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Structure–functional intimacies of transient receptor potential channels

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Abstract. Although a unifying characteristic common to all transient receptor potential (TRP) channel functions remains elusive, they could be described as tetramers formed by subunits with six transmembrane domains and containing cation-selective pores, which in several cases show high calcium permeability. TRP channels constitute a large superfamily of ion channels, and can be grouped into seven subfamilies based on their amino acid sequence homology: the canonical or classic TRPs, the vanilloid receptor TRPs, the melastatin or long TRPs, ankyrin (whose only member is the transmembrane protein 1 [TRPA1]), TRPN after the nonmechanoreceptor potential C (nonpC), and the more distant cousins, the polycystins and mucolipins. Because of their role as cellular sensors, polymodal activation and gating properties, many TRP channels are activated by a variety of different stimuli and function as signal integrators. Thus, how TRP channels function and how function relates to given structural determinants contained in the channel-forming protein has attracted the attention of biophysicists as well as molecular and cell biologists. The main purpose of this review is to summarize our present knowledge on the structure of channels of the TRP ion channel family. In the absence of crystal structure information for a complete TRP channel, we will describe important protein domains present in TRP channels, structure–function mutagenesis studies, the few crystal structures available for some TRP channel modules, and the recent determination of some TRP channel structures using electron microscopy.

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1. Introduction

The transient receptor potential (TRP) channel story began in the late 1960s with the report of Cosens & Manning (1969) of a spontaneously occurring *Drosophila* mutant with an abnormal response to light stimuli. In the mutant fly, the photoreceptor potential shows a transient response during prolonged illumination. Twenty years later, this finding lead to the discovery of the first TRP ion channel from *Drosophila* (Montell & Rubin, 1989). Montell & Rubin (1989) reported that *trp* encoded a large membrane protein containing 1275 amino acids displaying eight putative transmembrane (TM) spanning segments. The amino acid sequence did not present any clear homology with any other known ion channel-forming protein. It was realized later that a new gene dubbed *trp-like* (*trpl*) showed structural homologies with *Drosophila trp* and also with the superfamily of voltage-gated channel genes, raising the possibility that *trpl* encoded subunits of a calcium or of a nonspecific cation channel (Phillips *et al.* 1992). At the same time, Hardie & Minke (1992) demonstrated that in *trp* flies the permeability ratio P_{Ca}/P_{Na} is reduced about 10-fold, strongly suggesting that the *trp* gene coded for a highly Ca^{2+}-permeable channel. Despite the claims that TRP and TRPl were *Drosophila* photoreceptor-specific proteins (e.g. Montell & Rubin, 1989), Petersen *et al.* (1995) identified partial sequences of TRP homologues from *Xenopus* and mice using cDNA libraries, thereby showing that TRP-related proteins were also expressed outside of the invertebrates. Moreover, in the same year, the full sequence of the first human homologue (TRPC1) was reported (Wes *et al.* 1995; Zhu *et al.* 1995). *trpc1*, sharing a 37% identity with the *Drosophila trp* gene, is present in most human tissues. Today, 18 years after the first TRP channel was obtained, we can list more than 85 channels within this family (invertebrate and vertebrate combined; Montell, 2005b) organized by their sequence homology into seven subfamilies (Fig. 1): (1) The TRPC (classical) subfamily encompasses channels presenting a large number of different activation modes. Some are store-operated and activated by stretch, whereas others are $G_{q/11}$ receptor-operated channels activated by the production of diacylglicerol or redox processes. TRPC proteins also control growth cone guidance in both mammalian and amphibian model systems. (2) The TRPM (melastatin-related) subfamily contains channels

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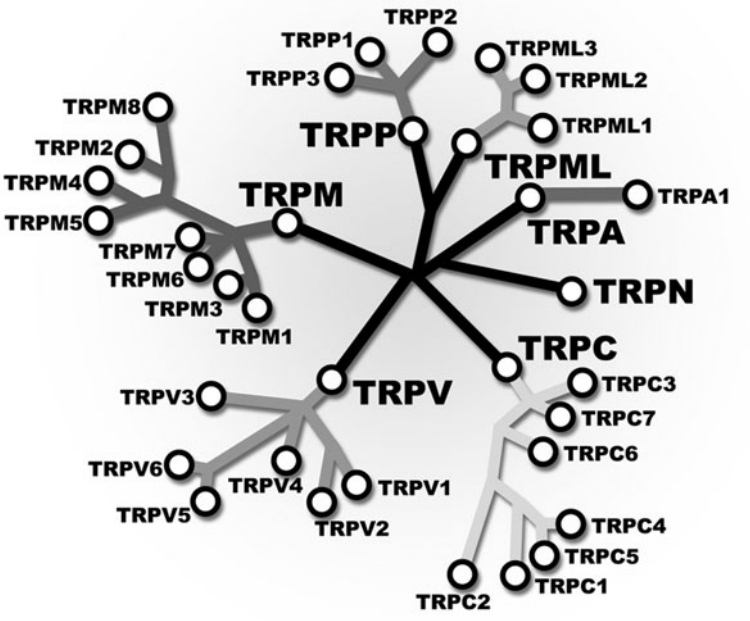


Fig. 1. Phylogeny of TRP channels. The phylogenetic tree shows the relationship between different TRP subfamilies. Sequence identity can be as low as 20%. TRPC2 is a pseudogene in human but not in rats. TRPN channels have not been identified in mammals.

designated as chanzymes since their C-terminal contains catalytic domains able to show protein kinase activities (TRPM6 and TRPM7). As discussed below, some members of this subfamily play an important role in detecting temperature changes. (3) The TRPV (vanilloid) subfamily has members activated by temperature. They are also involved in sensing osmolarity changes and mechanical stimuli. (4) TRPA (ankyrin) contains only one member, TRPA1, which is activated by noxious cold and a myriad of different chemical compounds including allyl isothiocyanate (AITC), the pungent principle of wasabi and other mustard oils. TRPA1 appears to have a central role in the pain response to volatile irritants like the one found in tear gas. (5) TRPN (NOMP-C homologues) has a single member that can be found in worms, flies, and zebrafish. TRPN proteins have a role in mechanotransduction. (6) The TRPP (polycystin) subfamily is formed by the polycystic kidney disease (PKD) proteins or polycystins. Heteromeric complexes TRPP2–TRPP1 form Ca^{2+}-permeable nonselective cation channels. (7) The TRPML (mucolipin) subfamily consists of channels that appear to be only present in intracellular vesicles and two of its members (TRPML1 and 2) have been implicated in hearing. Mutations in the TRPP1 or TRPP2 are responsible for such human diseases as autosomal-dominant PKD, and mutations in TRPML1 are associated with neurodegenerative lysosomal storage disorder, mucolipidosis type 4. (For general reviews on TRP channels, see Clapham, 2003; Montell, 2005a; Ramsey *et al.* 2006a; Venkatachalam & Montell, 2007; for a review on *Drosophila* TRPs, see Montell, 2005b; for a review on updated nomenclature of TRP channel families, see Clapham *et al.* 2005.)

We now know that TRP channels appeared relatively early in evolution and we can trace their expression from invertebrates to vertebrates, including humans (Harteneck *et al.* 2000; Venkatachalam & Montell, 2007). As TRP channels are directly involved in sensory perception

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such as vision, taste, olfaction, hearing, touch, thermal perception, and nociception, a recurrent theme is the idea that multicellular organisms use TRP channel-dependent pathways to sense their surroundings and, at more cellular level, TRP channels allow cells to respond to changes in their local environment. The critical role they play as sensors in cellular physiology make them incredibly attractive puzzles (Clapham, 2003; Voets *et al.* 2005).

Despite the large body of literature on TRP channels that has accumulated during the past 30 years (more than 4000 articles since 1969; Liedtke & Heller, 2007), very little is known about the mechanisms that control the gating process in these amazing molecular machines, and much work remains to be done in the fields of biophysics and protein structure (Gaudet, 2007).

2. TRP channel assembly

2.1 General TRP channel architecture

TM segment prediction algorithms indicate that TRP channels are related to the superfamily of voltage-gated cation channels. The putative organization of these channels consists in 6TM with the carboxy- and amino-terminal facing the intracellular side of the membrane (Fig. 2) (Harteneck *et al.* 2000; Ramsey *et al.* 2006b). Family relatives are voltage-activated *shaker*-like channels, cyclic nucleotide gated channels, and hyperpolarization-activated cyclic nucleotide-gated (HCN) channels (Harteneck *et al.* 2000). Although originally predicted to be 8 TM (Montell & Rubin, 1989), shortly thereafter TRP channel researchers reached a consensus about their 6TM organization and similarities with voltage-dependent K^+ and Ca^{2+} channels (Phillips *et al.* 1992; Wes *et al.* 1995; Harteneck *et al.* 2000; Ramsey *et al.* 2006b). According to the proposed 6TM architecture, the pore region would be a hydrophobic region somewhere between fifth and sixth segment (for a review, see Owsianik *et al.* 2006). Studies based on biochemical and optical based methods strongly suggest that TRP channels are formed by four subunits (Jahnel *et al.* 2001; Kedei *et al.* 2001; Amiri *et al.* 2003), and that active channels could assemble as homo- or heterotetramers (Hoenderop *et al.* 2003; Helwig *et al.* 2005; Schaefer, 2005).

TRP channels are diverse in their design and their features change from family to family. Figure 2 shows a schematic view of this diversity, and in the following sections we will give an overview of the structure–function relationships present in this remarkable superfamily of ion channels.

2.2 Viewing TRP channels through the electron microscope

Advances in our knowledge of the crystal structure of TRP channels has been hampered by the lack of adequate expression systems needed to obtain large amounts of protein, and attempts to overexpress these proteins in bacteria have so far been unsuccessful. However, by using mammalian cell (HEK293) transfection or yeast (*Saccharomyces cerevisiae*), sufficient amounts of protein have been collected to make it possible to determine the structures of three TRP channels by electron microscopy (albeit at low resolution): TRC3 (Mio *et al.* 2005, 2007), TRPM2 (Maruyama *et al.* 2007), and TRPV1 (Moiseenkova & Wensel, 2009) (Fig. 3). More recently, Moiseenkova & Wensel (2009) gave a critical appraisal of the different techniques used to provide the first steps in the elucidation of TRP channel structure and speculate on the future of the field. Of particular interest is that the structures of TRPC3 and TRPM2 proved to be very different from that of TRPV1. The TRPC3 channel structure was determined using a single particle reconstruction from images coming from a cryoelectron microscopy (Fig. 3). The

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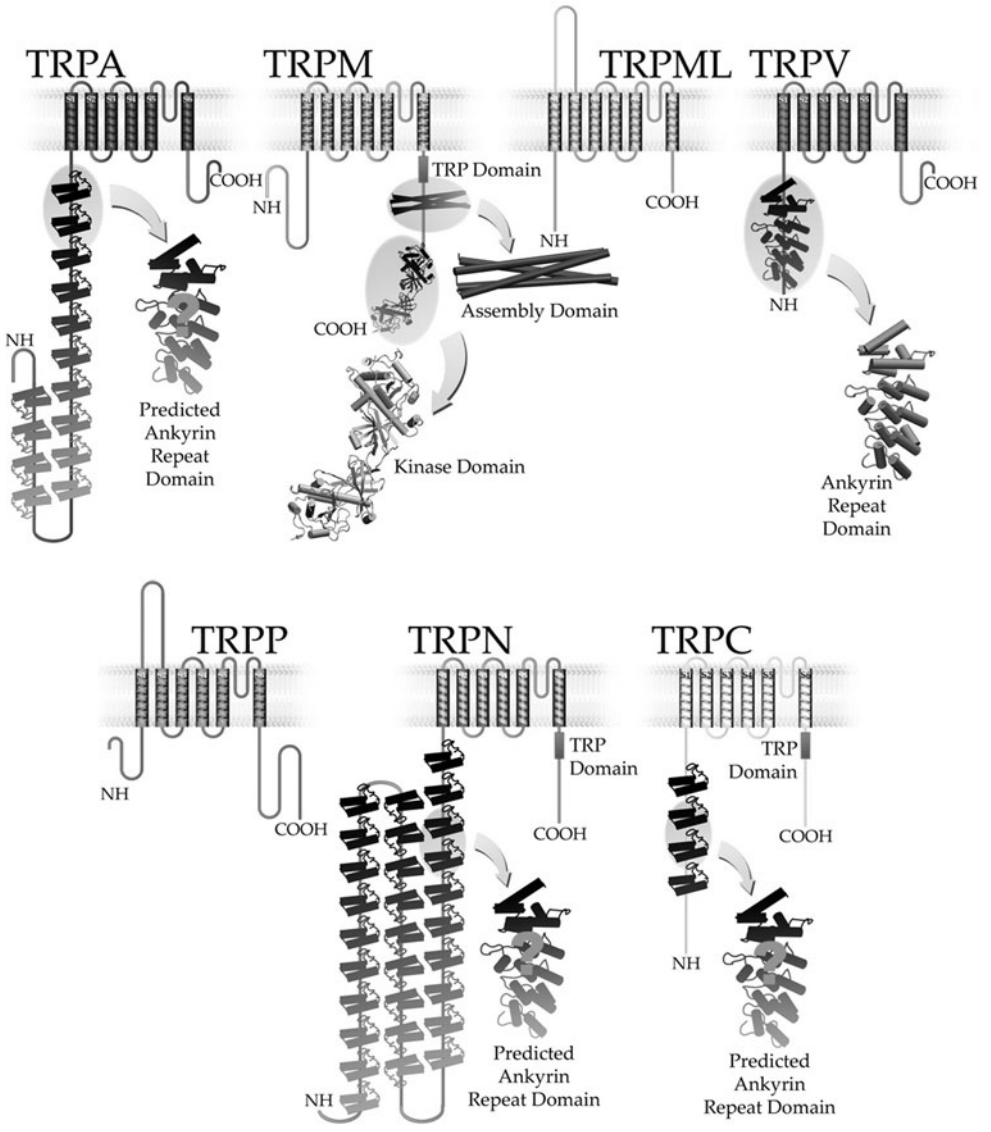


