher rise and decay time constants. An alternative Ca^{2+} binding site has been proposed within a cluster of acidic residues in the distal C-terminus of TRPA1, that has been found to be important for Ca^{2+}-mediated modulation of the agonist-induced responses (Sura et al. 2012).

9.4.3 TRPA1 as a Molecular Determinant in Noxious Cold Sensitivity

Cold-sensitivity of mammalian TRPA1 has been matter of intense debate. The seminal study of Story and co-workers described that mammalian cell lines and *Xenopus* oocytes expressing recombinant TRPA1 channels responded to intense cooling (Story et al. 2003). However, these results were not corroborated by similar experiments carried out by Jordt and co-workers one year later (Jordt et al. 2004). These authors studied thermal and chemical responses of cultured TG neurons, and they did not find a direct correlation between the cells responding to mustard oil and to noxious cold (Jordt et al. 2004). Later studies have argued either in favour (Bandell et al. 2004; Kwan et al. 2006; Sawada et al. 2007; Karashima et al. 2009) or against (Zurborg et al. 2007; Knowlton et al. 2010; Dunham et al. 2010) the cold sensitivity of TRPA1 and its contribution to cold sensing *in vivo*.

An alternative gating mechanism has been proposed, where the thermal sensitivity of TRPA1 would be an effect of cold-induced intracellular Ca^{2+}-increases required for channel activation in HEK293 cells (Zurborg et al. 2007; Caspani and Heppenstall 2009). Nevertheless, Karashima and co-workers found that heterologously expressed TRPA1 channels were able to respond to cold in a Ca^{2+}-independent fashion (Karashima et al. 2009). This cooling activation followed the same scheme of shifting $V_{1/2}$ to more negative voltages, described as a general mechanism of thermal activation for other thermoTRP channels (Karashima et al. 2009).

It has been recently reported that differences in cold-sensitivity of TRPA1 channels, which appears to be largely species-dependent since mouse and rat TRPA1 can be activated by cold in heterologous expression systems but not its orthologous of monkey and human, would be related to differences in a single residue within the S5 domain. Moreover, this residue (G878 in rodent and V875 in primate) also appears underlie the different effects of menthol on the channel observed across species (Chen et al. 2013). Interestingly, Jabba and coworkers recently reported that three single point mutations (S250, M258 and D261) switch mouse TRPA1 channels from cold- to hot-sensitive without affecting its chemical sensitivity (Jabba et al. 2014).

The contribution of TRPA1 to cold-sensing *in vivo* has been studied using genetically modified animals. Thermal sensitivity of two different lines of transgenic TRPA1 null mice has been evaluated. Bautista and coworkers developed a TRPA1-deficient mouse through deletion of the putative pore-loop domain of the channel (Bautista et al. 2006). On the other hand, Kwan and coworkers, generated a TRPA1 null mouse deleting the exons downstream of the pore region, by insertion of an IRES cassette and polyadenylation sequences (Kwan et al. 2006). Although disruption of the TRPA1 gene abolishes the behavioral responses to chemical activators of the channel, it has no mayor effects in the response or prevalence of cold-sensitive neurons (Bautista et al. 2006; Kwan et al. 2006). Bautista and coworkers found that behavioral responses to noxious and innocuous cold stimuli between knockout and control mice were similar. In contrast, other studies using knockout mice show low-

er sensitivity to noxious and innocuous cold stimuli than wild type animals (Kwan et al. 2006; Gentry et al. 2010). Using the TRPA1 knockout mice developed by David Corey and his group, Karashima and coworkers reported a decreased cold sensitivity of null mutant mice, supporting the idea that TRPA1 plays a role in noxious cold detection *in vivo* (Karashima et al. 2009). A recent study on the TRPA1 knockout mice developed by Kwan and coworkers showed a considerable loss of nerve endings in the skin compared to wild type animals, which may account for the reduced responses to mechanical and cold stimuli of these mice *in vivo* (Andersson et al. 2013).

On the other hand, it has also been suggested that TRPA1 is a mediator of hypersensitivity to cold under pathological conditions (del Camino et al. 2010; Chen et al. 2011). It has also been reported that TRPA1 could play a role defining the thermal threshold in heat nociception (Hoffmann et al. 2013). In cultured vagal sensory neurons, Fajardo and coworkers showed a clear overlap between cold sensitivity of these neurons and TRPA1 pharmacology, and a large reduction in the population of cold-sensitive neurons from nodose ganglia in TRPA1 knockout mice compared with wild-type animals (Fajardo et al. 2008).

Thus, TRPA1 emerges as a critical component of the molecular machinery involved in the response to noxious agents in primary somatosensory neurons, with an important role in the release and detection of inflammatory mediators, and a species-dependent contribution to cutaneous thermal sensitivity under physiological conditions

9.5 TRPC5 Channel in Cold Transduction

Transient **R**eceptor **P**otential **C**anonical **5** (TRPC5) (Fig. 9.5c) is a Ca^{2+} -permeable nonselective cation channel predominantly expressed in central nervous system, but is also found in primary somatosensory neurons and other cells and tissues, including vascular smooth muscle cells, endothelial cells, T-lymphocytes, monocytes cardiac cells, adrenal medulla, mammary glands and yolk sac, among others (reviewed by (Jiang et al. 2011; Zholos 2014)). This channel was cloned by Okada and colleagues in 1998 and formerly named TRP5 (Okada et al. 1998). Its full length mRNA sequence was obtained from the screening of a Oligo(dT)-primed cDNA library constructed with poly(A) RNA from adult mouse brain. This channel shows a wide diversity of opening mechanisms and activators. TRPC5 is mainly activated through receptors coupled to Gq, PLC and/or Gi proteins, but can be directly activated by a large list of agonist of the most diverse nature. This channel may also open in a store-dependent manner that requires other partner proteins, including other TRP channels (see (Zholos 2014)). It has been reported that TRPC5 also displays cold sensitivity in the mild to intense cold range (Zimmermann et al. 2011). Heterologous expression of TRPC5 in HEK293 cells generates cold-sensitive cation currents at temperatures below 37 °C that can be potentiated by the PLC-Gq cascade activated by carbachol (Zimmermann et al. 2011). In addition to its sensitivity to cold, the expression pattern of this thermoTRP channel in primary somatosensory

neurons also pointed to a role in cold thermotransduction. Specifically, this channel is expressed in ~32% of mouse DRG neurons of small and medium diameter, in the nerve endings, axons and soma of these neurons, and in the regions I/II/III of superficial laminae of the dorsal horn of the spinal cord (Zimmermann et al. 2011).

A TRPC5 deficient mouse strain was developed by Riccio and co-workers in 2009, using a targeting construct that allows the deletion of the genomic region encoding amino acids 412–459 of the TRPC5 gene and the introduction of a frame shift and a stop codon (Riccio et al. 2009). Calcium imaging experiments using cultured DRG neurons from TRPC5 knockout mice revealed a significant reduction in the percentage of cold-sensitive neurons, with a decrease in both the menthol-sensitive and the menthol-insensitive populations of cold-responding neurons (Zimmermann et al. 2011). These results are in agreement with the reduction in the total population of TRPM8-possitive neurons detected by immunostaining. However, in several behavioral tests, TRPC5 knockout mice have no impairment in their responses to cold in a wide range of temperatures, suggesting that TRPC5 is not required for innocuous and noxious cold detection (Zimmermann et al. 2011). The authors suggest that cold-sensitivity of TRPC5 could be involved in other adaptations, such as localized metabolic changes, vascular changes, retraction of neurites, or initiation of transcriptional programs, rather than cold thermotransduction.

9.6 Concluding Remarks

In summary, an increasing number of cellular, molecular, electrophysiological and behavioral studies support the idea that, among the thermoTRP channels activated by cold temperatures, TRPM8 is the main component of the molecular machinery responsible for cold sensitivity in primary somatosensory neurons. The uncontested involvement of TRPM8 in innocuous cold transduction is also being extended nowadays to noxious cold detection. TRPA1 appears to have a species-dependent contribution to cutaneous noxious-cold sensing under physiological conditions, and it is critical to the cold-sensitivity of visceral vagal afferents. On the other hand, further studies are necessary to determine if TRPC5 has a significant contribution to cold sensing. The molecular basis underlying cold-sensitivity of these thermoTRP channels is an exciting open question, and the whole picture of the role that these cold-activated thermoTRP channels play beyond thermosensation is still emerging.