Fig. 2. The seven TRP subfamilies and the gross architecture of their subunits. TRP channels are tetramers consisting of subunits containing six transmembrane (TM) segments (S1–S6). By analogy to K^+ channels the central pore is assumed to be formed by the hairpin located in between TM S5 and S6. A variable number of ankyrin domains are present in the N-terminal of most TRP channels with the exception of TRPP and TRPM subfamilies. The crystal structure of this domain has been elucidated in the case of TRPV1, 2, and 6. TRPM6 and 7 are defined as chanzymes owing to the presence of a C-terminal a typical protein kinase domain. TRP and coiled coil domains are present in the C terminus domain (for more details, see text).

bell-shape structure obtained in this manner has an enormous volume mostly conformed by the cytoplasmic domain. This large cytoplasmic domain is made of a sparse external shell that appears as girdles connected through low-density panels. This type of structure explains how the relatively low molecular weight channel-forming protein can present such a large volume. Using electron microscopy in negative stain, a large volume bullet-shape structure was also

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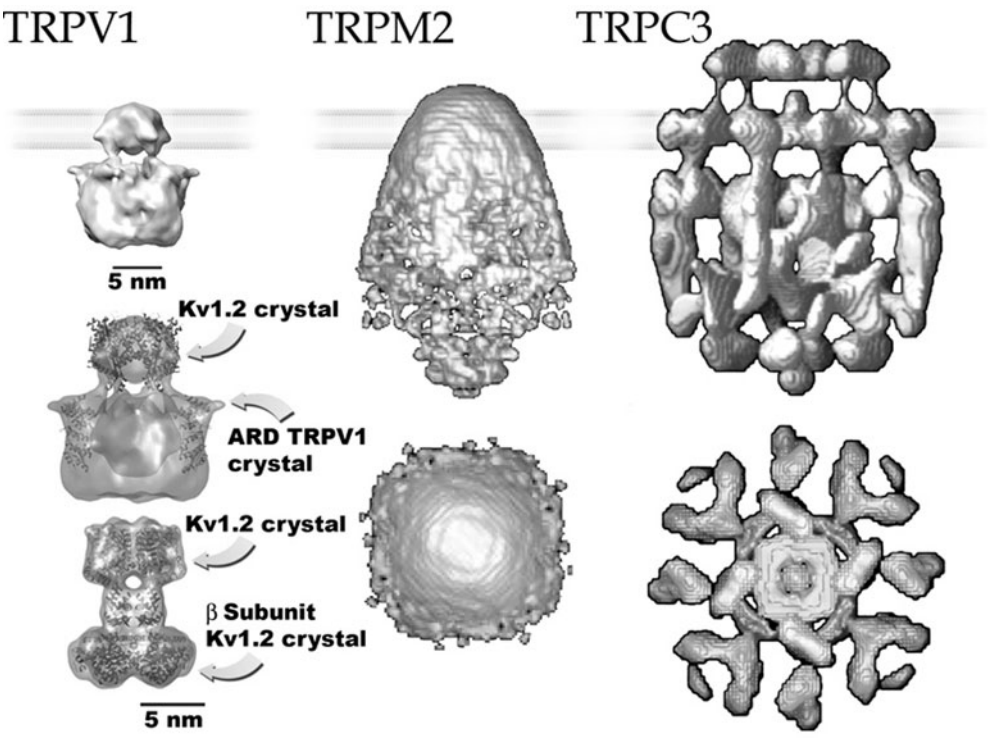


Fig. 3. Comparison of structures of TRP channels derived from electron microscopy studies. Cryoelectron microscopy structures: TRPV1, 19 Å (Moiseenkova *et al.* 2008); TRPC3, 15·3 Å (Mio *et al.* 2007). A comparison between the TRPV1 and the Kv1.2/β subunit structure (PDB code 2A79) is shown. The crystal structure of Kv1.2 and TRPV1 ankyrin domains were superimposed on the TRPV1 structure obtained using electron microscopy and T domain of Kv1.2 and β subunit were superimposed on the Kv1.2 structure obtained by cryomicroscopy. Negative stain structures, resolution: TRPM2, 37 Å (Maruyama *et al.* 2007).

observed for TRPM2. By contrast, the structure of TRPV1 is in many ways reminiscent of that of the Kv1.2 (Long *et al.* 2005; cf. second and third structures in the first column of Fig. 3), which includes a large 'hanging gondola' most probably made up of the N and C terminus of the channel (Fig. 3). The TRPV1 volume is several times smaller than that of TRPC3 or TRPM2. It has been argued that the large expansion of the TRP channel cytoplasmic surface allows for the association with several macromolecules. Because TRPV1 has also evolved to be a specialized sensor of various signals, and is likely to be a macromolecular complex, one can only agree with Moiseenkova & Wensel (2009) that, 'it is not obvious why the TRPC3 and TRPV1 channel structures look so different.'

2.3 TRP channel multimerization properties

Homo- or heteromultimerization was first tested when *Drosophila* TRP and TRPL channels were coexpressed in *Xenopus* oocytes (Gillo *et al.* 1996). When coexpressed TRP and TRPL form active heteromers with a characteristic conductance but low constitutive activity; they also can be coimmunoprecipitated from a preparation of *Drosophila* eye (Gillo *et al.* 1996; Xu *et al.* 1997). Coexpression of TRP and TRPL leads to a store-operated, outwardly rectifying current distinct from that caused by either TRP or TRPL alone (Minke *et al.* 1996). TRP-gamma, another subunit

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involved in the photoreceptor response in *Drosophila*, also interacts with TRPL forming a phospholipase C-sensitive channel (Xu *et al.* 2000).

Regarding mammalian TRP channels, the first report on heteromultimerization came from coimmunoprecipitation experiments on TRPC1 and TRPC3 complexes (Xu *et al.* 1997). Functional evidence of TRPC heteromultimerization came later from TRPC1 and TRPC3 coexpression experiments, which resulted in a diacylglycerol-sensitive Ca^{2+}-regulated cation currents different from the currents produced by the original subunits (Lintschinger *et al.* 2000). Clapham and coworkers demonstrated that TRPC1 is able to coassemble with TRPC4 and TRPC5 (Strübing *et al.* 2001). Interestingly, coexpression of TRPC1 and TRPC4 or TRPC5 gives origin to novel outwardly rectifying (TRPC4 and C5 are doubly rectifying channels), nonselective cation conductances, and the recombinant TRPC1/TRPC5 channel was activated by G_q-coupled receptors and its activity was independent of calcium store depletion. The characteristics of these heteromeric channels resemble those of native G_q-coupled receptor-activated currents present in the hippocampus and the frontal cortex, giving support to the hypothesis that TRPC heteromers form receptor-modulated currents in the mammalian brain. An attempt to determine the combinatorial rules within the TRPC subfamily was made by Hofmann *et al.* (2002) using a variety of different experimental approaches. They concluded that TRPC2 is utterly unable to form heteromers with the other TRPC proteins, whereas the other TRPC subunits can form heterodimers, but only with phylogenetically close relatives: TRPC1/TRPC4/TRPC5 representing one group and TRPC3/TRPC6/TRPC7 representing the other. The rules of TRPC channel assembly put forward by Hofmann *et al.* (2002) appeared to apply *in vivo* since Strübing *et al.* (2003) were able to detect the presence of the complex formed by TRPC1/TRPC4/TRPC5 in the prenatal brain. Although the investigations of Hofmann *et al.* (2002) ruled out heteromerization between distant relatives in the TRPC subfamily, the situation regarding heteromerization is far from clear. The evidence provided by several groups indicates that distant TRPC members can form heteromeric channels. Thus, TRPC3 can assemble with TRPC1 (Lintschinger *et al.* 2000; Liu *et al.* 2005), and more recently Poteser *et al.* (2006) reported the existence of a TRPC3/TRPC4 complex able to form redox sensitive channels in endothelial cells.

Regarding the molecular determinants involved in tetramerization, Lapage *et al.* (2006) used chimeras between TRPC4 and TRPC6 to demonstrate that there are two domains responsible for the association between TRPC4 and TRPC6 (members of distinct subgroups of TRPCs). TRPC4 coimmunoprecipitated with TRPC6 subunits containing the N-terminal ankyrin and coil-coiled domain (residues 1–304) of TRPC4 (domain 1) (Fig. 2). TRPC6/TRPC4 chimeras containing part of the S4–S5 linker and C-terminal tail (residues 516–974) of TRPC4 (domain 2) are also able to interact and form tetramers with TRPC4 subunits.

It is also known that TRPVs can form hetero-oligomers. How and where TRPV channels form heteromultimers are important questions since the coassembly of different TRPV subunits with widely different gating properties may have important repercussions for the coding and regulation of thermosensation. The early work done using TRPV3 channels and based on coimunoprecipitation and electrophysiology suggested that TRPV3 and TRPV1 form heteromultimers (Smith *et al.* 2002). More convincing data regarding the interaction between different TRPV subunits were obtained by Hoenderop *et al.* (2003) using a variety of different techniques. In a study that included chemical cross-linking, sucrose gradient centrifugation, coimmunoprecipitation, and electrophysiological analyses of concatemeric channels, Hoenderop *et al.* (2003) confirmed that TRPV channels assemble as tetramers and demonstrated that TRPV5/6 form

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heteromultimers with novel ion transport properties. These results were further confirmed by a systematic study on TRPVs interaction carried out by Helwig *et al.* (2005). Using fluorescence energy transfer (FRET) and a biochemical approach, they showed that TRPV5/6 are able to form heteromultimers. However, their data indicated that besides TRPV1/2 and TRPV5/6, all the other members of the TRPV subfamily show a *preference* to form homo-oligomers (Helwig *et al.* 2005). Moreover, *in vivo* experiments carried out in rat cerebral cortex cells provided evidence that TRPV1 and TRPV2 interactions do take place in native tissue (Liapi & Wood, 2005). Cheng *et al.* (2007) studied TRPV1–4 interactions using spectra-FRET, an approach similar to that used by Helwig *et al.* (2005) but is more sensitive. The spectra-FRET approach allows the detection of strong fluorescent signals when the signals are specifically collected from the plasma membrane, thereby efficiently decreasing the intracellular signals. The results confirmed previous reports of heteromeric interactions of TRPV1/2. However, in contrast to the results obtained by Helwig *et al.* (2005), they also found FRET values corresponding to heteromization when they included TRPV3 and TRPV4. Heteromerization of TRPV1–4 was further confirmed by combining their energy transfer results with single channel recordings. Heteromeric channels show particular intermediate conductance levels and different gating kinetic properties.

There is just one example of a TRPM family channel heteromultimer, which is the case of TRPM6 and TRPM7 channels with the unique characteristic of harboring serine/threonine kinase domains in the distal part of their C termini. TRPM6 appears to be crucial for maintaining the normal Mg^{2+} homeostasis since mutations in the *trpm6* gene causes autosomal recessive hypomagnesemia with secondary hypocalcemia. The study of Chubanov *et al.* (2004) strongly suggested that TRPM6 does not conduct currents by itself (cf. Voets *et al.* 2004). Instead, an association between TRPM7 and TRPM6 was required to incorporate channel complexes in the plasma membrane, and disruption of the complex by the naturally occurring TRPM6 mutation S141L may be the cause of hypomagnesaemia in humans. The association of TRPM6 and TRPM7 channels was also studied by Schmitz *et al.* (2005), who found that there is a cross talk between their activities and the need of TRPM7 for TRPM6 functional membrane expression. A more recent study (Li *et al.* 2006), however, clearly demonstrates that TRPM6, TRPM7, and heteromultimers of these two channels of the melastatin subfamily form well-defined ion channels with different ionic permeabilities, and with distinctive single channel conductance. These different findings have not been explained but they could be reconciled by the observation of Topala *et al.* (2007) of low TRPM7-like endogenous currents in HEK293 cells.

The molecular nature of the interactions that lead to TRP channel heteromultimerization is unclear (for a review, see Schaefer, 2005), and whether the formation of heteromeric TRP channel is physiologically relevant remains to be clarified.

2.3.1 The mysterious TRP domain

Some TRP subfamilies display a conserved sequence on their proximal C-terminal region that has been used as a signature sequence for TRP channels (Fig. 4). This ~25-amino acid intracellular region, just after the S6 region, is called the *TRP domain* (Montell, 2001). This domain contains the *TRP box*, a highly conserved region first reported by Zhu *et al.* (1995) as the 'EWKFAR motif' and was found in 11 of the 13 TRP channel sequences known at that time (all of them members or relatives of the mammalian TRPC family). The TRP *box* is defined by the consensus sequence WKFQR (Fig. 4), where *W* is the only conserved residue among the TRP families. Positive charges on positions 2 and 5 of that sequence are shared by almost all

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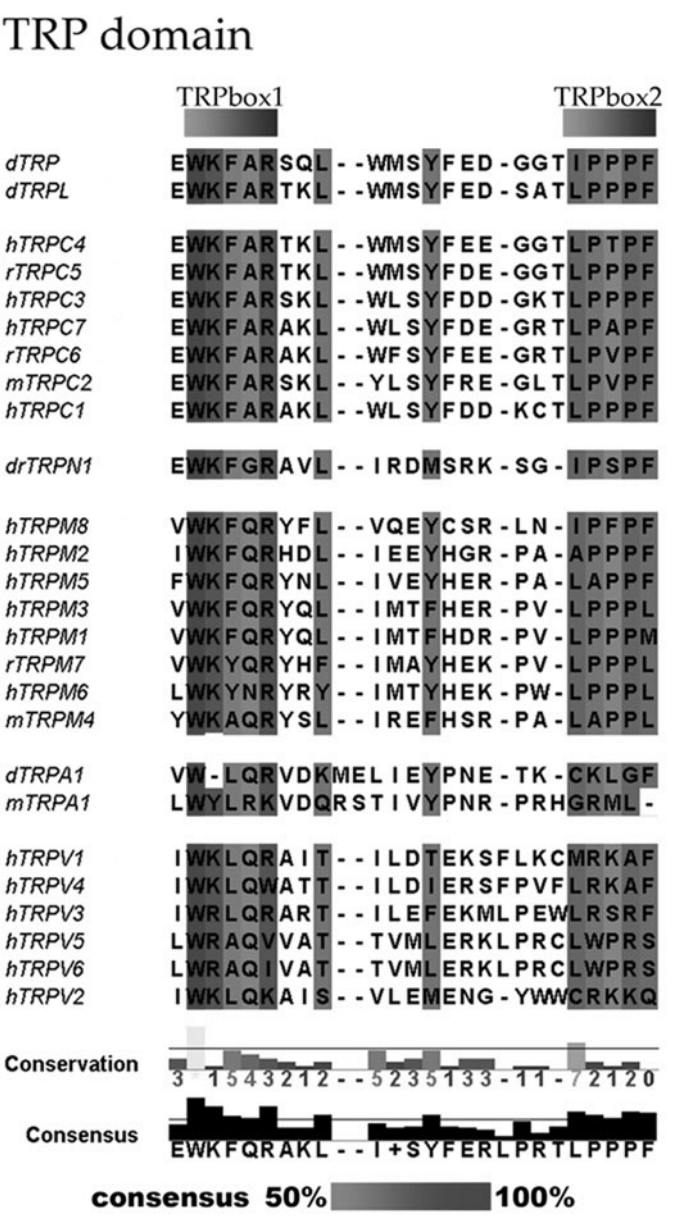


Fig. 4. A signature sequence for TRP channels: the TRP domain. This domain contains – in some cases – a highly conserved region the 'EWKFAR motif' (see the TRPC family). The TRP *box* is defined by the consensus sequence WKFQR, where *W* is the only conserved residue among TRP families. In channels belonging to the TRPC and TRPM subfamilies, there is a second conserved sequence on the C terminus end of the TRP domain; this second box has the consensus sequence LPPPF.

TRPs that present a TRP domain. It is possible to distinguish a *second* conserved sequence on the carboxy-terminal end of the TRP domain in the channels belonging to the TRPC and TRPM subfamilies. This second box is proline-rich, sharing the consensus sequence LPPPF (leucine on position one is highly conserved). The main role of the TRP domain remains elusive, but it has been suggested that it may participate in channel assembly (García-Sanz, 2004) and PIP_2 binding

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(Rohacs *et al.* 2005; Rohacs, 2006; Brauchi *et al.* 2007). Recently, using chimeras between TRPV channels, it has been proposed that the TRP domain may operate as both a tetramerization domain and a transduction domain somehow coupled to channel gating (García-Sanz *et al.* 2007). García-Sanz *et al.* (2007) showed that the incorporation of certain amino acids that alter the interactions inside the TRP domain may have an important effect on the activity of the channel by affecting the activation energy of channel gating. Interestingly, they propose that the TRP domain works as a 'negative allosteric modulator' holding the channel in the closed state. We note here that a small portion of the C-terminal of the Ca^{2+}-activated K^+ channel (hSlo) behaves very similarly to the TRP domain of TRPV channels. The interaction of that domain (tail) with the other portion of the hSlo channel inhibits channel opening, and this inhibition is relieved by Ca^{2+} binding to the tail via an allosteric mechanism (Schreiber *et al.* 1999).

2.4 Molecular determinants of channel assembly

Structurally, TRPs appear to share a common TM scaffold with four subunits around a central aqueous pore. Recent work shows that TRP channels from different families do not share the same assembly mechanism or tetramerization region (García-Sanz *et al.* 2004; Hoenderop *et al.* 2003; Erler *et al.* 2006; Lapage *et al.* 2006; Tsuruda *et al.* 2006). N and C termini and protein moieties located between TM segments 1–6 appear to be involved in TRPV1 channel tetramerization and the N terminus of TRPV4 makes a significant contribution to the assembly of TRPV4 (García-Sanz, 2004; Hoenderop *et al.* 2003). Ankyrin-repeat domains (ARDs) located in the N terminus of TRPV5 and TRPV6 are involved in channel tetramerization (Erler *et al.* 2004; Chang *et al.* 2004). However, in these cases, channel assembly appears not to be determined by direct interaction of ARDs as previously proposed but rather by interaction of the ARDs with other structures still to be determined (Phelps *et al.* 2008). Coiled coil domains located on the distal portion of TRPM8 C-terminal (Erler *et al.* 2006; Tsuruda *et al.* 2006), TRPM2 (Mei *et al.* 2006), and TRPM7 (Fujiwara & Minor, 2008) appear to be important in the assembly process of these channels (but see Phelps & Gaudet, 2007 and below). On the other hand, TRPCs apparently use a combination of N-terminal interactions through ankyrin–coiled coil domains together with C-terminal interactions to form active tetramers (Lapage *et al.* 2006).

3. N-terminal domain

3.1 Ankyrin domains

The 33-amino acid ankyrin repeat (AR) is among the most abundant repeat motifs and is present in different types of proteins (Mosavi *et al.* 2004). Probably because its high specificity, AR are involved in a varied set of cellular functions including regulation of cell cycle, transcription, cell–cell signaling, development, and transport. ARs act as building bricks that pile up side by side, forming a modular and highly efficient and specific protein-binding surface. AR structure is well defined: each repeat folds into two antiparallel helices followed by a beta-hairpin (Mosavi *et al.* 2004; Sedgwick & Smerdon, 1999; see below).

TRP channels from TRPA, TRPN, TRPV, and TRPC subfamilies present ankyrin domain repeats on their amino-terminal region (Fig. 2; Montell, 2005a; Gaudet, 2008). ARDs are highly conserved in TRPV channels and the six members of this subfamily contain a six-repeat ARD.

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The complete structure resembles the shape of a cupped hand were the helices form a palm and the hairpins (or sometimes loops) represent the fingers (Fig. 5*a*). The ARDs of TRPVs are important for the integrity of the channel, as their deletion impairs assembly and trafficking to the cell membrane (Jung *et al.* 2002; Chang *et al.* 2004; Erler *et al.* 2004). TRPV1 N-terminal has been shown to be involved in its calmodulin regulation and peptides from the N-terminal bind to calmodulin in a Ca^{2+}-dependent manner (Numazaki *et al.* 2003; Rosenbaum *et al.* 2004). Interestingly, protein kinase C (PKC) potentiation of the thermosensitive TRPV1 activity is linked to interactions with SNARE complex-related proteins through its N-terminal domain. Morenilla-Palao *et al.* (2004) found that TRPV1 interacts specifically (*in vitro* and *in vivo*) with two vesicular proteins, Snapin and Synaptotagmin IX, and that this interaction allows the recruitment of receptors located in vesicular compartments to the plasma membrane by an orchestrated exocytosis process. TRPV2 N-terminal was found to interact with the recombinase gene activator protein, which associates with the channel during biosynthesis and early trafficking (Barnhill *et al.* 2004; Stokes *et al.* 2005). The N-terminal region in TRPV2 also interacts with the adapter protein, Acyl CoA binding domain protein, (ACBD)3, allowing the recruitment of protein kinase A (PKA) into the channel micro environment (Stokes *et al.* 2005).

Two separate groups determined the crystal structure for TRPV2-ARD (McCleverty *et al.* 2006; Jin *et al.* 2006), and Lishko *et al.* (2007) solved the crystal structure of the TRPV1-ARD (residues 101–364) (Fig. 5*b*). Although previous predictions place four ARD's on the N-terminal sequence of TRPVs, the crystal structure clearly shows the presence of six-repeat ARD motifs that appear to be conserved throughout the TRPV subfamily (Fig. 5*a*). ARDs on TRPV channels do not follow a standard pattern. Repeats 1–3 have long and flexible fingers, whereas repeats 5 and 6 have long outer helices. Moreover, a large counterclockwise turn was observed in the stacking of repeats 4 and 5. These modifications affect the overall ARD structure/symmetry and may alter its functional behavior. TRPV1-ARD and TRPV2-ARD do not form hetero-oligomers but apparently ARDs play a role in promoting tetrameric assembly of TRPV5 and TRPV6 (Chang *et al.* 2004; Erler *et al.* 2004). It would be of interest to explore the nature of the interaction in other TRPV channels and especially in TRPA1 as ARDs are being used for interactions with agonists (see below). Interestingly, the TRPV2-ARD structure predicted that phosphorylation sites in TRPV1's N terminus (S116, Y200) should be on the surface of interaction of ARD with other proteins. Phosphorylation events could, therefore, modulate the binding of regulatory factors to the ARD (Jin *et al.* 2006).

TRPV1 presents at least two mechanisms that lead to the modulation of its responsiveness to noxius stimuli: desensitization and tachyphylaxis. Although both are forms of desensitization, desensitization proper is a short-term phenomenon and in the case of TRPV1, it is produced upon acute channel activation by capsaicin or protons. By contrast, tachyphylapsis is a loss of channel sensitivity after multiple separate challenges with an agonist. The crystal structure of TRPV1-ARD solved by Lishko *et al.* (2007) provided several hints about how the tachyphylapsis phenomenon comes about. In contrast to TRPV2-ARD, the TRPV1-ARD structure revealed a binding site able to accommodate ATP, and biochemical studies demonstrated that calmodulin binds to the same site. The site is noncanonical, being formed by a concave surface of repeats 1–3, and containing several hydrophobic and positively charged residues. In particular, R115, K155, and K160 interact with the ATP triphosphate moiety. Mutations K155A and K160A completely eliminate nucleotide interaction with the TRPV1-ARD, sensitization by ATP, and a remarkable reduction in tachyphylapsis. These apparently contradictory results were explained by Lishko *et al.* (2007) on the basis of a model that assumes that in the sensitized state

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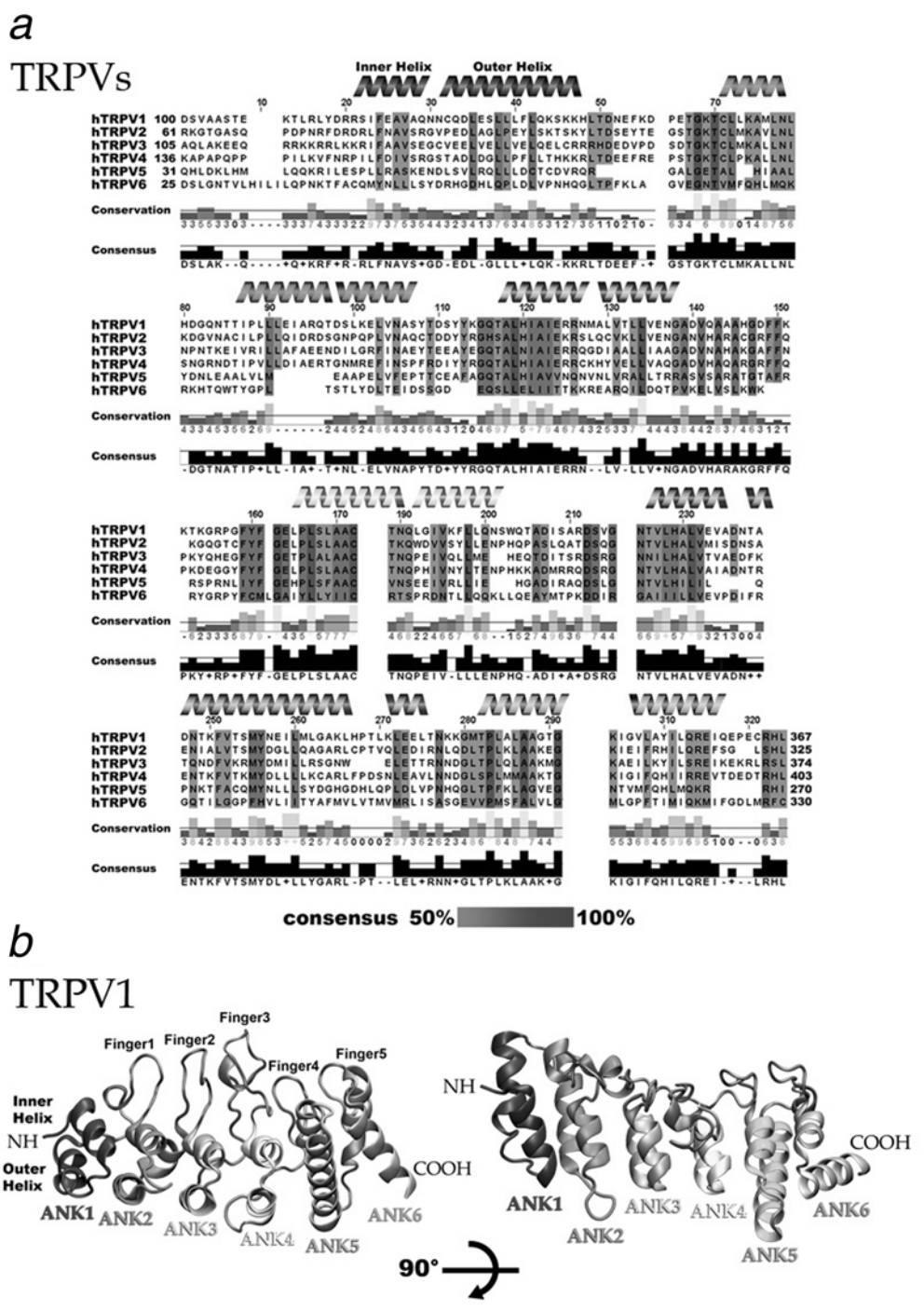


Fig. 5. Ankyrin repeat domains in the TRPV subfamily. (*a*) Alignment of the ARD of mammalian TRPV channels. Residues conforming the inner and outer helices of the ankyrin domains are indicated as helices above each repeat. (*b*) Crystal structure of the ARD domain of TRPV1. Mofified from Lishko *et al.* (2007) and Phelps *et al.* (2008).

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TRPV1-ARD is bound to ATP and that the Ca^{2+} influx through the open channel causes release of ATP and binding of the Ca–calmodulin complex to the TRPV1-ARD, which induces desensitization.