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References

Almaraz L, Manenschijn JA, de la Pena E, Viana F (2014) Trpm8. Handb Exp Pharmacol 222:547–579

Andersson DA, Gentry C, Light E, Vastani N, Vallortigara J, Bierhaus A, Fleming T, Bevan S (2013) Methylglyoxal evokes pain by stimulating TRPA1. PLoS One 8:e77986

Axelsson HE, Minde JK, Sonesson A, Toolanen G, Hogestatt ED, Zygmunt PM (2009) Transient receptor potential vanilloid 1, vanilloid 2 and melastatin 8 immunoreactive nerve fibers in human skin from individuals with and without Norrbottnian congenital insensitivity to pain. Neuroscience 162:1322–1332

Babes A, Zorzon D, Reid G (2004) Two populations of cold-sensitive neurons in rat dorsal root ganglia and their modulation by nerve growth factor. Eur J Neurosci 20:2276–2282

Babes A, Ciobanu AC, Neacsu C, Babes RM (2011) TRPM8, a sensor for mild cooling in mammalian sensory nerve endings. Curr Pharm Biotechnol 12:78–88

Bandell M, Story GM, Hwang SW, Viswanath V, Eid SR, Petrus MJ, Earley TJ, Patapoutian A (2004) Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. Neuron 41:849–857

Bang S, Hwang SW (2009) Polymodal ligand sensitivity of TRPA1 and its modes of interactions. J Gen Physiol 133:257–262

Barabas ME, Kossyreva EA, Stucky CL (2012) TRPA1 is functionally expressed primarily by IB4-binding, non-peptidergic mouse and rat sensory neurons. PLoS One 7:e47988

Baraldi PG, Preti D, Materazzi S, Geppetti P (2010) Transient receptor potential ankyrin 1 (TRPA1) channel as emerging target for novel analgesics and anti-inflammatory agents. J Med Chem 53:5085–5107

Bautista DM, Movahed P, Hinman A, Axelsson HE, Sterner O, Hogestatt ED, Julius D, Jordt SE, Zygmunt PM (2005) Pungent products from garlic activate the sensory ion channel TRPA1. Proc Natl Acad Sci U S A 102:12248–12252

Bautista DM, Jordt SE, Nikai T, Tsuruda PR, Read AJ, Poblete J, Yamoah EN, Basbaum AI, Julius D (2006) TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. Cell 124:1269–1282

Bautista DM, Siemens J, Glazer JM, Tsuruda PR, Basbaum AI, Stucky CL, Jordt SE, Julius D (2007) The menthol receptor TRPM8 is the principal detector of environmental cold. Nature 448:204–208

Belmonte C, Brock JA, Viana F (2009) Converting cold into pain. Exp Brain Res 196:13–30

Bessou P, Perl ER (1969) Response of cutaneous sensory units with unmyelinated fibers to noxious stimuli. J Neurophysiol 32:1025–1043

Brauchi S, Orio P, Latorre R (2004) Clues to understanding cold sensation: thermodynamics and electrophysiological analysis of the cold receptor TRPM8. Proc Natl Acad Sci U S A 101:15494–15499

Braun HA, Bade H, Hensel H (1980) Static and dynamic discharge patterns of bursting cold fibers related to hypothetical receptor mechanisms. Pflugers Arch 386:1–9

Brock JA, Pianova S, Belmonte C (2001) Differences between nerve terminal impulses of polymodal nociceptors and cold sensory receptors of the guinea-pig cornea. J Physiol 533:493–501

Campero M, Serra J, Ochoa JL (1996) C-polymodal nociceptors activated by noxious low temperature in human skin. J Physiol 497(Pt 2):565–572

Campero M, Serra J, Bostock H, Ochoa JL (2001) Slowly conducting afferents activated by innocuous low temperature in human skin. J Physiol 535:855–865

Caspani O, Heppenstall PA (2009) TRPA1 and cold transduction: an unresolved issue? J Gen Physiol 133:245–249

Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389:816–824

Chen J et al (2011) Selective blockade of TRPA1 channel attenuates pathological pain without altering noxious cold sensation or body temperature regulation. Pain 152:1165–1172

Chen J, Kang D, Xu J, Lake M, Hogan JO, Sun C, Walter K, Yao B, Kim D (2013) Species differences and molecular determinant of TRPA1 cold sensitivity. Nat Commun 4:2501
Clapham DE (2003) TRP channels as cellular sensors. Nature 426:517–524
Colburn RW, Lubin ML, Stone DJ, Jr, Wang Y, Lawrence D, D'Andrea MR, Brandt MR, Liu Y, Flores CM, Qin N (2007) Attenuated cold sensitivity in TRPM8 null mice. Neuron 54:379–386
Craig AD, Krout K, Andrew D (2001) Quantitative response characteristics of thermoreceptive and nociceptive lamina I spinothalamic neurons in the cat. J Neurophysiol 86:1459–1480
Croze S, Duclaux R, Kenshalo DR (1976) The thermal sensitivity of the polymodal nociceptors in the monkey. J Physiol 263:539–562
Daniels RL, Takashima Y, McKemy DD (2009) Activity of the neuronal cold sensor TRPM8 is regulated by phospholipase C via the phospholipid phosphoinositol 4,5-bisphosphate. J Biol Chem 284:1570–1582
Darian-Smith I, Johnson KO, Dykes R (1973) "Cold" fiber population innervating palmar and digital skin of the monkey: responses to cooling pulses. J Neurophysiol 36:325–346
de la Peña E, Malkia A, Cabedo H, Belmonte C, Viana F (2005) The contribution of TRPM8 channels to cold sensing in mammalian neurones. J Physiol 567:415–426
del Camino D, Murphy S, Heiry M, Barrett LB, Earley TJ, Cook CA, Petrus MJ, Zhao M, D'Amours M, Deering N, Brenner GJ, Costigan M, Hayward NJ, Chong JA, Fanger CM, Woolf CJ, Patapoutian A, Moran MM (2010) TRPA1 contributes to cold hypersensitivity. J Neurosci 30:15165–15174
Dhaka A, Murray AN, Mathur J, Earley TJ, Petrus MJ, Patapoutian A (2007) TRPM8 is required for cold sensation in mice. Neuron 54:371–378
Dhaka A, Earley TJ, Watson J, Patapoutian A (2008) Visualizing cold spots: TRPM8-expressing sensory neurons and their projections. J Neurosci 28:566–575
Doerner JF, Gisselmann G, Hatt H, Wetzel CH (2007) Transient receptor potential channel A1 is directly gated by calcium ions. J Biol Chem 282:13180–13189
Dragoni I, Guida E, McIntyre P (2006) The cold and menthol receptor TRPM8 contains a functionally important double cysteine motif. J Biol Chem 281:37353–37360
Dunham JP, Leith JL, Lumb BM, Donaldson LF (2010) Transient receptor potential channel A1 and noxious cold responses in rat cutaneous nociceptors. Neuroscience 165:1412–1419
Erler I, Al-Ansary DM, Wissenbach U, Wagner TF, Flockerzi V, Niemeyer BA (2006) Trafficking and assembly of the cold-sensitive TRPM8 channel. J Biol Chem 281:38396–38404
Fajardo O, Meseguer V, Belmonte C, Viana F (2008) TRPA1 channels mediate cold temperature sensing in mammalian vagal sensory neurons: pharmacological and genetic evidence. J Neurosci 28:7863–7875
Fujita F, Uchida K, Takaishi M, Sokabe T, Tominaga M (2013) Ambient temperature affects the temperature threshold for TRPM8 activation through interaction of phosphatidylinositol 4,5-bisphosphate. J Neurosci 33:6154–6159
Gentry C, Stoakley N, Andersson DA, Bevan S (2010) The roles of iPLA2, TRPM8 and TRPA1 in chemically induced cold hypersensitivity. Mol Pain 6:4
Hensel H (1981) Thermoreception and temperature regulation. Monogr Physiol Soc 38:1–321
Hensel H, Iahn M (1973) Cutaneous thermoreceptors. J Neurophysiol 14:377–385. (Handbook Sensory Physiology NY)
Hensel H, Zotterman Y (1951a) The effect of menthol on the thermoreceptors. Acta Physiol Scand 24:27–34
Hensel H, Zotterman Y (1951b) The response of the cold receptors to constant cooling. Acta Physiol Scand 22:96–105
Heppelmann B, Messlinger K, Neiss WF, Schmidt RF (1990) Ultrastructural three-dimensional reconstruction of group III and group IV sensory nerve endings ("free nerve endings") in the knee joint capsule of the cat: evidence for multiple receptive sites. J Comp Neurol 292:103–116
Hinman A, Chuang HH, Bautista DM, Julius D (2006) TRP channel activation by reversible covalent modification. Proc Natl Acad Sci U S A 103:19564–19568

Hjerling-Leffler J, Alqatari M, Ernfors P, Koltzenburg M (2007) Emergence of functional sensory subtypes as defined by transient receptor potential channel expression. J Neurosci 27:2435–2443

Hoffmann T, Kistner K, Miermeister F, Winkelmann R, Wittmann J, Fischer MJ, Weidner C, Reeh PW (2013) TRPA1 and TRPV1 are differentially involved in heat nociception of mice. Eur J Pain 17:1472–1482

Iriuchijima J, Zotterman Y (1960) The specificity of afferent cutaneous C fibres in mammals. Acta Physiol Scand 49:267–278

Jabba S, Goyal R, Sosa-Pagan JO, Moldenhauer H, Wu J, Kalmeta B, Bandell M, Latorre R, Patapoutian A, Grandl J (2014) Directionality of temperature activation in mouse TRPA1 ion channel can be inverted by single-point mutations in ankyrin repeat six. Neuron 82:1017–1031

Jaquemar D, Schenker T, Trueb B (1999) An ankyrin-like protein with transmembrane domains is specifically lost after oncogenic transformation of human fibroblasts. J Biol Chem 274:7325–7333

Jiang LH, Gamper N, Beech DJ (2011) Properties and therapeutic potential of transient receptor potential channels with putative roles in adversity: focus on TRPC5, TRPM2 and TRPA1. Curr Drug Targets 12:724–736

Jordt SE, Bautista DM, Chuang HH, McKemy DD, Zygmunt PM, Hogestatt ED, Meng ID, Julius D (2004) Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. Nature 427:260–265

Kang K, Pulver SR, Panzano VC, Chang EC, Griffith LC, Theobald DL, Garrity PA (2010) Analysis of Drosophila TRPA1 reveals an ancient origin for human chemical nociception. Nature 464:597–600

Karashima Y, Damann N, Prenen J, Talavera K, Segal A, Voets T, Nilius B (2007) Bimodal action of menthol on the transient receptor potential channel TRPA1. J Neurosci 27:9874–9884

Karashima Y, Talavera K, Everaerts W, Janssens A, Kwan KY, Vennekens R, Nilius B, Voets T (2009) TRPA1 acts as a cold sensor in vitro and in vivo. Proc Natl Acad Sci U S A 106:1273–1278

Karashima Y, Prenen J, Talavera K, Janssens A, Voets T, Nilius B (2010) Agonist-induced changes in Ca(2+) permeation through the nociceptor cation channel TRPA1. Biophys J 98:773–783