Solved at 1.7 Å resolution, the crystal structure of the ARD of TRPV6 showed many similarities with that of TRPV1 and TRPV2 (Phelps *et al.* 2008), and revealed structural characteristics unique to TRPV ARDs. For example, unusually long finger loops and amino acid residues conserved in TRPV proteins diverge from the AR sequence consensus, causing a twist between the fourth and 5th repeat. There are, however, several important differences between these ARD domains that should be noted since they may have important functional consequences. In particular, unlike the TRPV1-ARD, the TRPV6-ARD is unable to bind ATP or calmodulin (CaM) to any appreciable extent – suggesting that CaM modulates TRPV channels using more than one mechanism. Moreover, there is ample evidence that CaM is able to interact with a site in the C terminus of TRPV6. CaM facilitates channel inactivation, a process that can be overcome by a phosphorylation of the CaM binding site mediated by PKC (Niemeyer *et al.* 2001).

3.2 TRP channel activation through covalent modification of N-terminal cysteines

TRPA1 is activated by a number of pungent natural compounds used in cooking including wasabi and mustard, which owe their pungency to isothyocyanate compounds (see Fig. 13*e*) (Bandell *et al.* 2004; Jordt *et al.* 2004). This list of compounds has recently been extended to include a series of environmental irritants such as air pollutants and chemotherapeutic agents (Bautista *et al.* 2006; Hinman *et al.* 2006; Macpherson *et al.* 2006, 2007; McNamara *et al.* 2007; Trevisani *et al.* 2007). A fascinating new discovery is that cysteine residues in TRP channels can be covalently modified by a great variety of chemically unrelated agonists, whose only similarity is that they are electrophyles, as part of their mechanism of activation (Hinman *et al.* 2006; Macpherson *et al.* 2007; Bang & Hwang, 2009). Thus, unlike other natural agonists such as menthol or capsaicin that appear to act in a lock-and-key fashion, TRPA1 agonists act by covalently modifying their target cysteines and binding 'irreversibly' to the channel. Mutagenesis experiments showed that modification of only three cysteines of the 31 present in the cytoplasmic aspect of mouse TRPA1 led to agonist activation. All three cysteines are located in the N-terminal region of mouse TRPA1 channels (C415, C422, and C622; Macpherson *et al.* 2007). Two of these cysteines are inside one of the 18 ARD present in TRPA1 (C415, C422), and C622 is in the linker that joins together the last ARD with TM domain 1. Three cysteines were also found to be responsible for TRPA1 agonist activation by Hinman *et al.* (2006) and they mapped to C619, C639, and C663, all of them located in the ARD-TM1 linker of the human TRPA1 channel. Thus, the results obtained by Macpherson *et al.* (2007) and Hinman *et al.* (2006) share only one cysteine residue in common, C619; this cysteine residue in the human TRPA1 corresponds to C622 in mouse TRPA1. Recalling that isothiocyanates also modify primary amines, Hinman *et al.* (2007) found that a residual response of the triple cysteine mutant to AITC was due to a covalent modification of a lysine side chain since the mutant K708R produces TRPA1 channels that are completely insensitive to AITC.

How the free energy of ligand binding is converted into mechanical work (pore opening) is still a mystery, but the results of Chen *et al.* (2008) may have identified some of the pieces of the puzzle. Using a high-throughput screen, Chen *et al.* (2008) found electrophilic thioaminal-containing compounds that activated the rat TRPA1 but inhibited the human TRPA1. Notably, the opposite functional responses to these compounds are due to differences in some key amino

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acid residues contained in the S6 TM segments. Alanine 946 and methionine 949 are found to be critical for channel activation in rTRPA1 whereas the equivalent residues in hTRPA1 (serine 943 and isoleucine 946) are responsible for channel block. Moreover, point mutations of residue A946 can lead to activation (rA946G), inhibition (rA946T), or no response (rA946D). These data strongly suggest that the pore region of TRPA1 is involved in the coupling between the covalent binding of electrophilic compounds and channel gating.

In summary, all the data presented above strongly suggest that TRPA1 works as a chemical nociceptor in the nervous system, able to detect chemicals that mediate tissue damage and raise the voice of alarm to the whole organism by allowing the propagation of the pain response.

TRPA1 is not the only TRP channel to be activated by covalent modification of cysteines mediated by pungent compounds and, actually, it was known that allicin, one of the constituents of garlic, was able to activate both TRPA1 and TRPV1 (MacPherson *et al.* 2005; cf. Bautista *et al.* 2006). This observation was recently confirmed by Salazar *et al.* (2008), who further showed that the allicin behaves as a TRPV1 agonist through the covalent modification of a single cysteine localized in the N-terminal of the channel at position C157. Keeping Section 3.1 in mind, it is important to note that this residue is located near the amino acids that form part of the ATP binding site, a region that can undergo substantial structural conformational coupled to channel gating.

3.3 TRPM homology regions (MHRs)

Unlike the AR expressing channels, TRPMs share four high homology regions in their N-terminal cytoplasmic domain (Clapham, 2003). Although MHRs are highly homologous within TRPM channels, they do not show similarities outside of the TRPM family. As stated above, TRPM6 MHR mutation on Serine 141 (S141L) severely alters channel behavior, preventing proper channel assembly and causing hereditary hypomagnesemia (Walder *et al.* 2002; Schlingmann *et al.* 2002; Chubanov *et al.* 2004). Given the low similarity to known protein domains, it is difficult to speculate about the structural organization of MHR domains.

4. TM regions

Primary sequence analyses suggested that the 6TM domain architecture is the most probable for TRP channels (Montell, 2005a; Ramsey *et al.* 2006a). Although there is evidence supporting this hypothesis, it is all circumstantial and we cannot discard at present the existence of some exceptions since predictions for TRP channel TM domains often return more than 6TMs (Ramsey *et al.* 2006a). An appropriate example to illustrate this point is the voltage- and calcium-activated potassium channel (BK). This potassium channel belongs to the S4 superfamily that includes the six TM domains of voltage-dependent K^+ (Kv) channels but has an extra TM called S0 joining the standard 6TM segments, which allows the N-terminal to face the extracellular medium (for a review, see Latorre & Brauchi, 2006). To our knowledge, the Vannier *et al.* (1998) report is the only detailed topological study performed on a TRP (TRPC3) channel. In the case of TRPC3, the h1 segment, a seventh N-terminal hydrophobic domain predicted for members of the TRPC, TRPM, TRPV families, is intracellular and does not constitute a real TM segment.

The general scaffold for a 6TM domain channel considers that the TM region would be divided in two main modules: the voltage sensor (S1–S4) and the pore region (S5–S6)

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(Gaudet, 2007; Latorre *et al.* 2007b). For the case of TRP channels, there is growing evidence supporting an S5–S6 conductive pore module. One line of evidence supporting this topology is that the pore sequence is reasonably conserved among members of the same TRP subfamily. Another argument is that mutations introduced on the S6 segment may produce dominant negative forms of TRP channels (Kuzhikandathil *et al.* 2001; Krapivinsky *et al.* 2006). The strongest evidence in support of the S5–S6 conductive pore module, however, is that mutations of the residues within this consensus region affect permeation properties of the channels (see 'Pore' section and Owsianik *et al.* 2006 for a review on permeation of TRPs).

Although the analogy with *Shaker* type of ion channels suggests that the region between the helices S1 and S4 may be responsible for voltage sensing, there is a lack of convincing data showing that the voltage dependence is related to this particular module. Even if the voltage sensor is located in the S1–S4 domain, there is no reason to believe that the coupling between the sensor displacement and the pore opening is similar to that of the *Shaker* channel.

TRP channels are known to be responsive to several natural as well as synthetic compounds (Voets *et al.* 2005) and, as pointed out by Dhaka *et al.* (2006), most of the compounds with oral sensory qualities different from olfaction and taste can be attributed to the opening of TRP channels. This behavior is understood, in general, as part of their role as molecular sensors for external stimuli. Interestingly, several binding sites have been found within the TM region (Bandell *et al.* 2006; Jordt & Julius, 2002). The mechanism allowing the transformation of the chemical energy of binding into the mechanical energy necessary to open the pore is still unknown, but an allosteric hypothesis which relates this process with the channels' voltage dependence has been suggested (Nilius *et al.* 2005; Voets *et al.* 2007; Latorre *et al.* 2007b).

4.1 Searching for the voltage sensor

The 6TM domain voltage-gated ion channels play a pivotal role in muscle contraction, neuronal excitability, and secretion. The voltage sensor domain (VSD) is the key element of voltage-gated ion channels for sensing TM potential, and has been studied for more than 50 years (Hodgkin & Huxley, 1952) at the levels of both, biophysics and protein structure (e.g. Bezanilla, 2000; Long *et al.* 2007).

The main feature of VSD is the special array of positive charges observed in S4 and, in particularlin voltage-dependent K^+ (Kv) channels, in the S2 TM segment (Noda *et al.* 1984; Papazian *et al.* 1995; Seoh *et al.* 1996; Jiang *et al.* 2003). These charges allow the voltage sensor to be displaced inside the membrane electric field in response to changes in membrane potential (Hodgkin & Huxley, 1952; Hille, 2001). The VSD is present in the membrane not only in voltage-dependent channels, but also in voltage-dependent enzymes (Murata *et al.* 2005) and incorporated into voltage-dependent proton channels (Sasaki *et al.* 2006; Ramsey *et al.* 2006b). Because some TRP channels are voltage-activated (Nilius *et al.* 2005; Latorre *et al.* 2007b), it is tempting to suggest the existence of a similar module in voltage-dependent TRP channels. In contrast to Kv channels, however, the voltage dependence of thermoTRPs is predicted to be very weak (Brauchi *et al.* 2004; Voets *et al.* 2004, 2007; Nilius *et al.* 2005; Latorre *et al.* 2007b). One possible explanation for the weak voltage dependence shown by thermoTRPs (apparent number of gating charges, $z = 0.6$–0.7 for TRPV1 and TRPM8, compared with $z = 13$ in Shaker K^+ channels) may be the scarcity of positive charges in the S4 domain: the S4 of the TRPV subfamily contains a single arginine residue and there are two arginines in the S4 of the TRPM subfamily (Fig. 6).

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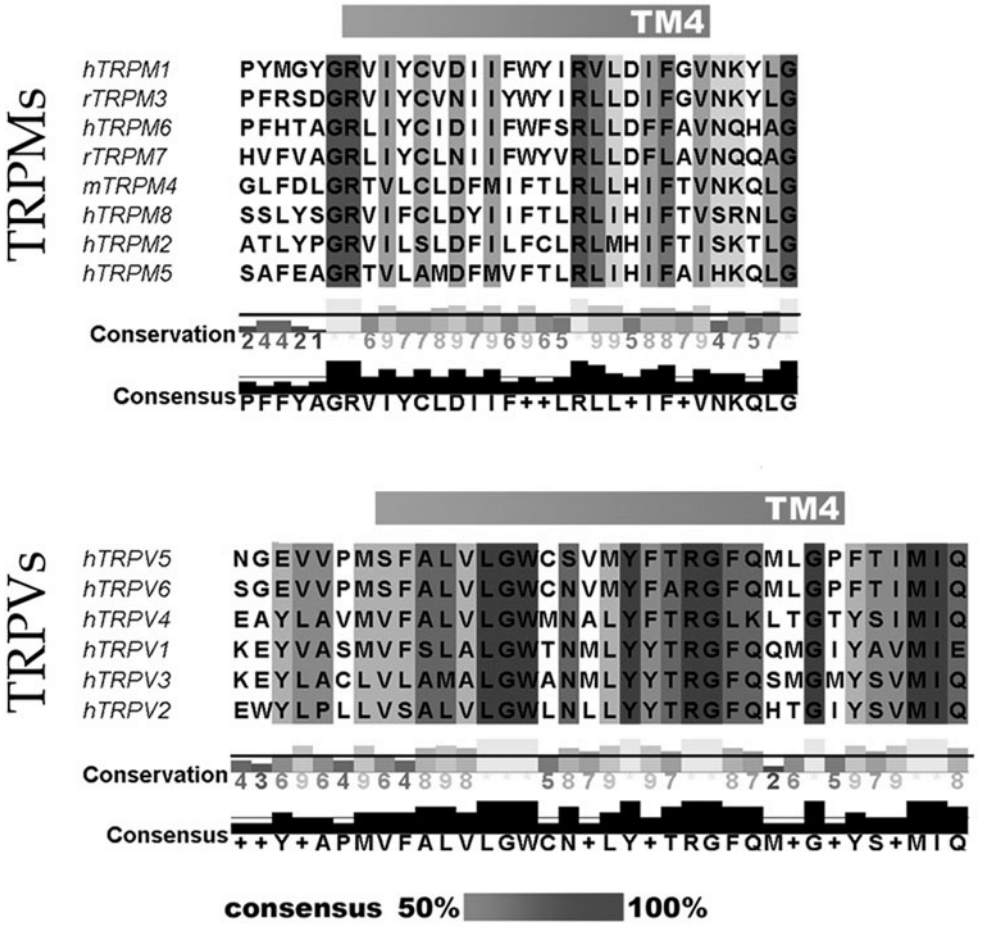


Fig. 6. Predicted TM segment 4 (S4) in human TRPM and TRPV subfamily. Alignments were performed by consensus using T-COFFEE v. (Notredame *et al.* 2000) and CLUSTALW. The sequences used in the alignments were extracted from NCBI GenBank.

The first hints that the positively charged residues in the S4 domain may be involved in voltage sensing were obtained by exchanging the arginines and lysines by neutral amino acids in the S4 domain of *Shaker* K^+ channels (Aggarwal & MacKinnon, 1996; Seoh & Bezanilla, 1996). The same mutagenesis strategy was followed by Voets *et al.* (2007), who neutralized all the charged residues, including histidine 845, contained in the S4 domain and in the S4–S5 linker of the TRPM8 channel. Using the limiting slope method (Almers, 1978; Sigg & Bezanilla, 1997), they found that the total apparent number of gating charges ($z\delta$) per TRP8 channel is, on average, 0.85 e^- and that neutralization of residues R842 or K866 was able to decrease the number of gating charges to about 0.6 e^-. Notably, the $z\delta$ obtained with the limiting slope method is very similar to that obtained by fitting the P_o–V curve to a Boltzmann function and there is a decrease in $z\delta$ obtained with the neutralization of a lysine K866 located in the S4–S5 linker. This first observation tends to support the use of a two-state model and, of course, for the sake of economy, this result validates its use. However, this simplest model does not explain the single channel electrical activity that comes in bursts separated by long quiescent periods.