Knowlton WM, Bifolck-Fisher A, Bautista DM, McKemy DD (2010) TRPM8, but not TRPA1, is required for neural and behavioral responses to acute noxious cold temperatures and cold-mimetics in vivo. Pain 150:340–350

Knowlton WM, Palkar R, Lippoldt EK, McCoy DD, Baluch F, Chen J, McKemy DD (2013) A sensory-labeled line for cold: TRPM8-expressing sensory neurons define the cellular basis for cold, cold pain, and cooling-mediated analgesia. J Neurosci 33:2837–2848

Kobayashi K, Fukuoka T, Obata K, Yamanaka H, Dai Y, Tokunaga A, Noguchi K (2005) Distinct expression of TRPM8, TRPA1, and TRPV1 mRNAs in rat primary afferent neurons with adelta/c-fibers and colocalization with trk receptors. J Comp Neurol 493:596–606

Kwan KY, Allchorne AJ, Vollrath MA, Christensen AP, Zhang DS, Woolf CJ, Corey DP (2006) TRPA1 contributes to cold, mechanical, and chemical nociception but is not essential for hair-cell transduction. Neuron 50:277–289

LaMotte RH, Thalhammer JG (1982) Response properties of high-threshold cutaneous cold receptors in the primate. Brain Res 244:279–287

Latorre R, Brauchi S, Madrid R, Orio P (2011) A cool channel in cold transduction. Physiology (Bethesda) 26:273–285

Linte RM, Ciobanu C, Reid G, Babes A (2007) Desensitization of cold- and menthol-sensitive rat dorsal root ganglion neurones by inflammatory mediators. Exp Brain Res 178:89–98

Lippoldt EK, Elmes RR, McCoy DD, Knowlton WM, McKemy DD (2013) Artemin, a glial cell line-derived neurotrophic factor family member, induces TRPM8-dependent cold pain. J Neurosci 33:12543–12552

Liu B, Qin F (2005) Functional control of cold- and menthol-sensitive TRPM8 ion channels by phosphatidylinositol 4,5-bisphosphate. J Neurosci 25:1674–1681

Macpherson LJ, Patapoutian A (2010) Channels: flies feel your pain. Nat Chem Biol 6:252–253

Macpherson LJ, Geierstanger BH, Viswanath V, Bandell M, Eid SR, Hwang S, Patapoutian A (2005) The pungency of garlic: activation of TRPA1 and TRPV1 in response to allicin. Curr Biol 15:929–934

Macpherson LJ, Dubin AE, Evans MJ, Marr F, Schultz PG, Cravatt BF, Patapoutian A (2007a) Noxious compounds activate TRPA1 ion channels through covalent modification of cysteines. Nature 445:541–545

Macpherson LJ, Xiao B, Kwan KY, Petrus MJ, Dubin AE, Hwang S, Cravatt B, Corey DP, Patapoutian A (2007b) An ion channel essential for sensing chemical damage. J Neurosci 27:11412–11415

Madrid R, Pertusa M (2014) Intimacies and physiological role of the polymodal cold-sensitive ion channel TRPM8. Curr Top Membr 74:293–324

Madrid R, Donovan-Rodriguez T, Meseguer V, Acosta MC, Belmonte C, Viana F (2006) Contribution of TRPM8 channels to cold transduction in primary sensory neurons and peripheral nerve terminals. J Neurosci 26:12512–12525

Madrid R, de la Peña E, Donovan-Rodriguez T, Belmonte C, Viana F (2009) Variable threshold of trigeminal cold-thermosensitive neurons is determined by a balance between TRPM8 and Kv1 potassium channels. J Neurosci 29:3120–3131

Malkia A, Madrid R, Meseguer V, de la Peña E, Valero M, Belmonte C, Viana F (2007) Bidirectional shifts of TRPM8 channel gating by temperature and chemical agents modulate the cold sensitivity of mammalian thermoreceptors. J Physiol 581:155–174

McCoy DD, Knowlton WM, McKemy DD (2011) Scraping through the ice: uncovering the role of TRPM8 in cold transduction. Am J Physiol Regul Integr Comp Physiol 300:R1278–R1287

McKemy DD, Neuhausser WM, Julius D (2002) Identification of a cold receptor reveals a general role for TRP channels in thermosensation. Nature 416:52–58

Montell C (2005) The TRP superfamily of cation channels. Sci STKE 2005:re3

Morenilla-Palao C, Pertusa M, Meseguer V, Cabedo H, Viana F (2009) Lipid raft segregation modulates TRPM8 channel activity. J Biol Chem 284:9215–9224

Morenilla-Palao C, Luis E, Fernandez-Pena C, Quintero E, Weaver JL, Bayliss DA, Viana F (2014) Ion channel profile of TRPM8 cold receptors reveals a role of TASK-3 potassium channels in thermosensation. Cell Rep 8:1571–1582

Myers BR, Sigal YM, Julius D (2009) Evolution of thermal response properties in a cold-activated TRP channel. PLoS One 4:e5741

Nagata K, Duggan A, Kumar G, Garcia-Anoveros J (2005) Nociceptor and hair cell transducer properties of TRPA1, a channel for pain and hearing. J Neurosci 25:4052–4061

Nilius B, Prenen J, Owsianik G (2011) Irritating channels: the case of TRPA1. J Phys 589:1543–1549

Nilius B, Appendino G, Owsianik G (2012) The transient receptor potential channel TRPA1: from gene to pathophysiology. Pflugers Arch 464:425–458

Noel J, Zimmermann K, Busserolles J, Deval E, Alloui A, Diochot S, Guy N, Borsotto M, Reeh P, Eschalier A, Lazdunski M (2009) The mechano-activated K + channels TRAAK and TREK-1 control both warm and cold perception. EMBO J 28:1308–1318

Okada T, Shimizu S, Wakamori M, Maeda A, Kurosaki T, Takada N, Imoto K, Mori Y (1998) Molecular cloning and functional characterization of a novel receptor-activated TRP Ca2+ channel from mouse brain. J Biol Chem 273:10279–10287

Okazawa M, Inoue W, Hori A, Hosokawa H, Matsumura K, Kobayashi S (2004) Noxious heat receptors present in cold-sensory cells in rats. Neurosci Lett 359:33–36

Orio P, Madrid R, de la Peña E, Parra A, Meseguer V, Bayliss DA, Belmonte C, Viana F (2009) Characteristics and physiological role of hyperpolarization activated currents in mouse cold thermoreceptors. J Physiol 587:1961–1976

Orio P, Parra A, Madrid R, Gonzalez O, Belmonte C, Viana F (2012) Role of Ih in the firing pattern of mammalian cold thermoreceptor endings. J Neurophysiol 108:3009–3023

Parra A, Madrid R, Echevarria D, Del OS, Morenilla-Palao C, Acosta MC, Gallar J, Dhaka A, Viana F, Belmonte C (2010) Ocular surface wetness is regulated by TRPM8-dependent cold thermoreceptors of the cornea. Nat Med 16:1396–1399

Pedretti A, Marconi C, Bettinelli I, Vistoli G (2009) Comparative modeling of the quaternary structure for the human TRPM8 channel and analysis of its binding features. Biochim Biophys Acta 1788:973–982

Peier AM, Moqrich A, Hergarden AC, Reeve AJ, Andersson DA, Story GM, Earley TJ, Dragoni I, McIntyre P, Bevan S, Patapoutian A (2002) A TRP channel that senses cold stimuli and menthol. Cell 108:705–715

Pertusa M, Madrid R, Morenilla-Palao C, Belmonte C, Viana F (2012) N-Glycosylation of TRPM8 Ion Channels Modulates Temperature Sensitivity of Cold Thermoreceptor Neurons. J Biol Chem 287:18218–18229

Pertusa M, González A, Hardy P, Madrid R, Viana F (2014) Bidirectional modulation of thermal and chemical sensitivity of TRPM8 channels by the initial region of the N-terminal domain. J Biol Chem 289:21828–21843

Phelps CB, Gaudet R (2007) The role of the N terminus and transmembrane domain of TRPM8 in channel localization and tetramerization. J Biol Chem 282:36474–36480

Pogorzala LA, Mishra SK, Hoon MA (2013) The cellular code for mammalian thermosensation. J Neurosci 33:5533–5541

Premkumar LS, Raisinghani M, Pingle SC, Long C, Pimentel F (2005) Downregulation of transient receptor potential melastatin 8 by protein kinase C-mediated dephosphorylation. J Neurosci 25:11322–11329

Reid G, Flonta ML (2001) Physiology. Cold current in thermoreceptive neurons. Nature 413:480

Reid G, Babes A, Pluteanu F (2002) A cold- and menthol-activated current in rat dorsal root ganglion neurones: properties and role in cold transduction. J Physiol 545:595–614

Riccio A, Li Y, Moon J, Kim KS, Smith KS, Rudolph U, Gapon S, Yao GL, Tsvetkov E, Rodig SJ, Van't Veer A, Meloni EG, Carlezon WA, Jr, Bolshakov VY, Clapham DE (2009) Essential role for TRPC5 in amygdala function and fear-related behavior. Cell 137:761–772

Rohacs T, Lopes CM, Michailidis I, Logothetis DE (2005) PI(4,5)P2 regulates the activation and desensitization of TRPM8 channels through the TRP domain. Nat Neurosci 8:626–634

Sarria I, Ling J, Zhu MX, Gu JG (2011) TRPM8 acute desensitization is mediated by calmodulin and requires PIP(2): distinction from tachyphylaxis. J Neurophysiol 106:3056–3066

Sawada Y, Hosokawa H, Hori A, Matsumura K, Kobayashi S (2007) Cold sensitivity of recombinant TRPA1 channels. Brain Res 1160:39–46