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The most simple kinetic model able to account for this type of channel gating needs to assume the existence of more than one closed and at least two open states (Latorre *et al.* 2007a). If this is the case, the determination of the $z\delta$ using the limiting slope may not be the best experimental approach since this method breaks down for kinetic models containing *more* than one open state connected through voltage-dependent transitions (Sigg & Bezanilla, 1997). The second result is surprising given that, assuming a Kv channel structure valid as a template for TRPM8, the S4–S5 linker would be excluded from the electric field. There is, however, no reason to dismiss the possibility that the structure of the TRPM8 is different from that of Kv channels and that the decrease in $z\delta$ when K856 is neutralized can be accounted for if the S4 is long enough to leave K856 inside the electric field (Latorre *et al.* 2007b). Direct measurements of VSD movements in the form of gating currents and/or fluorescence quenching are at present needed to prove what parts of the S1–S4 module are involved in voltage sensing.

A closer inspection of the predicted S4 segment of TRPV subfamily channels reveals only one basic residue and, unexpectedly, TRPV1, TRPV3, and TRPV4 are outward rectifiers (Nilius *et al.* 2005), and TRPV6 behaves as weak inward rectifier in the absence of Mg^{2+} (Voets *et al.* 2003). More surprising is the fact that TRPA1 is also voltage-dependent, albeit with a lower $z\delta$ ($\sim$0.4; Karashima *et al.* 2009), despite the fact that it does not have positively charged residues in its S4 segment. These observations strongly suggest that the actual location of the voltage sensor in TRP channels is still a mystery.

4.2 Agonist binding sites inside TM region

Mammalian TRP channels are also the target of several very diverse series of pungent and other chemical compounds (Caterina *et al.* 1997; Patapoutian *et al.* 2003; Hinman *et al.* 2006; Bandell *et al.* 2007; Macpherson *et al.* 2007). Temperature-sensitive TRP channels (thermoTRP) channels are the best example of TRP channels able to act as sensors of a myriad of chemical stimuli. The list of compounds includes capsaicin (active component of hot chili peppers), menthol, icilin, camphor, allicin (one of the active components of garlic), mustard oil, and others, and is increasing every day (Fig. 9) (Voets *et al.* 2005; Dhaka *et al.* 2006).

A comparison between mammalian and avian (heat-sensitive but capsaicin-insensitive) TRPV1 channels resulted in a map of the structural determinants for capsaicin interaction. The results revealed that residues Y511 and S512, located at the transition between the second intracellular loop and the third TM domain (Jordt & Julius, 2002), are required for capsaicin binding. Rabbits are also insensitive to capsaicin and the rabbit TRPV1 is about 100-fold less sensitive to vanilloid activation than the human or the rat clone. Comparison between the primary sequences of the rabbit, rat, and human TRPV1 channels confirmed the results of Jordt & Julius (2002) that the capsaicin and resiniferatoxin (RTX, an ultra potent vanilloid from the plant *Euphorbia resinifera*) binding sites lie between TM regions 3 and 4 (Gavva *et al.* 2004). Gavva *et al.* (2004) argue that since the residue Y511 is conserved across species, it cannot explain the difference in vanilloid sensitivity between rat and rabbit TRPV1, and found that a single nonconserved residue is able to restore capasaicin binding (I550T) located in S4. On the other hand, the double mutation I550T/L547M restores the high binding affinity of RTX.

The same species comparison approach revealed the amino acid residues essential for the binding of icilin a *super-cooling* agent to the cold receptor TRPM8 (Chuang *et al.* 2004). The determinants of icilin sensitivity include residues located in the intracellular loop connecting TM domains 2 and 3. Note that the residues important for icilin sensitivity reside in a position

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that also correspond to the capsaicin binding site in TRPV1, suggesting that the protein structures involved in agonist interaction appear to be conserved across TRP channel subfamilies. Interestingly, icilin is unable to activate TRPM8 in the absence of external Ca^{2+} (Fig. 11). Therefore, for full channel activation icilin requires the presence of another stimulus, for example, a decrease in temperature, and in this regard TRPM8 behaves a coincidence detector (Chuang *et al.* 2004). Using a high-throughput assay, which includes and compares thousands of random mutations, Bandell *et al.* (2006) identified two distinct sites in TRPM8 required for sensitivity of the TRPM8 agonist menthol. The main binding site was proposed to be located in the S2 segment (Y745), while the C-terminal site (TRP domain, residues Y1005, L1009) would be required for geometrical specificity and presumably for coupling to the gate opening. Nevertheless, Brauchi *et al.* (2007) found that a chimera made of a TRPM8 channel with the C-terminal of TRPV1 has essentially the same menthol sensitivity as the wild-type, suggesting that the C-terminal is not an absolute requirement for menthol binding. This conclusion is supported by the fact that binding studies using radiolabeled menthol demonstrated that the mutation L1009R in the TRPM8 C-terminal resulted in normal radiolabeled menthol binding (Voets *et al.* 2007). Voets *et al.* (2007) confirmed Y745 as an important residue for menthol binding, but also located a different set of residues on the S4 and inside the S4–S5 intracellular loop that affect the TRPM8 menthol sensitivity and the affinity to radiolabeled menthol. In particular, the mutation of residue R842A, located in S4, decreased both TRPM8 menthol sensitivity (as determined from activation curves) and binding using radiolabeled menthol.

4.3 Gates and dynamic properties of the selectivity filter

If the TRP channel pore follows an architectural design similar to that of the Kv channels, we expect to find a large internal vestibule able to accommodate large organic cations. If this were the case, quaternary ammonium (QA) ions can be used to investigate the gross architecture of this channel region and to determine the location of the structure (gate) in charge of hindering the passage of ions when the channels are closed (Armstrong, 1975). Jara-Oseguera *et al.* (2008) followed this strategy in the case of the TRPV1 channel and found that QA ions of different sizes, from tetraethylammonium (TEA) to tetrapentylammonium, were able to block the TRPV1 channel when applied internally. These results are consistent with a diameter for the inner vestibule of about 12 Å in its widest section. They further found that closed channels cannot be blocked and that the inner pore can accommodate a TEA molecule when the channel is in its closed configuration – TEA can be trapped inside the channel when it closes. Therefore, as is the case with Kv channels, the evidence suggests the existence of an internally located gate. Clearly, this type of experiments revealed a gate able to hinder the path to large cations of the size of TEA, but does not tell us if this gate also avoids permeation to small cations such as K^+, Na^+, or Ca^{2+}.

Salazar *et al.* (2009) have added a new twist to the TRPV1 gating story by discovering that TRPV1 closing implies the formation of *two* constrictions in the internal vestibule channel. The most intracellular constriction was found to be at the level of residue L681 of the S6 segment of the rTRPV1 being the one in charge of preventing the passage of large molecules and implying a decrease in the internal vestibule diameter from 12 to about 6 Å. About in the middle of the S6 segment (Y671), they found a second narrowing of the internal vestibule able to restrain the passage to small permeating ions. Experimentally, they used a cysteine-less TRPV1 mutant and the cysteine scanning mutagenesis technique to test the state-dependent accessibility of thiol

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reagents such as 2-(trimethylammonium) ethyl methenethiosulfonate (MTSET) and 2-aminoethyl methanethiosulfonate (MTSEA). These compounds were used as calipers to determine the approximate diameter of the first constriction these charged molecules encounter when coming from the intracellular side of the channel; with a diameter of 3.6 Å, MTSEA is much smaller that MTSET (∼6 Å). State-dependent accessibility was found for MTSET when using the L681C mutant, but not for MTSEA, setting the maximum diameter of the most intracellular constriction at ∼6 Å. On the other hand, state-dependent accessibility for the small ion Ag^+ can be only detected beyond S6 residue Y671, strongly suggesting that the gating access for small permeant cations is located in this region of the S6 segment. Notably, the S6 segment of TRPV1 lacks the region proline-X-proline found in Kv (Webster *et al.* 2004) or the glycine residues found in some Ca^{2+}-activated K^+ channels (Jiang *et al.* 2002) that allow the S6 helix to attain the bend configuration found in the open channel structures. The PVP region in the S6 of Kv channels aligns well with a conserved tyrosine residue in the S6 of TRPV1 and other channels of the TRPV family. Whether this residue can play the role of a gating hinge remains to be explored further.

Since the early seventies it has been clear that K^+ and Na^+ channels should contain in some region of their conduction machinery a structure known as a selectivity filter, which is able to endow the channel with the ability to discriminate the nature of the ion trying to go through once the gate opens (for a nice historical account, see Armstrong, 1975). A fantastic picture of this domain was given by Zhou *et al.* (2001). The determination at 2.0 Å resolution, allowed us to see even 'a K^+ ion disrobing' (as excitedly stated by Armstrong, 1975) in the selectivity filter of the KcsA channel. In K^+ channel from different families, the selectivity filter sequence constitutes a distinctive signature since the GYGD motif is present in every potassium selective pore. By contrast, TRP channels do not show a clear consensus on their putative selectivity filter sequence. Moreover, most of them do not discriminate between monovalent cations, and for that reason they are categorized as nonselective cation channels (exceptional cases are TRPV5 and TRV6, which are calcium selective). Dogma dictates, on the other hand, that permeability properties of the pore are one structural–functional fingerprint for a given channel and should remain constant under different physiological or experimental conditions. Apparently, as we discuss below, a completely different picture appears to be true for several TRP channels. (The interested reader should consult Owsianik *et al.* 2006, for a detailed revision regarding TRP channels permeation properties.)

We would like to focus this section on TRP channels pore dilation process. We consider this a remarkable new finding that will force researchers to revise their hypothesis, built considering static pore architectures. Additionally, we note that this finding provides another regulatory element to ion channel function and an element that should be considered in future models for activation.

The work on ATP-gated ion channels of the P2X family suggested that prolonged agonist exposure increased the pore size, leading to enhanced permeability of the channel. This phenomenon, known as pore dilation, has been largely relegated to P2X receptors until now. However, a general mechanism underlying pore dilation on P2X channels remains controversial. Several models have been proposed, including direct changes in the selectivity filter, formation of new channel multimers, and recruitment of a nonselective pore (Fujiwara & Kubo, 2004; Locovei *et al.* 2007; Egan *et al.* 2006).

During the past few years, various researchers have provided evidence for the existence of a large pore in the case of TRPV1 channels, allowing permeation of large organic cations including

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spermine, $NMDG^+$, Yo-Pro^{2+}, quaternary amonium ions, gentamycin, and QX-314. These results suggest either a big static pore or changes on their pore diameter during activation (Ahern *et al.* 2006; Meyers *et al.* 2003; Jara-Oseguera *et al.* 2008; Binshtok *et al.* 2007; Myrdal & Steyger 2005; Hellwig *et al.* 2004). Moreover, the detailed study of Chung *et al.* (2005) showed that the permeability properties of TRPV3 channels change upon stimulation with 2-aminoethyl diphenylborinate (2-APB), diphenylboronic anhydride, and temperature. Several properties such as rectification, permeability, Ca^{2+} blocking, gating kinetics, and temperature dependence are affected after persistent stimulation.

TRPV5, one of the calcium selective TRP channels, presents a pore dilation process that is dependent of internal and external pH. Using a well conducted set of experiments, Yeh *et al.* (2005) demonstrated that the pore of TRPV5 channels changes its diameter (giving an estimate of 6–12 Å expansion), that the pore helix twist upon stimulation, and that a residue located on the C-terminal region is responsible for intracellular pH-induced pore dilation. In the same way, TRPV1 undergoes time- and agonist-dependent pore dilation after stimulation, allowing permeation of large organic cations such as Yo-Pro^{2+} and $NMDG^+$. The channel also presents differential ionic selectivity changes when stimulated by different agonists. One explanation for the dynamic changes in P_{Ca}/P_{Na} may be that it results from altered rates of Ca^{2+} interactions with the Ca^{2+} binding sites that are induced by activation-dependent conformational changes, rather than being a consequence of changes in TRPV1 pore size. As in TRPV5, a C-terminal modification in TRPV1 (PKC-dependent phosphorilation) affects selectivity (Chung *et al.* 2008). In the case of TRPV1, different activation modalities affect Ca^{2+} permeability; for example, Ca^{2+}-driven currents evoked by protons are smaller than those evoked by capsaicin. Site-directed mutagenesis experiments showed that charges D646, E648, and E651, all located on the outer mouth of the pore, are involved in such a process (Samways *et al.* 2008). Pore dilation phenomena appears not to be a property of only TRPV channels since it has recently been shown that TRPA1 activation results in dynamic changes of pore permeability to $NMDG^+$ and Yo-pro^{2+} uptake. Moreover, TRPA1 agonist such AITC induced Yo-Pro^{2+} uptake in a concentration-dependent fashion (Chen *et al.* 2009). These changes in TRP channel permeability may have effects on channel function through calcium-dependent desensitization and also on other neighboring proteins that are modulated by intracellular calcium. Interestingly, in cells expressing TRPM8 channels, neither menthol nor icilin induced permeability changes (Chen *et al.* 2009). The fact that pore dilation occurs in TRPA1, but not in TRPM8, may provide an opportunity to understand which are the molecular determinants of pore dilation.

5. C-Terminal domain

5.1 TRP domain, intracellular linkers, PIP2, and allosteric control

As discussed above, a two-state model (Nilius *et al.* 2004; Voets *et al.* 2007) is the simplest scheme to describe the effects of temperature on TRP channels opening. It is clear, however, that this model represents an oversimplification of the full gating intricacies of voltage-dependent thermoTRPs. In fact, although this two-state scheme describes fairly well the overall equilibrium behavior of voltage-dependent thermoTRPchannels, some results cannot be adequately accounted for by the two-state model. These include results showing that both TRPV1 and TRPM8 can gate independently of voltage (Brauchi *et al.* 2004; Matta & Ahern, 2007) and that the voltage-dependent transitions are poorly temperature-dependent (Latorre *et al.* 2007b). These

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facts are well explained by the use of allosteric models of channel activation (Latorre *et al.* 2007a, b; Matta & Ahern, 2007), and we will describe them in more detail in Section 6. Here, we will try to give a mechanistic vision of the possible nature of the coupling factors that participate in the gating of these channels.

A recent report from García-Sanz *et al.* (2007) further investigates the role of the TRP domain among TRPV channels. The authors found that chimeras constructed by replacing the TRP domain of TRPV1 with the TRP domain of the other members of this subfamily (TRPV2–TRPV4) affects channel function by altering the sensitivity to the stimuli. TRPV1 channels containing the TRP domain of either TRPV5 or TRPV6 reached the plasma membrane but did not show electrical activity. Notably, the authors suggest that the TRP domain might work as the coupling structure between the stimuli and the activation gate. As discussed earlier in this review, the TRP domain appears to be the, or part of the, tetramerization domain forming coiled coil structures (García-Sanz *et al.* 2004). Based on this observation and the results obtained with the TRP domain chimeras, García-Sanz *et al.* (2007) have proposed a model in which this channel region acts as a negative allosteric modulator. In other words, by forming a coiled coil structure the TRP domain keeps the channel closed. This hypothesis is attractive because if the stimuli (temperature, agonists, etc.) modify the interaction surfaces between subunits, they would probably induce an expansion of the 'gating ring ' formed by the C-terminals that would be translated into channel opening. This mechanism would be similar to that proposed for the MthK channel in which the gating ring uses the energy of Ca^{2+} binding to perform the necessary mechanical work to open the pore (Jiang *et al.* 2002).

We described TRP channels as 6TM proteins, in which voltage sensing occurs somewhere between segments S1 and S4. That region and perhaps some part of the membrane facing pore region (S5–S6 helices) also participate in agonist binding and, in a still unknown manner, couples the chemical energy of binding to the gating process. Additionally, we know that in both TRPV1 and TRPM8 there is a region inside the C-terminal domain that is responsible for, or at least has the role in, coupling the thermal energy and the PIP2 binding energy to channel opening (Vlachova *et al.* 2003; Rohacs *et al.* 2005; Brauchi *et al.* 2006, 2007). Predictions (Clapham, 2003) and subsequent work on PIP2 binding site in TRPM8 (Rohacs *et al.* 2005) identify the amino acids involved in channel activation by this lipid in the TRP domain. Functional analyses on chimeric channels, molecular dynamics, and docking methods were used to demonstrate that the TRP domain region also serves as a PIP2 binding site in TRPV1 (Brauchi *et al.* 2007). Molecular modeling shows a close interaction of PIP2 phosphates outside the C-terminal domain specifically with amino acids R575 and R579 located in the S4–S5 linker (of the neighbor subunit), a region of critical importance in the coupling of voltage sensing properties and gate function on potassium channels (Fig. 7; Long *et al.* 2005). Interaction of PIP2 with those charges may, in turn, decrease the electrical energy necessary to displace the voltage sensor, thereby promoting the observed leftward shift in the conductance–voltage curve. In this scenario, the C-terminal domain and the VSD are connected trough PIP2, suggesting that phospholipid binding might work as a chemical switch that modulates the activity of the channel. We note here that charged residues located in the S4–S5 intracellular linker of TRPM8 affects voltage sensitivity (although they are supposedly outside the dielectric field!), menthol, and temperature activation (Voets *et al.* 2007). Thus, we speculate that the complex – linker S4–S5/PIP2/TRP domain – may work as an allosteric modulator of TRPV channels, using the phosphate coordination with charged residues as the structure that controls their polymodal activation (Fig. 7). This concept might be extended, at least, to TRPMs and possibly to other TRP channels.

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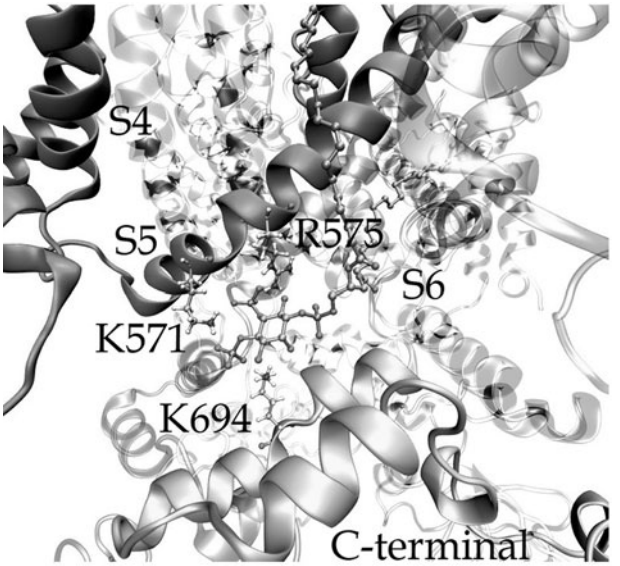


Fig. 7. Homology model for the TRPV1 channel reveals a PIP2-binding site. Ribbon diagram of the TRPV1 channel depicting one subunit (S4, S5), a neighboring one (C-terminal), and one bound PIP2 molecule. The model predicts that K571 and R579 in the S4–S5 linker of one subunit as well as K694 in the C-terminal from the neighboring subunit are in close contact with the PIP2 molecule. TRPV1 homology models were built by using as reference structure the crystal structures of Kv1.2 (PDB:2A79) and HCN2 (PDB:1Q43) as templates for TM and C-terminal regions respectively. The construction of topology file of the PIP_2 molecule is described by Brauchi *et al.* (2007).

5.2 Coiled coil domains and TRP tetramerization

Coiled coil is a protein structure domain in which α-helices wrap around each other to form a particularly stable structure. This structure, first proposed by Crick (1952), can form when the α-helices have most of their hydrophobic amino acids on one side (for the solution structure of a C-terminal tetramerization domain, see Nooren *et al.* 1999). In this case, α-helices can twist around each other with their hydrophobic side facing inward forming a supercoil twisted structure. Thus, coiled coil structures function as oligomerization domains for a variety of proteins including structural proteins, transcription factors, motor proteins, and ion channels. The coiled coil structure is highly conserved among different living organisms. Sequences with a propensity to assume coiled coil structures are characterized by a heptad repeat pattern of amino acids (*abcdefg*)*n*, where *a* and *d* are hydrophobic, and *e* and *g* are charged or polar (see Fig. 8*a*). Coiled coils may interact with each other and with other coiled coil domains (Nooren *et al.* 1999). Coiled coil domains have been implicated in subunit interaction and assembly of ion channels, including potassium channels (e.g. Jenke *et al.* 2003; Schwake *et al.* 2006). The TRP channels TRPV1 (García-Sanz *et al.* 2004), TRPM8 (Erler *et al.* 2006; Tsuruda *et al.* 2006), and TRPM2 (Mei *et al.* 2006) have also been found to assemble in what may be considered a coiled coil-dependent fashion.

Coiled coil domains present in the C terminus region immediately after the channel pore were found to be crucial for the proper assembly and function of TRPV1 channels (García-Sanz *et al.*

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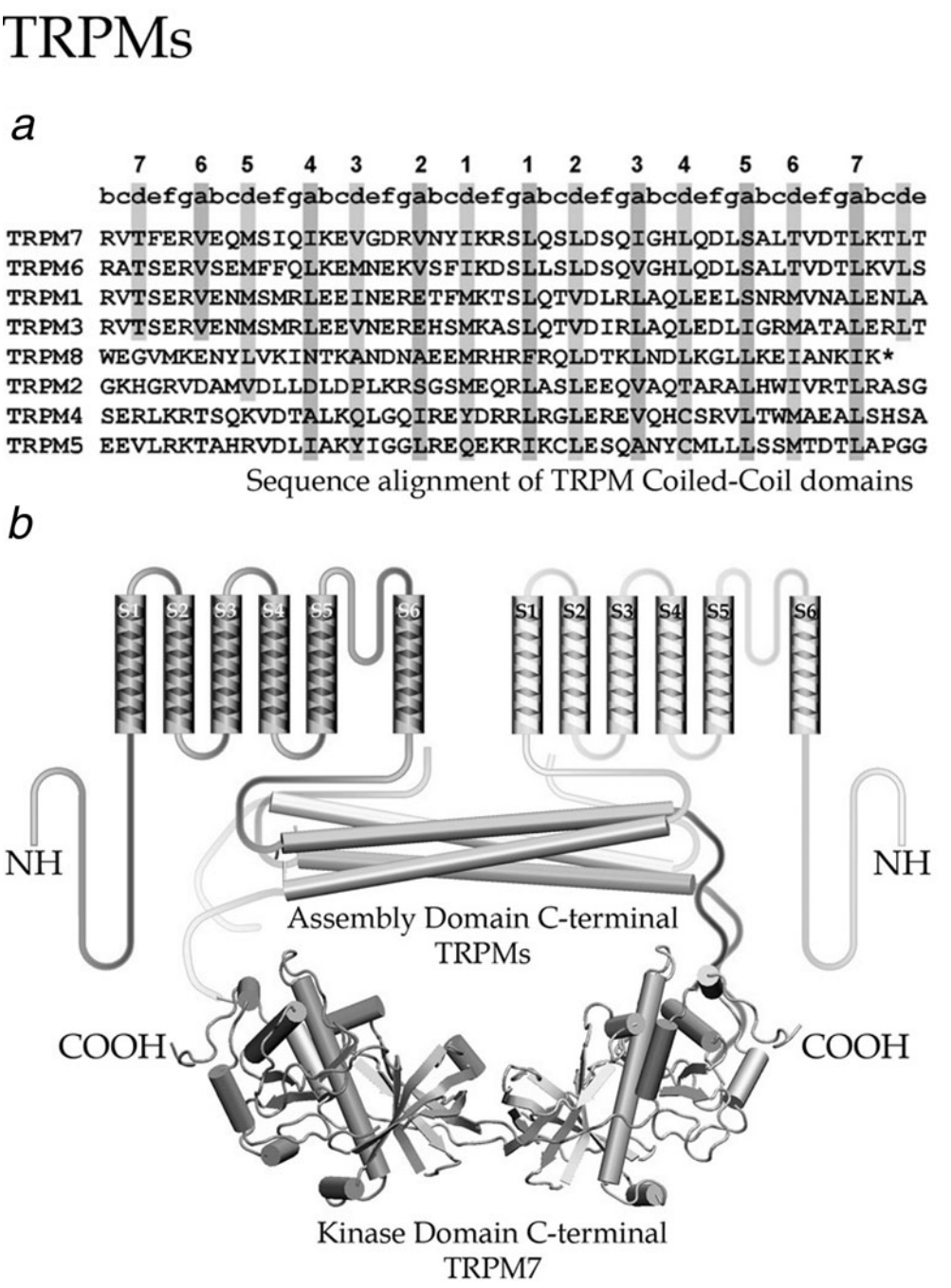


Fig. 8. The coiled coil structure of the distal C terminus in TRPM channels. (*a*) Sequence alignments of TRPM coiled coil domains. Heptad repeat pattern of amino acids *abcdefg* is shown on top of the coiled coil domains and hydrophobic amino acids *d* and *a* denoted by vertical rectangles. Note that the coiled coil sequences are ordered according to their length and hydrophobic matching producing the two groups: TRPM7, 6, 1, 3 and TRPM8, 2, 4, 5. (*b*) The TRPM7 TM domains of two subunits, their respective C-terminals containing the antiparallel coiled coil assembly domain and the dimeric kinase domains are shown. Modified from Fujiwara & Minor (2008).

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2004). The region between Glu 684 and Arg 721 of the TRP domain of TRPV1 adopts a coiled coil shape that is conserved within the TRPV family. A series of deletion experiments and the use of a dominant negative form of TRPV1 suggest that this region is critical for channel self-association. Coiled coil domains are part of TRPM channels but, in contrast to TRPV1, they are located in the distal part of the C-terminal (Fig. 8*a*; Launay *et al.* 2004; Mei *et al.* 2006; Erler *et al.* 2006; Tsuruda *et al.* 2006; Fujiwara & Minor, 2008). Truncation of the first 177 amino acid residues of the N-terminal and of the last 160 residues containing the coiled coil domain of the C-terminal domains of the TRPM4 showed that only the latter behaves as a dominant negative, preventing homomultimerization between the wild-type TRPM4 and the ΔC-TRPM4 (Launay *et al.* 2004). These results only suggest that the last 160 amino acids of the C-terminal region of TRPM4 are required for multimerization but do not necessarily imply that the coiled coil region of the TRPM4 channel is directly involved in channel assembly. Evidence that this structure is involved in channel function was given by Tsuruda *et al.* (2006), who showed that the coiled coil domain of TRPM8, comprising residues 1064–1104 (last 40 amino acids; Fig. 8*a*), works as dominant negative when coexpressed with WT channels, and was necessary for channel maturation and trafficking. In addition, when the coiled coil domain is expressed by itself, it is capable of forming tetramers. A dominant-negative can be also produced by attaching the coiled coil region to the S6 TM domain of TRPM8; finally, a single mutation L1089P within a central *a* position in the coiled coil region results in nonfunctional channels (Erler *et al.* 2006).

The crystal structure of the coiled coil domain of TRPM7 has recently been elucidated (Fujiwara & Minor, 2008). Despite the fact that TRP channels appear to have a fourfold rotational symmetry around the pore, the homotetrameric crystal structure of TRPM7 coiled coil shows a twofold symmetry. In solution, the TRPM7 coiled coil is a tetramer assembled with an antiparalallel orientation (Fig. 8*b*): two α-helical strands going in one direction and two strands going in the opposite direction. This twofold symmetry, however, matches the structural symmetry of the kinase domain (Fig. 8*b*), a dimer held together by an exchange of the N terminus α-helix of each monomer (Yamagushi *et al.* 2001) which follows the C terminus of the TRPM7 coiled coil. Notably, an analysis of the primary structure of the coiled coil domains of other members of this subfamily suggest that they can be divided into two groups: TRPM1, 3, 6, 7, and TRPM2, 4, 5, 8 (see alignments in Fig. 8). The large differences in the coiled coil lengths and the lack of matching between hydrophobic core compositions suggests that the quaternary structure of the coiled coil domain of these two groups will be different. These observations predict that it is unlikely that heterotetramers will form between groups. In other words, coiled coil domains impose restrictions to the type of channel that can be assembled.