Simone DA, Kajander KC (1996) Excitation of rat cutaneous nociceptors by noxious cold. Neurosci Lett 213:53–56

Simone DA, Kajander KC (1997) Responses of cutaneous A-fiber nociceptors to noxious cold. J Neurophysiol 77:2049–2060

Story GM, Peier AM, Reeve AJ, Eid SR, Mosbacher J, Hricik TR, Earley TJ, Hergarden AC, Andersson DA, Hwang SW, McIntyre P, Jegla T, Bevan S, Patapoutian A (2003) ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. Cell 112:819–829

Sura L, Zima V, Marsakova L, Hynkova A, Barvik I, Vlachova V (2012) C-terminal acidic cluster is involved in Ca^{2+} -induced regulation of human transient receptor potential ankyrin 1 channel. J Biol Chem 287:18067–18077

Takahashi N, Mizuno Y, Kozai D, Yamamoto S, Kiyonaka S, Shibata T, Uchida K, Mori Y (2008) Molecular characterization of TRPA1 channel activation by cysteine-reactive inflammatory mediators. Channels (Austin) 2:287–298

Takashima Y, Daniels RL, Knowlton W, Teng J, Liman ER, McKemy DD (2007) Diversity in the neural circuitry of cold sensing revealed by genetic axonal labeling of transient receptor potential melastatin 8 neurons. J Neurosci 27:14147–14157

Talavera K, Gees M, Karashima Y, Meseguer VM, Vanoirbeek JA, Damann N, Everaerts W, Benoit M, Janssens A, Vennekens R, Viana F, Nemery B, Nilius B, Voets T (2009) Nicotine activates the chemosensory cation channel TRPA1. Nat Neurosci 12:1293–1299

Taylor-Clark TE, Undem BJ, Macglashan DW, Jr, Ghatta S, Carr MJ, McAlexander MA (2008) Prostaglandin-induced activation of nociceptive neurons via direct interaction with transient receptor potential A1 (TRPA1). Mol Pharmacol 73:274–281

Taylor-Clark TE, Ghatta S, Bettner W, Undem BJ (2009) Nitrooleic acid, an endogenous product of nitrative stress, activates nociceptive sensory nerves via the direct activation of TRPA1. Mol Pharmacol 75:820–829

Trevisani M, Siemens J, Materazzi S, Bautista DM, Nassini R, Campi B, Imamachi N, Andre E, Patacchini R, Cottrell GS, Gatti R, Basbaum AI, Bunnett NW, Julius D, Geppetti P (2007) 4-Hydroxynonenal, an endogenous aldehyde, causes pain and neurogenic inflammation through activation of the irritant receptor TRPA1. Proc Natl Acad Sci U S A 104:13519–13524

Tsavaler L, Shapero MH, Morkowski S, Laus R (2001) Trp-p8, a novel prostate-specific gene, is up-regulated in prostate cancer and other malignancies and shares high homology with transient receptor potential calcium channel proteins. Cancer Res 61:3760–3769

Tsuruda PR, Julius D, Minor DL, Jr (2006) Coiled coils direct assembly of a cold-activated TRP channel. Neuron 51:201–212

Vetter I, Hein A, Sattler S, Hessler S, Touska F, Bressan E, Parra A, Hager U, Leffler A, Boukalova S, Nissen M, Lewis RJ, Belmonte C, Alzheimer C, Huth T, Vlachova V, Reeh PW, Zimmermann K (2013) Amplified cold transduction in native nociceptors by M-channel inhibition. J Neurosci 33:16627–16641

Viana F, de la Peña E, Belmonte C (2002) Specificity of cold thermotransduction is determined by differential ionic channel expression. Nat Neurosci 5:254–260

Voets T, Droogmans G, Wissenbach U, Janssens A, Flockerzi V, Nilius B (2004) The principle of temperature-dependent gating in cold- and heat-sensitive TRP channels. Nature 430:748–754

Voets T, Owsianik G, Janssens A, Talavera K, Nilius B (2007a) TRPM8 voltage sensor mutants reveal a mechanism for integrating thermal and chemical stimuli. Nat Chem Biol 3:174–182

Voets T, Owsianik G, Nilius B (2007b) TRPM8. Handb Exp Pharmacol 179:329–344

Vriens J, Nilius B, Voets T (2014) Peripheral thermosensation in mammals. Nat Rev Neurosci 15:573–589

Wang YY, Chang RB, Waters HN, McKemy DD, Liman ER (2008) The nociceptor ion channel TRPA1 is potentiated and inactivated by permeating calcium ions. J Biol Chem 283:32691–32703

Xing H, Ling J, Chen M, Gu JG (2006) Chemical and cold sensitivity of two distinct populations of TRPM8-expressing somatosensory neurons. J Neurophysiol 95:1221–1230

Yudin Y, Rohacs T (2012) Regulation of TRPM8 channel activity. Mol Cell Endocrinol 353:68–74

Zhang X, Mak S, Li L, Parra A, Denlinger B, Belmonte C, McNaughton PA (2012) Direct inhibition of the cold-activated TRPM8 ion channel by Galphaq. Nat Cell Biol 14:851–858

Zholos AV (2014) TRPC5. Handb Exp Pharmacol 222:129–156

Zimmermann K, Leffler A, Babes A, Cendan CM, Carr RW, Kobayashi J, Nau C, Wood JN, Reeh PW (2007) Sensory neuron sodium channel Nav1.8 is essential for pain at low temperatures. Nature 447:855–858

Zimmermann K, Lennerz JK, Hein A, Link AS, Kaczmarek JS, Delling M, Uysal S, Pfeifer JD, Riccio A, Clapham DE (2011) Transient receptor potential cation channel, subfamily C, member 5 (TRPC5) is a cold-transducer in the peripheral nervous system. Proc Natl Acad Sci U S A 108:18114–18119

Zurborg S, Yurgionas B, Jira JA, Caspani O, Heppenstall PA (2007) Direct activation of the ion channel TRPA1 by Ca^{2+}. Nat Neurosci 10:277–279

Zygmunt PM, Hogestatt ED (2014) TRPA1. Handb Exp Pharmacol 222:583–630

Chapter 10
Mathematical Modeling of TRPM8 and the Cold Thermoreceptors

Erick Olivares and Patricio Orio

Abstract The role of TRPM8 channel in thermotransduction involves several aspects of complexity that make it difficult to understand intuitively. First, it is activated by several stimuli (cold, voltage, agonists and intracellular signaling) that interact with each other, raising the question of how these interactions occur. Experimental evidence in this type of polymodal channel may be misinterpreted if the consequences of a working hypothesis are not considered carefully. Second, in parallel with the identification of TRPM8 as the main molecular transducer of cold temperatures in cold thermoreceptors of the somatosensory system, a list of other ion channels have been shown to be involved in the activity of cold-sensitive neurons and nerve endings. The variety of firing patterns observed at cold sensitive nerve endings arises from a complex interaction of ion channels that operate on different time scales. Mathematical modeling has been instrumental in understanding these phenomena, showing the consequences of the hypotheses raised. Here we review some of the models that have been proposed in these two areas: the activation of TRPM8 and TRPV1 by voltage and temperature, and the generation of firing patterns of cold thermoreceptors. We finish this chapter with a mathematical model showing how the calcium-dependent adaptation of TRPM8 may account for the response of cold thermoreceptors to rapid changes in temperature.

Keywords Mathematical modeling · Cold thermoreceptors · TRPM8

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10.1 Introduction

Mathematical modelling has been a useful tool for understanding neural behavior since the beginning of the neurosciences. Physically-tractable mathematical laws naturally arising from the electrical properties of cell membranes and their ion channels may be used to build models with a biological meaning. In turn, these are useful for interpreting experimental data and designing experiments. On the other hand, the physical laws that govern the behavior of ion channels are not completely clear but the mathematical representations are helpful for understanding them.

Typically, a mathematical model serves one or more purposes. The most common one is to test a hypothesis on the underlying mechanisms of an observable phenomenon. If the postulated mechanisms can be translated into a set of mathematical expressions, these expressions should reproduce the phenomenon; otherwise a different hypothesis will have to be considered. The model can make predictions that may be then tested experimentally, and in this way the underlying hypothesis is further challenged.

There are two issues related to the mathematical representation of the cold-activated channel TRPM8. First, its activation by temperature, voltage, and chemical agonists very early raised the question of whether these agents were acting on the same or different parts of the protein. The same question applies to other polymodal thermoTRP channels, among them, the related heat-sensitive TRPV1 channel. Various answers to the question were obtained, expressed by different mathematical representations and with predictions that could be assessed experimentally. Once the predictions were tested by the appropriate experiments, it was concluded that the different stimuli must be sensed by separate parts of the protein. In the second place, is the issue of the role of TRPM8 in the context of thermosensitivity. Cold-sensitive nerve endings and fibers have a complex behavior, firing action potentials in a variety of patterns depending on the temperature. Also, their activity is different depending on whether the temperature is raising or decreasing, being in fact better detectors of *temperature changes* than of *absolute temperature values*. Through the years, before and after the molecular cloning of TRPM8 was attained, many other ion channels have been involved in the function of cold thermoreceptor neurons. How do they all orchestrate to originate the complex responses of cold thermoreceptors? As we shall see in the second part of this chapter, mathematical modeling can help in the design of a hypothesis to be explored experimentally.

10.2 Allosteric Models and ThermoTRP Channel Activation

The activation of TRPM8 and TRPV1 channels raised the debate on the strictness of the coupling between the activators (temperature, membrane depolarization, etc.) and the pore gate. *Strict coupling* implies that whenever an activator is present the channel will open and this is the basic assumption for two-state models (Fig. 10.1a).

On the other hand, *allosteric coupling* (Fig. 10.1b, c) assumes that activation of the voltage (or other) sensor(s) does not lead directly to channel opening but rather to an increase of the open state probability. The difference may be subtle, but the two models have profound differences in their mechanistic interpretations and the prediction of channel behavior under certain conditions.

In a linear, strict coupling model, a maximum open probability of 1 is always attainable. This is seen from the open probability of a 2-state channel controlled by a voltage sensor:

$$P_O = \frac{O}{O+C} = \frac{1}{1+\exp\left(-\frac{zF\left(V-V_0\right)}{RT}\right)}$$

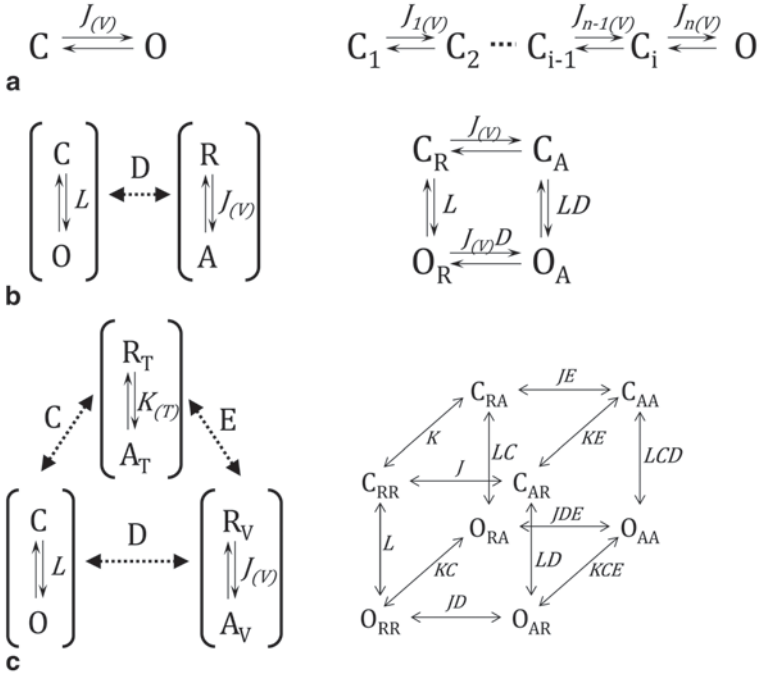

Fig. 10.1 **a** Simple two-state model. The voltage sensor and the channel gate are assumed to be the same molecular entity or two strictly coupled structures. Whenever the voltage sensor activates, the channels opens, and vice versa. **b** Allosteric activation by voltage. *Left*, two independent equilibriums that interact allosterically. *R* and *A* represent the resting and activated state of the voltage sensor. *C* and *O* are the closed and open conformation of the pore gate. Only the equilibrium constant for voltage sensor activation, *J*, is voltage dependent. *Right*, when the combinations of the two equilibriums are considered, a 4-state diagram results. C_n and O_n represent closed and open conformations of the pore, respectively, with the voltage sensors in the 'n' state. **c** Allosteric activation by voltage and temperature. A third equilibrium is added where R_T and A_T represent the resting and active conformations of the temperature sensor, respectively. Two new allosteric factors are introduced (*C* and *E*) to account for the possible interactions between structures. The expanded representation needs to be rendered in 3D (*right*)

In this expression and assuming $z > 0$, in the limit $V \to +\infty$ we find that $P_O \to 1$. Conversely when $V \to -\infty, P_O \to 0$. Similar expressions with the same consequences are found for schemes with many states, provided that the open state is only reached when all the sensors are activated (and conversely, the channel always opens when the sensors are active).

An allosteric gating scheme can be thought as two or more unconnected state transitions whose rate (and equilibrium) constants are modified depending on the state of the other equilibria. Fig. 10.1b depicts the simplest example of a two-state pore allosterically gated by a two-state voltage sensor. When the voltage sensor is in the resting state, (C_R or O_R), the channel opens with a (probably very low) equilibrium constant L. When the sensor is in the activated state (C_A or O_A), this equilibrium constant is multiplied by an allosteric factor D, thus incrementing the probability of the open state. Conversely, when the pore gate is in the open state, the equilibrium constant for the voltage sensor activation (J) is multiplied by the same factor D, thus fulfilling the microscopic reversibility principle.

In the allosteric scheme depicted in (Fig. 10.1b), the open probability is

$$P_O = \frac{O_R + O_A}{C_R + C_A + O_R + O_A} = \left(1 + \frac{1+J}{L\left(1+JD\right)}\right)^{-1}$$

In this case, if we assume that J increases with depolarization, in the limit $V \to +\infty$ we find that $J >> 1 \Rightarrow P_O \to (1+LD^{-1})^{-1}$. Conversely, when $V \to -\infty$ we find that $P_O \to (1+L^{-1})^{-1}$, an expression that approaches L when $L << 1$. Thus, this scheme implies that minimum and maximum values for the open probability, different than 0 and 1, exist regardless of the state of the sensors. Fig. 10.2 graphically shows this difference in behavior of both strictly coupled and allosterically coupled models.

Other sensors for different stimuli are added to the model either as additional states or as another equilibrium that also interacts allosterically. Figure 10.1c shows the allosteric model originally proposed by Brauchi et al. (2004) for the activation by voltage and temperature, both in its allosteric representation and its expanded form that takes the shape of a cube. In this case a similar analysis of the minimum and maximum open probabilities results, where now the maximum open probability attainable by voltage stimulus depends not only on L and D but also on the temperature. At the same time, the maximum open probability that is reached by a temperature stimulus depends on the voltage.

In allosteric gated channels the number of states grows exponentially if other sensors such as for chemical agonists are included, and this type of scheme cannot be easily expressed in two dimensions. For instance, Matta and Ahern (2007) expanded the model to include activation by ligands. The expanded form requires four dimensions to be drawn with all the possible transitions. In the most recent and detailed study of TRPM8 activation by voltage and temperature, Raddatz et al. (2014) propose a three-tiered scheme with 75 states to account for all of their experimental observations (and this does not include activation by agonists). However, when seen in its allosteric depiction (as in Fig. 10.1c, left), it is evident that this type of activa-

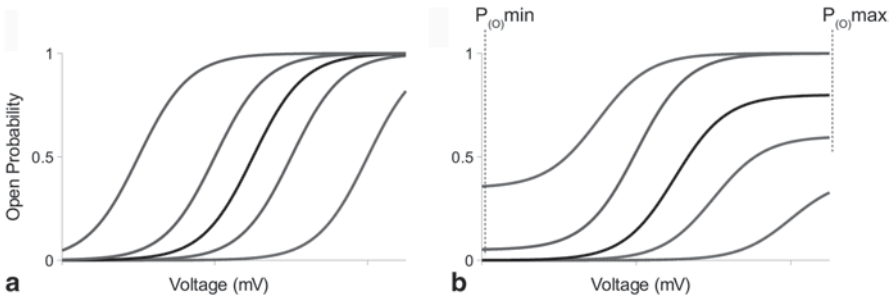

Fig. 10.2 Different behavior of strict coupling versus allosteric coupling. **a** A two-states model for voltage activation (strict coupling between sensor and gate) will always have maximum $P_{(O)}=1$ and minimum $P_{(O)}=0$. Another agonist or activator can only change the $V_{1/2}$ of the G/V relationship shifting the curve to the left or to the right. **b** On the other hand, with allosteric activation by voltage minimum and maximum $P_{(O)}$ is not restricted to 0 and 1. In the model depicted in Fig. 10.1c, when the temperature sensor is resting ($K(T)<<1$) the minimum $P_{(O)}$ (when $J(V)<<1$) is $(1+1/L)^{-1}$ and the maximum open probability that can be reached by depolarization ($J(V)>>1$) is $(1+1/LD)^{-1}$. On the contrary, when the temperature sensor is fully active ($K(T)>>1$) the minimum $P_{(O)}$ is $(1+1/LC)^{-1}$ and the maximum is $(1+1/LCD)^{-1}$. The values of L, C and D will determine whether these probabilities are significantly different from 0 or 1 for a macroscopic current analysis, but nevertheless they should always be measurable with single channel recordings

tion scheme has a relatively small number of parameter (compared to the number of states) and captures the proposed intramolecular interactions with simplicity.

The experimental behavior of a channel predicted by an allosteric gating scheme has several landmarks that have been described and extensively studied for the BK and HCN channels (Altomare et al. 2001; Horrigan and Aldrich 2002). They include the existence of several open states evidenced as multiple mean open times or current relaxations with multiple exponentials and the movement of gating charges between open states. However, as mentioned before, the most notable prediction are the minimum and maximum open probabilities that can be reached by hyperpolarization or depolarization. Depending on the values of L and D this may or may not be evident from macroscopic current recordings. For instance, the BK channel shows a maximum P_O almost equal to 1 $\left(LD \gg 1\right)$ and no minimum open probability because the equilibrium constant L has a weak voltage dependence that has been shown to be independent of the voltage sensors. Still, this phenomenon is evident at $P_O < 10^{-6}$ (Horrigan et al. 1999).

(Brauchi et al. 2004) were the first to postulate an allosteric gating scheme for TRPM8, as their recordings showed that cold increases its maximum P_O. This was not noticed by (Voets et al. 2004) as they based their analysis and fitting only on macroscopic current recordings. On the other hand, Brauchi and coworkers measured actual P_O values with single channel recordings and non-stationary noise analysis. The observation was confirmed later for a wider range of temperatures and voltages, together with the finding of a *minimum* P_O at negative voltages which is also increased by cold (Raddatz et al. 2014). Matta and Ahern (2007) reported

the same for both TRPM8 and TRPV1 (in the case of TRPV1, heating increases maximum P_O) also from single channel recordings. Moreover, Matta and Ahern showed that the minimum P_O of both channels is dramatically increased by the presence of chemical agonists (menthol for TRPM8, capsaicin and resiniferatoxin for TRPV1). The analysis presented by Voets et al. (2007) of the activation of TRPM8 by agonists led them to propose an allosteric gating scheme for the effect of menthol, while retaining a two-state mechanism for the activation by voltage (implying a strict coupling). The authors did not determine actual values of P_0 and based their conclusions on normalized macroscopic G/V curves.