It should be noted, however, that deletions of the C-terminal region prevent TRPM8 function but not tetramerization. Indeed, TRPM8 lacking the whole C-terminal region are able to reach the membrane and form tetramers but are nonfunctional (Phelps & Gaudet, 2007). Even in the absence of N- or C-terminal regions, the channel is able to tetramerize and reach the membrane. Combining immunostaining and confocal microscopy, electrophysiology, and biochemistry, Phelps & Gaudet (2007) also showed that, to be functional, TRPM8 need the coiled coil domain located at the end on the C-terminal as well as a region between the amino acids 40 and 86 of the intracellular N-terminal. It should be noted here that, contrary to what is found in TRPM8, deletion of the TRPM2 coiled coil domain indeed affects assembly and trafficking (Mei *et al.* 2006).

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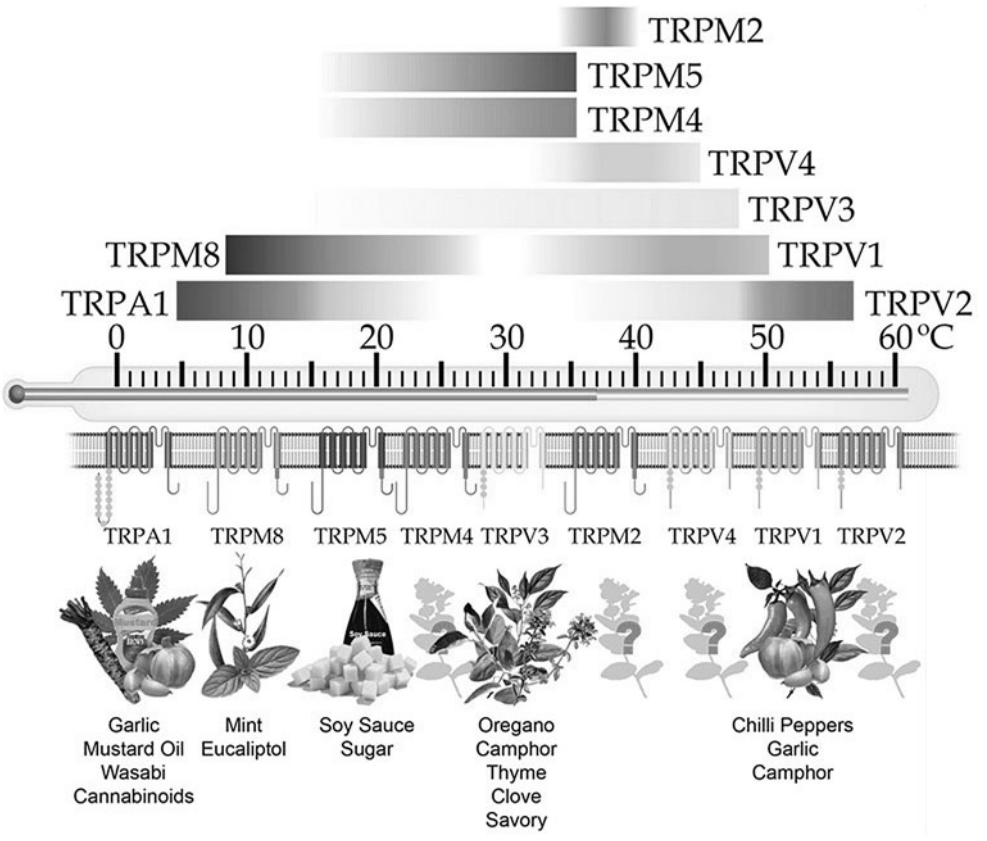


Fig. 9. Schematic representation of the thermoTRPs that function in temperatures ranging from noxious heat to noxious cold. Proposed membrane topology and functionally important domains are represented. Various plant compounds activate the thermoTRPs: TRPV1 is activated by capsaicin, the pungent ingredient in chilly peppers; TRPM8 is activated by menthol, the cooling compound from mint; TRPA1 is activated by various pungent compounds such as allicin, the active ingredient in garlic, and isothiocyanates, the pungent ingredients found in wasabe.

6. ThermoTRP channels

One of the most fascinating features of TRP channels is the presence in at least three different families of channels exquisitely sensitive to temperature, the thermoTRP channels. Understanding the molecular nature of the temperature-dependent conductance offers a challenge to biophysicists, as attractive as was the unveiling of the molecular nature of the voltage sensor in voltage-dependent channels. In this and the next section, we describe some of the biophysical properties which we speculate account for the temperature sensitivity of these fantastic molecular machines, and we end the review by giving a description of what we have learned from the use of knockout models.

6.1 A brief detour on thermoTRP channels

Nine members of the mammalian TRP ion channels show different temperature thresholds: TRPV1–4, TRPM2, TRPM4, and TRPM5 are heat-activated, whereas TRPM8 and TRPA1 are activated by cold (Fig. 9) (Talavera *et al.* 2005; Dhaka *et al.* 2006; Huang *et al.* 2006; Togashi *et al.*

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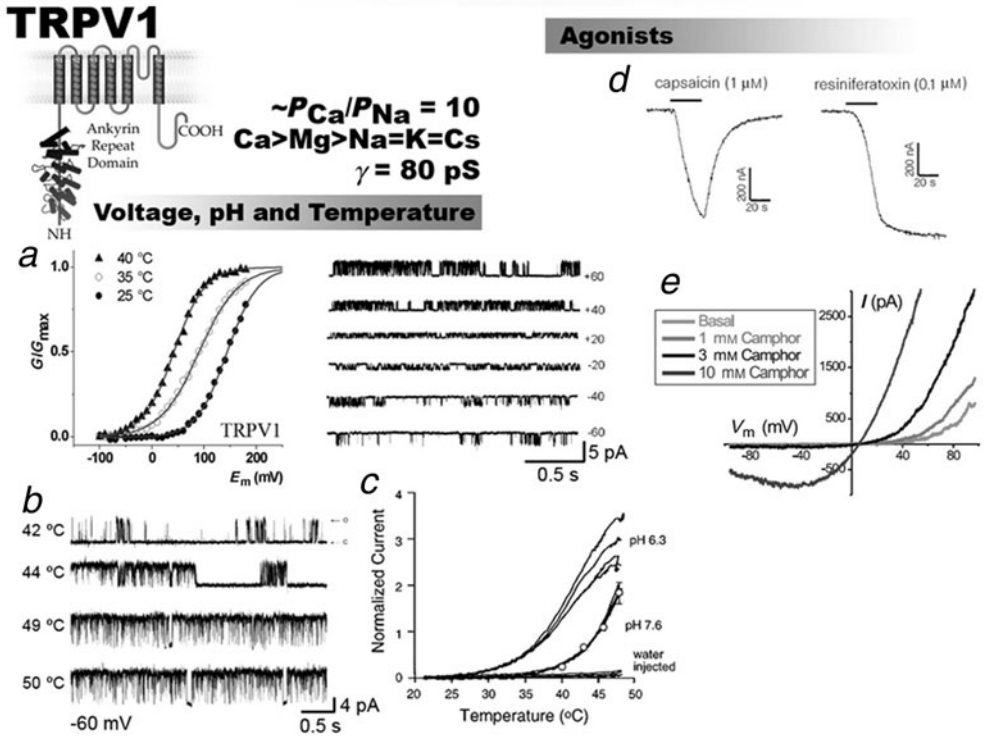


Fig. 10. Polymodal nature of TRPV1. (*a*) Left panel. Plots showing the normalized conductance in function of voltage at the indicated temperatures. Solid lines correspond to the best fit to Boltzmann functions (Brauchi *et al.* 2006). Right panel. single-channel currents recorded at different membrane potentials from an oocyte heterologously expressing TRPV1. Notice the progressive reduction in P_o with hyperpolarisation (Premkumar *et al.* 2002). (*b*) Heat-evoked single channel activity in oocytes expressing TRPV1. The temperature was elevated from 25 to 50 °C. Measurable activity was detected at ~42 °C. Note the marked increase in burst duration as temperature is increased. Data were obtained from an inside-out patch at a holding potential of −60 mV (Liu *et al.* 2003). (*c*) Protons lower the threshold and increase the magnitude of heat-evoked currents in TRPV1-expressing cells. Currents were normalized to responses at 45 °C (pH 7·6) (Tominaga *et al.* 1998). (*d*) VR1 responds to purified vanilloids and pepper extracts. Activation of TRPV1 by capsaicin and resinferatoxin. Membrane currents were recorded in the whole-cell voltage-clamp configuration. Bars denote duration of agonist application (Caterina *et al.* 1997). (*e*) Concentration dependence of the activation of TRPV1 by camphor (Xu *et al.* 2005).

2006; for a recent review on ThermoTRP channels tissue distribution, agonists, antagonists, and modulators, see Mandadi & Roufugalis, 2008). The first member of this class of channels that was cloned was TRPV1 (Caterina *et al.* 1997). TRPV1 is expressed mainly in small diameter nociceptive neurons of the trigeminal and dorsal root ganglia (DRG). Capsaicin (the active compound of 'hot' chili peppers), protons, depolarizing voltages, and noxious thermal stimuli (~42 °C) all activate TRPV1 (Fig. 10) (Caterina *et al.* 1997; Voets *et al.* 2004). The channel is cationic with a relatively high Ca^{2+} permeability (P_{Ca}/P_{Na} ~10). TRPV1 can be activated by protons at room temperature when the pH is decreased below 6. The decrease in temperature threshold for activation promoted by protons is hypothesized to be involved in inflammatory pain because tissue acidification accompanies ischemia and inflammation (Tominaga *et al.* 1998). TRPV1 is also activated by pungent extracts of onion and garlic, compounds that also activate the TRPA1 channel (Macpherson *et al.* 2005; Salazar *et al.* 2008). TRPV2 has a temperature

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threshold of about 53 °C and is expressed in DRG neurons of intermediate size (Caterina *et al.* 1999). Its expression, however, is not restricted to the nervous system; it is also found in the spleen and the small intestine. This channel is also permeable to Ca^{2+} ($P_{Ca}/P_{Na} \sim 3$) but is capsaicin-insensitive and is not activated by protons. Unlike the other thermoTRPs, TRPV3 and TRPV4 are mainly expressed in keratinocytes (Chung *et al.* 2004). TRV3 is also expressed in the tongue and nose, and TRPV4 in the central nervous system (CNS). Both channels are activated at innocuous temperatures (33–35 °C) and are therefore activated at body temperature (Güler *et al.* 2002; Peier *et al.* 2002a; Smith *et al.* 2002; Watanabe *et al.* 2002; Xu *et al.* 2002). Camphor, a natural compound that modulates sensation of warmth in humans, activates TRPV3 in keratinocytes but not sensory neurons (Moqrich *et al.* 2005). Components of plants such as carvacrol, eugenol, and thymol strongly activate and sensitize TRPV3 (Xu *et al.* 2006). TRPM2 is expressed mainly in the brain but is also present in the heart, liver, and pancreas (Perraud *et al.* 2001; Togashi *et al.* 2006). The channel discriminates poorly between monovalent and divalent cations, and contains a C-terminal domain homologous to the ADP ribose phosphohydrolase (Nudix domain; Perraud *et al.* 2001). TRPM2 is constitutively activated at body temperature (threshold $\sim$35 °C at $-$60 mV) and its activation is strongly potentiated by cyclic ADP ribose. By regulating Ca^{2+} entry into pancreatic β cells depending on the availability of cyclic ADP ribose, TRPM2 appears to play an important role in regulating insulin secretion. TRPM5 is a cationic channel impermeable to Ca^{2+} that is selectively expressed in taste receptor cells and has a key role in the perception of bitter, sweet, and umami taste (Pérez *et al.* 2002; Hofmann *et al.* 2003). TRPM5 is voltage- and temperature-dependent; current increases steeply in the 15–35 °C range (Talavera *et al.* 2005). The temperature dependence of this channel may explain why taste perception in human is modulated by temperature and, in particular, why the perception of sweet is enhanced as temperature is increased. TRPM8, which was cloned independently by two groups and characterized as a cold- and menthol-sensitive receptor (hence its other name, CMR1) (Fig. 11) (McKemy *et al.* 2002; Peier *et al.* 2002a), is expressed selectively in a small population of primary sensory neurons. It is directly activated by slight cooling (threshold $\sim$22–30 °C) and depolarizes sensory neurons (McKemy, 2005; Reid, 2005). TRPM8 is also activated by the super-cooling agent icilin and by eucalyptol, albeit with lower efficacy and potency than menthol (McKemy *et al.* 2002). Another compound of interest and of pharmacological importance is 2-aminoethoxydiphenyl borate since it activates TRPV1, TRPV2, and TRPV3, but inhibits the activity of TRPM8 induced by menthol (Hu *et al.* 2004). Strong cooling activates ($<$17 °C) TRPA1 channels and has been suggested to be important in the transduction of strong (painful) cooling stimuli (Story *et al.* 2003; Bandell *et al.* 2004); however, the involvement of this channel in the cold sensitivity of sensory neurons has been questioned (Babes *et al.* 2004; Jordt *et al.* 2004; but see Sawada *et al.* 2007; Karashima *et al.* 2009; see below). Isothiocyanate compounds, the active component of wasabi and mustard, activates TRPA1 channels, identifying this channel as a molecular target for the pungent action of mustard oils (Bandell *et al.* 2004; Jordt *et al.* 2004).