Not everything is about maximum and minimum open probabilities. Latorre and his group (Latorre et al. 2007; Raddatz et al. 2014) have shown that activation of TRPM8 by voltage, despite being very fast, shows a brief delay after the onset of a depolarizing pulse. Moreover, this delay is shortened by a depolarizing pre-pulse. This phenomenon, known as the Cole-Moore shift, cannot be observed in a two-states scheme for voltage activation and is a clear indication of multiple closed states (Cole and Moore 1960). More evidence supporting the existence of multiple open and closed states comes from single channel dwell time analysis in the TRPV1 channel (Liu et al. 2003), the bursting nature of the TRPM8 single channel activity (Zakharian et al. 2010), and the bi-exponential time course of TRPM8 current deactivation (Raddatz et al. 2014).

Finally, from a structural point of view, for a channel to be allosterically activated its sensors have to be different protein domains, also different from the pore gate. Though voltage and temperature sensors are far from being identified, a modular structure has been revealed in thermoTRP channels (Latorre et al. 2006; Brauchi et al. 2006), with growing evidence suggesting the existence of different activation domains for voltage, temperature and PIP_2 in TRPM8.

Thus, it appears difficult to sustain a strictly coupled two-state mechanism for voltage activation of thermo-TRP channels. A two-state model is simpler than allosteric models and reproduces satisfactorily the macroscopic conductance/voltage relationships and their modulation by temperature and agonists. However, the debate between strict and allosteric coupling of the voltage sensor and the pore gate has implications that go beyond the best fit of the experimental data. If the purpose of the model is to reproduce the channel behavior (for inserting it into a conductance-based neuron model, for instance) the choice will always be the simplest model. But when it comes to drawing mechanistic or structural conclusions, the model has to be challenged at every thinkable condition, especially at maximum and minimum $P_{(O)}$. Evidence for allosteric coupling has been found even in the family of Kv channels, largely thought to have the strictest coupling between the voltage sensor and the pore gate (Lu 2002). Still, allosteric coupling seems to be more convenient for channels activated by more than one stimulus, because the dynamic range for the effect of each agonist can be tuned independently.

10.3 TRPM8 Within the Big Picture of Cold Thermosensation

Since the cloning of TRPM8 (McKemy et al. 2002; Peier et al. 2002), this channel has been viewed as the main molecular sensor responsible for cold sensation. Its pivotal role was truly confirmed with the characterization of TRPM8 knockout mice (Chung and Caterina 2007; Colburn et al. 2007; Dhaka et al. 2007; Bautista et al. 2007). However, more than 50 years of physiological characterization of cold thermoreceptors preceded the molecular findings (Hensel and Zotterman 1951; Iggo 1969; Hensel and Wurster 1970; Dykes 1975; Bade et al. 1979). Based on electrophysiological and pharmacological evidence, other ion channels such as TREK-1/TRAAK have been proposed to play the role of transducing cold into electrical potentials, and growing molecular evidence shows that TRPM8 is a piece to be fit into a more complex picture.

10.3.1 Cold Receptors as Pattern-Generating Machines

Mammalian thermoreceptor nerve endings that respond in the innocuous cold temperature range show a different behavior from other somatosensory receptors such as heat-thermoreceptors, mechanoreceptors or nociceptors. They are characterized by a regular ongoing spiking activity at normal skin temperature, that can be of tonic or bursting nature, and that is accelerated upon cooling the receptive field and suppressed by warming (Braun et al. 1980; Brock et al. 2001). The change of firing rate upon a temperature change suffers a strong adaptation in about a minute, relaxing to a steady state firing pattern that depends on the temperature (Fig. 10.3a, b). At low steady temperatures, the interval between spikes is increased but at the same time bursting is promoted so that single spiking events are rarely seen. On the contrary, at temperatures above 30–32°C single spikes prevail and 'skipping' events are evidenced as intervals that are twice the mean (or higher multiples) (Braun et al. 1980). It has been hypothesized that these regular firing patterns arise from a slow oscillation of the membrane voltage, on top of which action potentials are generated. Low temperature would prolong the period of this oscillation (by the typical effect of temperature on channel kinetics), thus allowing more action potentials per period at low temperatures (Braun et al. 1980). Current experimental evidence suggest that the slow oscillation is driven by a combination of slow TTX-resistant sodium channels (Brock et al. 2001) (probably Nav1.9 (Herzog et al. 2001)), and T-type voltage activated calcium channels (Schäfer et al. 1982). In addition, the HCN1 channel plays a role in setting the correct period of the slow oscillation. This channel is the main molecular counterpart of a hyperpolarization-activated current (I_h) present in cold-sensitive neurons (Orio et al. 2009), and its blockade alters the firing pattern of cold-sensitive neurons (Orio et al. 2012).

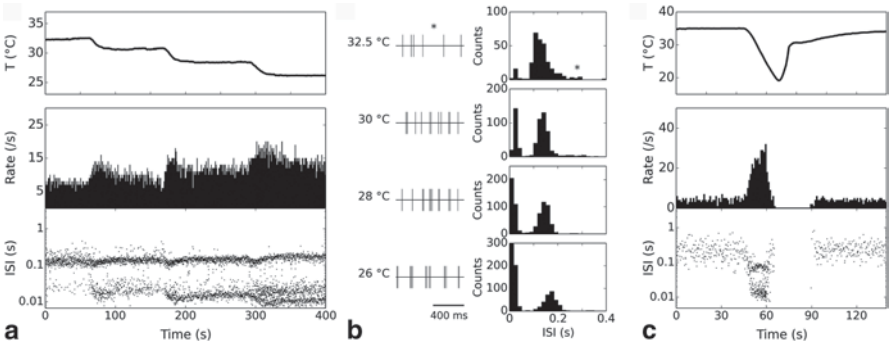

Fig. 10.3 Static and dynamic response of cold thermoreceptors. **a** Response of a corneal thermoreceptor ending to a step-like temperature protocol. Below the temperature trace, the firing rate and the Inter-Spike Intervals (*ISI*) are shown. *ISIs* are shown in logarithmic scale for a better depiction of bursts (low *ISI* values). **b** Representative firing patterns (*left*) and *ISI* histograms (*right*) from the data shown in a. Data for the histograms were taken after the transient responses has decayed. *Asterisks* denote skipping events, corresponding to intervals that are multiples of the main peak of the histogram. **c** Dynamic response of the thermoreceptor to an acute temperature pulse. Note the transient response of a bursting nature and how the nerve ending stops firing during the period of fast temperature raise. (Raw data obtained from Orio et al. 2012)

This variety of firing patterns is reproduced by mathematical models (Longtin and Hinzer 1996; Braun et al. 1998; Huber et al. 2000) based on a general model of slow wave bursting (Plant and Kim 1976). In this type of model, regular spiking or bursting is driven by a slow membrane potential oscillation and all that it takes to change the firing pattern with temperature decreases is to consider the usual effects of temperature on ion channels (Q_{10} values of 3 for kinetics and 1.3 for conductance). Huber and Braun's model was further modified to account for the experimental observations regarding I_h and HCN channels (Orio et al. 2012). In the latter work, the authors use mathematical modeling to support the hypothesis that the behavior of cold thermoreceptors from HCN1-knockout mice is best explained by compensation with the related channel HCN2 rather than by the absence of I_h. As a result, a new model of cold-sensitive nerve ending is proposed that not only reproduces the varying stationary firing patterns of cold thermoreceptors but also the role that HCN channels play in it. The model proposed by Orio and coworkers also introduces a TREK-1-like current, which is a background potassium channel that closes with decreasing temperature (Maingret et al. 2000; Kang et al. 2005). The decrease of a potassium conductance upon cooling was reported previously (Reid and Flonta 2001; Viana et al. 2002) and mice lacking both TREK1 and the similar TRAAK channels have altered cold perception (Noël et al. 2009).

Cold sensitivity in coldt hermoreceptors is dampened by the expression of I_{KD}, an inhibitory outward slow-inactivating K^+-current (Viana et al. 2002; Madrid et al 2009; see also Chap. 9 by González et al. in this book). The low activation threshold of I_{KD} and its slow inactivation implies that this current acts as an excitability brake that counteracts the depolarizing effect of cold in primary sensory neurons.

10.4 The Dynamic Response of Cold Thermoreceptors: A Model with TRPM8

The behavior of cold-sensitive nerve endings involves the participation of several ion channels, and the whole picture of cold thermotransduction has yet to be unveiled. Where does TRPM8 fit in the cold transduction phenomenon? The mathematical models described before only reproduce the shifting patterns of nerve impulses that are observed when the cold receptors are already adapted to a steady temperature (the static response). They lack, however, the dynamic response, i.e. the acute changes in firing rate upon changes in temperature (Fig. 10.3c).

The TRPM8 channel is necessary for the response of cold thermoreceptors to acute changes in temperature. However, this does not explain all aspects of the dynamic response, such as the adaptation to a sustained stimulus or the absence of activity when heating. Yet the same ion channel may be at the center of all these phenomena. TRPM8 undergoes desensitization dependent on extracellular calcium (McKemy et al. 2002; Peier et al. 2002; Reid et al. 2002), by at least two mechanisms: one related to PIP_2 depletion by activated phospholipase C (Rohács et al. 2005; Daniels et al. 2009) and another one by Ca^{2+}-Calmodulin which can revert the activation by PIP2 (Sarria et al. 2011). The temporal scale of this process matches that of the adaptation of cold receptors, suggesting that TRPM8 desensitization can underlie the dynamic response in cold nerve endings. To test this hypothesis, we developed a model in which we inserted TRPM8 with a Ca^{2+}-dependent adaptation into the existing Huber & Braun model.

As mentioned before, in the Huber & Braun model two slow currents (voltage activated sodium/calcium current and calcium activated potassium current) induce a slow subthreshold oscillation of the membrane potential. On top of this oscillation, a pair of fast depolarizing and repolarizing conductances originate action potentials whenever the membrane potential goes beyond the threshold. The kinetics of the channels, and thus the period of the oscillation, are modulated by temperature. This produces the variety of firing patterns observed in cold receptors. We inserted the TRPM8 conductance into the Huber & Braun model expecting it to maintain the variety of firing patterns, and additionally, that it could show the dynamic response of cold receptors.

Under these assumptions we have a modified Huber & Braun model in which the voltage membrane is given by

$$C_m \frac{dV}{dt} = -I_d - I_r - I_{sd} - I_{sr} - I_{M8} - I_{leak} + I_n$$

Where C_m is membrane capacitance; I_d, I_r, I_{sd} and I_{sr} are the depolarizing, repolarizing, slow depolarizing and slow repolarizing conductances, respectively; I_{M8} is the current mediated by the TRPM8 channel, I_{leak} corresponds to a unspecific leak current and I_n is a noise term. With the exception of I_{M8}, all the currents are present in the Huber & Braun model, and are described as follows:

$$I_i = \rho(T)\, g_i a_i \left(V - E_i\right) \quad i = d, r, sd, leak\ \rho$$

$$I_{M8} = g_{M8} a_{M8} \left(V - E_i\right)$$

Where $a_i \in [0,1]$ is an activation term, g_i is the maximal conductance parameter, E_i is the reversal potential for the current and $\rho(T)$ is a temperature scaling factor for the currents.

The sr current is a calcium-activated channel, therefore its activation term actually reflects the internal calcium concentration. According to this, the current is expressed as

$$I_{sr} = \rho(T)\, g_{sr} \frac{a_{sr}^2}{a_{sr}^2 + 0.4^2} (V - E_{sr})$$

The activation terms a_r and a_{sd} follow the differential equation

$$\frac{da_i}{dt} = \phi(T) \frac{(a_i^{\infty}(V) - a_i)}{\tau_i} \qquad a_i^{\infty}(V) = \frac{1}{1 + \exp\left(-s_i (V - V_{h,i})\right)} \qquad i = r, sd$$

Where $\phi(T)$ is a temperature factor for channel kinetics, and s_i and $V_{h,i}$ are the parameters for the voltage-dependent activation of the currents. a_{sr} represents the internal calcium concentration, which increases with the inward I_{sd} current (a mixed sodium/calcium current) and decays according to the equation

$$\frac{da_{sr}}{dt} = \phi(T) \frac{-\eta I_{sd} - \kappa a_{sr}}{\tau_{sr}}$$

a_d and a_{M8} have very fast kinetics compared to the other channels, therefore they are always equal to their steady-state values:

$$a_d = a_d^{\infty}(V) = \frac{1}{1 + \exp\left(-s_d \left(V - V_{h,d}\right)\right)}$$

$$a_{M8} = a_{M8}^{\infty}(V, T) = \frac{1}{1 + \exp\left(-\frac{z_{M8} F}{RT} \left(V - V_h(T) - \delta V\right)\right)}$$

For the TRPM8 channel we have taken the simple two-state model proposed by Voets and colleagues (Voets et al. 2004; Voets et al. 2007) where z_{M8} is the voltage dependency and F, R, T have their usual physical constants meaning. The voltage for half-activation V_h is a function of temperature:

$$V_h(T) = \frac{CRT - \left(E_{a,C} - E_{a,O}\right)}{z_{M8} F}$$

Where C is a constant related to the preexponential factors of the rate constants, and $E_{a,C}$ and $E_{a,O}$ are the activation energies for channel closing and opening, respectively. We introduced an additional shift δV that depends on intracellular calcium according to the equations

$$\frac{d\delta V}{dt} = \frac{\delta V^{\infty}\left(Ca^{2+}\right) - \delta V}{\tau_{\delta V}}$$

$$\delta V^{\infty}\left(Ca^{2+}\right) = \delta V_{min} + \frac{\left(\delta V_{max} - \delta V_{min}\right)\left[Ca^{2+}\right]}{\left[Ca^{2+}\right] + K_{Ca,M8}}$$

The intracellular calcium concentration is modeled considering that calcium influx to the cell is a fraction of I_{M8} and calcium removal follows a simple decay:

$$\frac{d\left[Ca^{2+}\right]}{dt} = -\frac{p_{Ca} I_{M8}}{2Fd} - \frac{\left[Ca^{2+}\right]}{\tau_{Ca}}$$

The temperature-dependent factors for conductance and kinetics are given, respectively, by:

$$\rho(T) = 1.3^{\frac{T-25}{10}}$$

$$\rho(T) = 3.0^{\frac{T-25}{10}}$$

Finally, the noise term was implemented as a low-pass filtered noise using an Ornstein-Uhlenbeck process with mean 0:

$$\frac{dI_n}{dt} = \frac{-I_n + D\xi(t)}{\tau_n}$$

Where $\xi(t)$ is a normally distributed random variable (white noise) with zero mean and variance = 1.

Figure 10.4 shows the response of the model to an acute cold pulse using the following set of parameters:

$$g_d = 2.8\ mS/cm^2; g_r = 2.8\ mS/cm^2; g_{sd} = 0.2\ mS/cm^2;$$
$$g_{sr} = 0.2\ mS/cm^2; g_{M8} = 2.74\ mS/cm^2; g_{leak} = 0.23\ mS/cm^2;$$
$$E_d = E_{sd} = 50\ mV; E_r = E_{sr} = -90\ mV; E_{leak} = -60\ mV; E_{M8} = 0\ mV$$

$$\tau_r = 2\ ms;\ \tau_{sd} = 10\ ms;\ \tau_{sr} = 35ms;$$
$$V_{h,d} = V_{h,r} = -25\ mV; V_{h,sd} = -40\ mV; s_d = s_r = 0.25\ mV^{-1};$$
$$s_{sd} = 0.11\ mV^{(-1)};\ \eta = 0.014\ cm^2/\mu A;\ \kappa = 0.18,$$

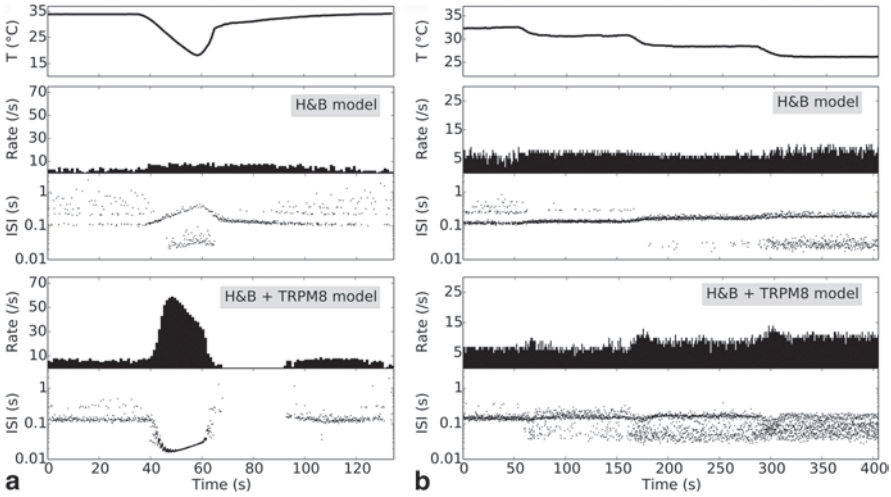

Fig. 10.4 Static and dynamic response of a thermoreceptor model including an adapting *TRPM8*. **a** Response of the original Huber & Braun (*H&B*) model (*middle*) and the *H&B + TRPM8* model (*bottom*) to an acute cold pulse (*top*; the same temperature trace as in Fig. 10.3c). Note that although the *H&B* model shows a slight increase in firing rate at lower temperatures, this response is instantaneous with respect to temperature and does not distinguish whether the temperature is decreasing or decreasing. The *H&B* model shows a dramatic difference in firing rate depending on the direction of temperature change. **b** Response of the models to the temperature stimulus shown in (Fig. 10.3a). Although both models show a changing static firing pattern, the brief transient responses after each decrease in temperature are observed only in the *H&B + TRPM8* model

$$z_{M8} = 0.82; C = 67.123; E_{a,C} = 173000J; E_{a,O} = 15700J;$$
$$\tau_{\delta V} = 18600\ ms; \delta V_{max} = 490\ mV; \delta V_{min} = -440mV;$$
$$K_{Ca,M8} = 25\ nM; p_{Ca} = 8.86 \times 10^{-6}; d = 1\ \mu m; \tau_{Ca} = 27800\ ms$$

$$\tau_n = 1ms; D = \sqrt{0.0005 / dt}$$

Equations were solved in the Neuron simulation environment (Hines and Carnevale 2001) with a fixed integration time step $dt = 0.05\,ms$.

It is important to note that we have chosen a two-state model to model TRPM8, in spite of the already discussed evidence in favor of multi-state models to reproduce its voltage- and temperature-dependence. The reason to do this is simple, and was already given at the end of the first section: the purpose of the model here is just to reproduce the behavior of the channels. A close look at some figures in the work of Raddatz and co-workers (Raddatz et al. 2014) reveals that *within the physiological values of voltage and temperature* (15–40 °C; -90- 50 mV) the behavior of the channel is indistinguishable from a two-state model. Therefore, we chose the simplest model that fulfilled our purpose.

10.5 Concluding Remarks

Our model shows that the only element necessary to display a dynamic response in a model of cold thermoreceptor is a cold-activated channel (resembling TRPM8) with a slow activity-dependent adaptation. Moreover, and in tune with the discussion presented in the first part of this chapter, a simple two-state model of TRPM8 does the job, as all that is needed for this purpose is a phenomenological representation of the channel behavior within the physiological ranges of temperature and voltage.

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References

Altomare C, Bucchi A, Camatini E et al (2001) Integrated allosteric model of voltage gating of HCN channels. J Gen Physiol 117:519–532

Bade H, Braun HA, Hensel H (1979) Parameters of the static burst discharge of lingual cold receptors in the cat. Pflugers Arch 382:1–5. doi:10.1007/BF00585897

Bautista DM, Siemens J, Glazer JM et al (2007) The menthol receptor TRPM8 is the principal detector of environmental cold. Nature 448:204–208. doi:10.1038/nature05910

Brauchi S, Orio P, Latorre R (2004) Clues to understanding cold sensation: thermodynamics and electrophysiological analysis of the cold receptor TRPM8. Proc Natl Acad Sci U S A 101:15494–15499. doi:10.1073/pnas.0406773101

Brauchi S, Orta G, Salazar M et al (2006) A hot-sensing cold receptor: C-terminal domain determines thermosensation in transient receptor potential channels. J Neurosci 26:4835–4840. doi:10.1523/JNEUROSCI.5080-05.2006

Braun HA, Bade H, Hensel H (1980) Static and dynamic discharge patterns of bursting cold fibers related to hypothetical receptor mechanisms. Pflugers Arch 386:1–9. doi:10.1007/BF00584180

Braun HA, Huber MT, Dewald M et al (1998) Computer simulations of neuronal signal transduction: the role of nonlinear dynamics and noise. Int J Bifurc Chaos 8:881–889. doi:10.1142/S0218127498000681

Brock JA, Pianova S, Belmonte C (2001) Differences between nerve terminal impulses of polymodal nociceptors and cold sensory receptors of the guinea-pig cornea. J Physiol 533:493–501. doi:10.1111/j.1469-7793.2001.0493a.x

Chung M-K, Caterina MJ (2007) TRP channel knockout mice lose their cool. Neuron 54:345–347. doi:10.1016/j.neuron.2007.04.025

Colburn RW, Lou LM, Stone DJ et al (2007) Attenuated cold sensitivity in TRPM8 null mice. Neuron 54:379–386. doi:10.1016/j.neuron.2007.04.017

Cole KS, Moore JW (1960) Potassium ion current in the squid giant axon: dynamic characteristic. Biophys J 1:1–14

Daniels RL, Takashima Y, McKemy DD (2009) Activity of the neuronal cold sensor TRPM8 is regulated by phospholipase C via the phospholipid phosphoinositol 4,5-bisphosphate. J Biol Chem 284:1570–1582. doi:10.1074/jbc.M807270200

Dhaka A, Murray AN, Mathur J et al (2007) TRPM8 is required for cold sensation in mice. Neuron 54:371–378. doi:10.1016/j.neuron.2007.02.024

Dykes RW (1975) Coding of steady and transient temperatures by cutaneous "cold" fibers serving the hand of monkeys. Brain Res 98:485–500. doi:10.1016/0006-8993(75)90368-6

Hensel H, Wurster RD (1970) Static properties of cold receptors in nasal area of cats. J Neurophysiol 33:271–275
Hensel H, Zotterman Y (1951) The response of the cold receptors to constant cooling. Acta Physiol Scand 22:96–105. doi:10.1111/j.1748-1716.1951.tb00758.x
Herzog RI, Cummins TR, Waxman SG (2001) Persistent TTX-resistant Na+ current affects resting potential and response to depolarization in simulated spinal sensory neurons. J Neurophysiol 86:1351–1364
Hines ML, Carnevale NT (2001) NEURON: a tool for neuroscientists. Neuroscientist 7:123–135
Horrigan FT, Aldrich RW (2002) Coupling between voltage sensor activation, Ca2+ binding and channel opening in large conductance (BK) potassium channels. J Gen Physiol 120:267–305
Horrigan FT, Cui J, Aldrich RW (1999) Allosteric voltage gating of potassium channels I. Mslo ionic currents in the absence of Ca(2+). J Gen Physiol 114:277–304
Huber M, Krieg J, Dewald M et al (2000) Stochastic encoding in sensory neurons: impulse patterns of mammalian cold receptors. Chaos Solitons Fractals 11:1895–1903. doi:10.1016/S0960-0779(99)00126-5
Iggo A (1969) Cutaneous thermoreceptors in primates and sub-primates. J Physiol 200:403–430
Kang D, Choe C, Kim D (2005) Thermosensitivity of the two-pore domain K+ channels TREK-2 and TRAAK. J Physiol 564:103–116. doi:10.1113/jphysiol.2004.081059
Latorre R, Vargas G, Orta G, Brauchi S (2006) Voltage and temperature gating of thermoTRP channels. 287–302
Latorre R, Brauchi S, Orta G et al (2007) ThermoTRP channels as modular proteins with allosteric gating. Cell Calcium 42:427–438. doi:10.1016/j.ceca.2007.04.004
Liu B, Hui K, Qin F (2003) Thermodynamics of heat activation of single capsaicin ion channels VR1. Biophys J 85:2988–3006. doi:10.1016/S0006-3495(03)74719-5
Longtin A, Hinzer K (1996) Encoding with bursting, subthreshold oscillations, and noise in mammalian cold receptors. Neural Comput 8:215–255
Lu Z (2002) Coupling between Voltage sensors and activation gate in voltage-gated K+ channels. J Gen Physiol 120:663–676. doi:10.1085/jgp.20028696
Madrid R, de la Peña E, Donovan-Rodriguez T et al (2009) Variable threshold of trigeminal cold-thermosensitive neurons is determined by a balance between TRPM8 and Kv1 potassium channels. J Neurosci 29:3120–3131. doi:10.1523/JNEUROSCI.4778-08.2009
Maingret F, Lauritzen I, Patel AJ et al (2000) TREK-1 is a heat-activated background K(+) channel. EMBO J 19:2483–2491. doi:10.1093/emboj/19.11.2483
McKemy DD, Neuhausser WM, Julius D (2002) Identification of a cold receptor reveals a general role for TRP channels in thermosensation. Nature 416:52–58. doi:10.1038/nature719
Matta JA, Ahern GP (2007) Voltage is a partial activator of rat thermosensitive TRP channels. J Physiol 585:469–482. doi:10.1113/jphysiol.2007.144287
Noël J, Zimmermann K, Busserolles J et al (2009) The mechano-activated K+ channels TRAAK and TREK-1 control both warm and cold perception. EMBO J 28:1308–1318. doi:10.1038/emboj.2009.57
Orio P, Madrid R, de la Peña E et al (2009) Characteristics and physiological role of hyperpolarization activated currents in mouse cold thermoreceptors. J Physiol 587:1961–1976. doi:10.1113/jphysiol.2008.165738
Orio P, Parra A, Madrid R et al (2012) Role of Ih in the firing pattern of mammalian cold thermoreceptor endings. J Neurophysiol 108:3009–3023. doi:10.1152/jn.01033.2011
Peier AM, Moqrich A, Hergarden AC et al (2002) A TRP channel that senses cold stimuli and menthol. Cell 108:705–715
Plant RE, Kim M (1976) Mathematical description of a bursting pacemaker neuron by a modification of the Hodgkin-Huxley equations. Biophys J 16:227–244. doi:10.1016/S0006-3495(76)85683-4
Raddatz N, Castillo JP, Gonzalez C et al (2014) Temperature and voltage coupling to channel opening in transient receptor potential melastatin 8 (TRPM8). J Biol Chem. doi:10.1074/jbc.M114.612713

Reid G, Flonta M (2001) Cold transduction by inhibition of a background potassium conductance in rat primary sensory neurones. Neurosci Lett 297:171–174

Reid G, Babes A, Pluteanu F (2002) A cold- and menthol-activated current in rat dorsal root ganglion neurones: properties and role in cold transduction. J Physiol 545:595–614. doi:10.1113/jphysiol.2002.024331

Rohács T, Lopes CMB, Michailidis I, Logothetis DE (2005) PI(4,5)P2 regulates the activation and desensitization of TRPM8 channels through the TRP domain. Nat Neurosci 8:626–634. doi:10.1038/nn1451

Sarria I, Ling J, Zhu MX, Gu JG (2011) TRPM8 acute desensitization is mediated by calmodulin and requires PIP(2): distinction from tachyphylaxis. J Neurophysiol 106:3056–3066. doi:10.1152/jn.00544.2011

Schäfer K, Braun HA, Hensel H (1982) Static and dynamic activity of cold receptors at various calcium levels. J Neurophysiol 47:1017–1028

Viana F, de la Peña E, Belmonte C (2002) Specificity of cold thermotransduction is determined by differential ionic channel expression. Nat Neurosci 5:254–260. doi:10.1038/nn809

Voets T, Droogmans G, Wissenbach U et al (2004) The principle of temperature-dependent gating in cold- and heat-sensitive TRP channels. Nature 430:748–754. doi:10.1038/nature02732

Voets T, Owsianik G, Janssens A et al (2007) TRPM8 voltage sensor mutants reveal a mechanism for integrating thermal and chemical stimuli. Nat Chem Biol 3:174–182. doi:10.1038/nchembio862

Zakharian E, Cao C, Rohacs T (2010) Gating of transient receptor potential melastatin 8 (TRPM8) channels activated by cold and chemical agonists in planar lipid bilayers. J Neurosci 30:12526–12534. doi:10.1523/JNEUROSCI.3189-10.2010

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