6.2 Allosteric models and thermoTRP channel activation

In TRPM8 channels, we found that the data could be explained using an allosteric mechanism for both voltage and temperature dependence (Brauchi *et al.* 2004). This type of mechanism assumes the existence of *independent* temperature and voltage sensors both having resting (R) and activated (A) states coupled to channel opening. For clarity, we discuss first the simple model

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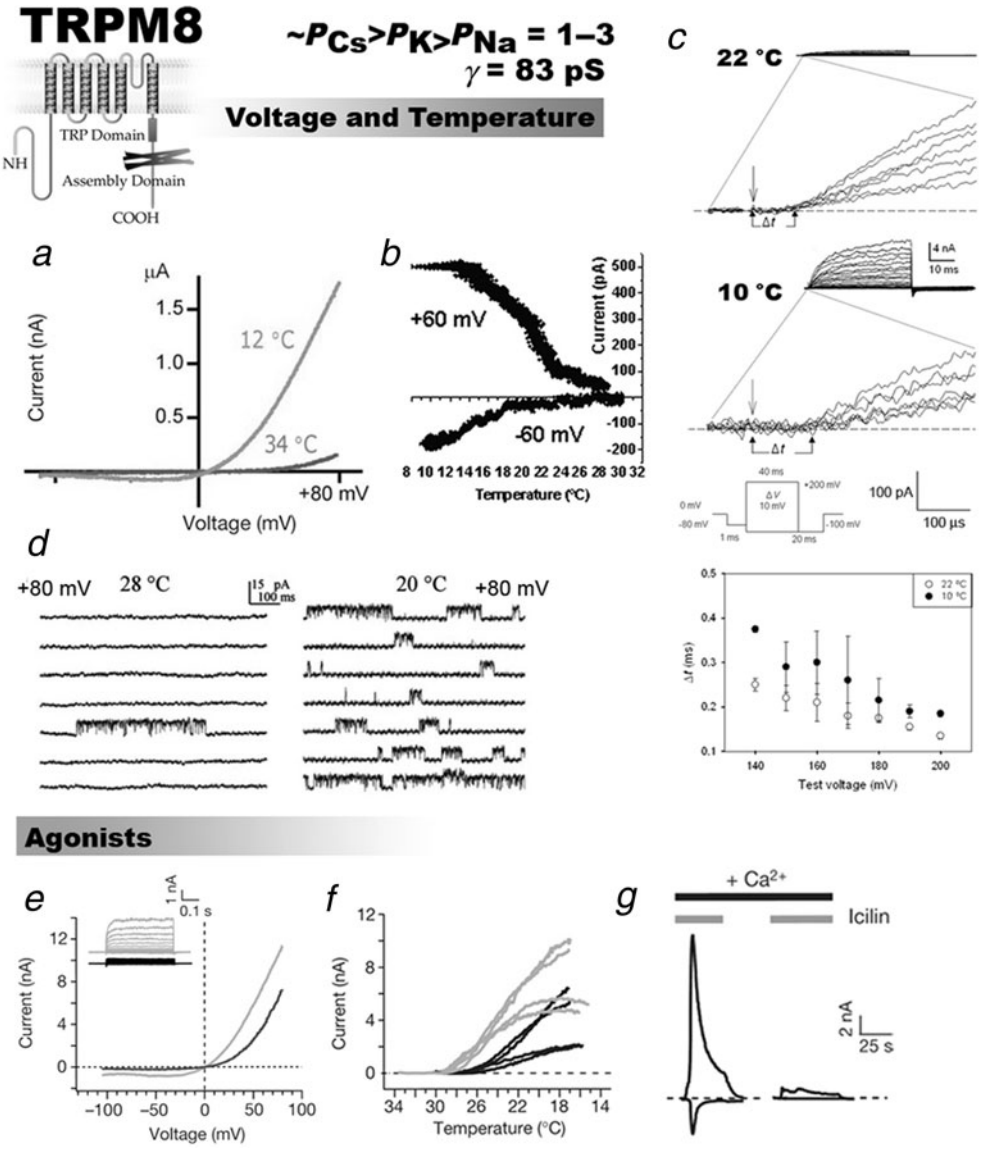


Fig. 11. Polymodal nature of TRPM8. (*a*) TRPM8 is activated by cold temperature (12 °C) and exhibits strong outward rectification (McKemy *et al.* 2002; Chuang *et al.* 2004). (*b*) Current–temperature recordings at holding potentials of −60 and +60 mV (Brauchi *et al.* 2004). Note that currents can be elicited with a threshold of about 18 °C at −60 mV, suggesting that temperature sensors can be activated when the voltage sensors are at rest (see panel *a*). (*c*) The time course TRPM8 channel activation is well described by an initial lag followed by an exponential current increase. Top: Family of macroscopic TRPM8 ionic currents at two (22 and 10 °C) different temperatures elicited in response to the variable depolarizing voltages shown in the pulse protocol. TRPM8 currents were measured using the whole-cell configuration of the patch clamp technique. Below each family of current records selected current records are shown in an enlarged time scale that allows appreciating the initial lag (Δt) of the ionic currents at the two temperatures studied. The single arrow indicates the time at which the test voltage pulse was applied. Below: Delay duration (Δt) is plotted *versus* test voltage pulse for three different experiments at two different temperatures. The delay has low temperature dependence ($Q_{10} = 1{\cdot}6$ at +200 mV). (*d*) TRPM8 single-channel recordings at two temperatures: 28 °C (low P_o) and 20 °C (high P_o). Short- and long-duration closed states can be discerned. Recordings were obtained in cell-attached conditions; patches were performed on HEK293

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depicted in Fig. 12*a*. In this case, in the absence of stimuli the channel is confined to the equilibrium between states C_0 and O_0, with a small equilibrium constant L that makes channel opening very improbable. The equilibrium between states C_0 and C_1 represents the activation of voltage sensors and is governed by the voltage-dependent constant J. These two equilibria are coupled by the allosteric factor D, so that when voltage sensors are active, the opening of the channel (equilibrium between states C_1 and O_1) has a higher equilibrium constant, LD. When the channel is open, the activation of voltage sensors (O_0–O_1 equilibrium) is JD. Therefore, when the temperature is high, the maximum open probability achievable by depolarization will be determined by the LD value.

It is important to note here that, being a homotetramer, the channel is likely to contain four voltage sensors. However, because there is no evidence about their molecular nature and whether they move independently, we are making a simplifying assumption that the voltage sensors move in concert.

In the more general case shown in Fig. 12*b*, temperature sensors and voltage sensors are coupled by an allosteric factor E and to channel opening by means of allosteric factors D and C respectively (Latorre *et al.* 2007a; Matta & Ahern, 2007). The model can be generalized to include, for example, the effect of ligand activation governed by an equilibrium constant Q and coupled to channel gating by an allosteric factor P. This type of model has received experimental support and some of the most important results are described below. In TRPV1 and TRPM8, the single channel activity occurs in bursts separated by gaps (Liu *et al.* 2003; Brauchi *et al.* 2004; Figs 10*b* and 11*c* respectively). In the case of TRPV1, temperature mainly affects burst length ($Q_{10} = 32$); the gaps ($Q_{10} = 7$) and single-channel dwell time histograms show the presence of 2–6 closed and 2–5 open components across temperature (Liu *et al.* 2003). The individual time constants of the different components show a much weaker temperature dependence with $Q_{10} < 3$, but their distribution is dramatically modified by temperature. For example, at a low temperature (40 °C), short closures are as frequent as long ones, whereas at 50 °C the long closures are almost absent from the single channel records, indicating that temperature mainly modifies the equilibrium between the different components rather than their transition rates. More importantly, short openings and long quiescent periods are coupled, indicating that the kinetic scheme is branched and that transitions between open and closed states occur in loops, as is the case of the allosteric models proposed by Brauchi *et al.* (2004) and Matta & Ahern (2007) (Fig. 12*a* and *b*).

As pointed out by Latorre *et al.* (2007a), having multiple states does not rule out the possibility that both temperature and voltage sensors move simultaneously (i.e., they have infinite co-operativity). There are at least four different sets of data that are in disagreement with this hypothesis but in concordance with the allosteric model in which voltage and temperature sensors are *independent structures* coupled to channel gating. First, a model with n identical sensors that are highly temperature- and voltage-dependent demands that the voltage at which

transfected cells (Latorre *et al.* 2007b). (*e*) Voltage activation curves. Responses evoked by menthol (50 mM, grey) and cold (16 to 8 °C, black) show strong outward rectification. Inset, baseline currents (black) and menthol-evoked responses (grey) in nominally Ca^{2+}-free bath solution at room temperature. (*f*) Temperature–response curves (from 33 to 16 °C) were determined for trigeminal neurons in the presence (grey) or absence (black) of 10 mM menthol. Menthol potentiated the size of cold-evoked currents and shifted thermal thresholds from $27{\cdot}1 \pm 0{\cdot}5$ to $29{\cdot}6 \pm 0{\cdot}3$ °C. (*g*) Activation and current desensitization of icilin (2 mM) dependent on calcium (McKemy *et al.* 2002).

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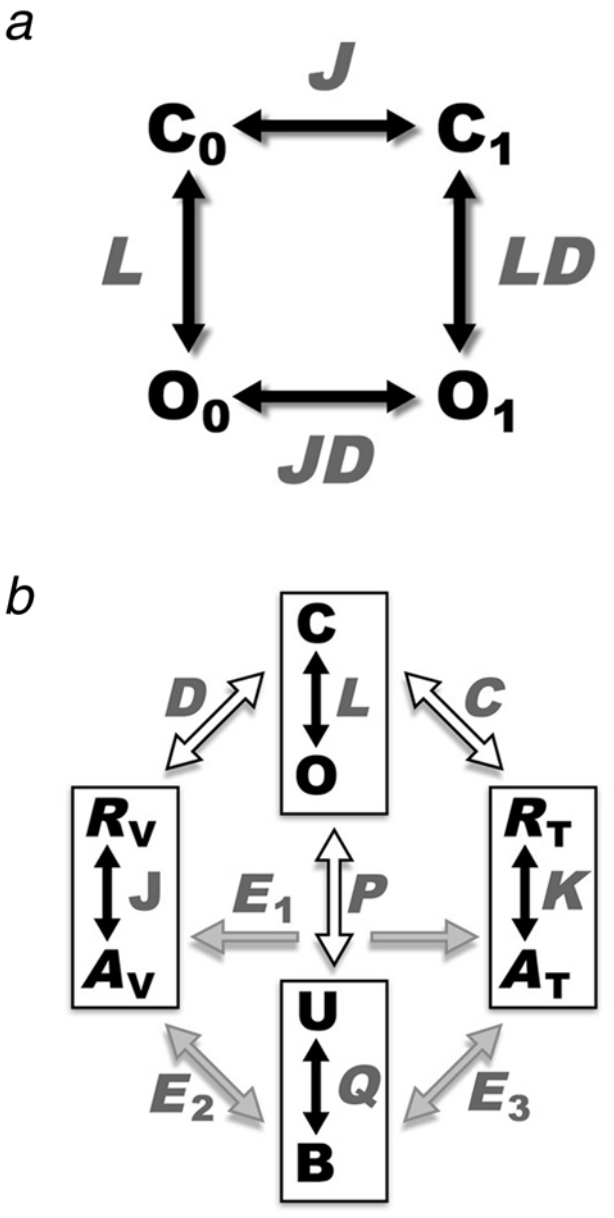


Fig. 12. An allosteric kinetic model to account for the thermoTRP channel gating. (*a*) We consider first the simple case in which the concentration of the agonist is zero and the system is at a temperature that is unable to activate the channel, in this case the gating mechanism includes an allosteric interaction between channel opening (C–O), voltage sensor activation (R_V–A_V). L is the C–O equilibrium constant when the voltage sensor is in the resting state (R_V) and J is the R_V–A_V voltage-dependent equilibrium. D is the allosteric interaction factor such as the C–O equilibrium constant that increases D-fold when the channel opens. (*b*) A general allosteric gating mechanism includes an allosteric interaction between channel opening (C–O), voltage sensor activation, temperature sensor activation (R_T–A_T) and agonist activation (U–B). L would be, in this case, the C–O equilibrium constant when voltage sensors and temperature sensors are in the resting state (R_V, R_T) and the agonist binding sites are unliganded (U); K is the R_T–A_T temperature-dependent equilibrium constant; and Q is the agonist binding constant defined as $Q=[A]/K_D$, where $[A]$ is the agonist concentration and K_D is the dissociation constant. Allosteric interactions of temperature sensors and agonist binding with channel opening are determined by allosteric factors P and C respectively.

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$P_o = 1/2$, $V_{1/2}$ be linear in the whole temperature range. In TRPM8, however, $V_{1/2}$ saturates at high and low temperatures (Latorre *et al.* 2007a); second, the time course of TRPM8 channel activation is well described by an initial lag followed by an exponential increase in ionic current. This delay is shortened as the prepulses reach more depolarized values (Fig. 11*c*) (Cole-Moore shift; Cole & Moore, 1960), indicating that the channel transits between several *voltage-dependent* closed states and that large hyperpolarizing voltages will populate those closed states further removed from the open state. The delay, and hence the voltage–dependent steps, show a very weak temperature dependence ($Q_{10} = 1.6$) strongly suggesting that the voltage and temperature sensors are different entities; third, the results of Brauchi *et al.* (2007) clearly demonstrate that voltage sensors can function normally in TRPM8 channel in which specific mutagenesis made in the C terminus abolish temperature sensitivity; and fourth, Matta & Ahern (2007) found that voltage does not fully activate TRPM8 and TRV1, as would be the case for channels intrinsically voltage-dependent and temperature and/or agonist act by modifying the voltage-dependent equilibria (see, e.g. Voets *et al.* 2004). Furthermore, TRPV1 channel in the presence of resiniferatoxin (Winter *et al.* 1990; Caterina *et al.* 1997), an extremely potent channel agonist, becomes essentially voltage-independent, a clear demonstration that this thermoTRP channel can gate independently of voltage (Matta & Ahern, 2007).

From the discussion above it is clear that voltage, temperature, and agonists act separately to modulate channel activity and that remains to be demonstrated quantitatively, which is the degree of allosteric coupling between different sensors. On the other hand, the molecular nature of the temperature sensor is still a mystery and the unveiling of the structures involved in temperature detection will be crucial for determining how the thermal energy is converted into mechanical (pore opening) energy.

6.3 In Search of the temperature sensor

Evidence suggests that the C-terminal plays a role either in containing or strongly modulating the temperature sensor (Brauchi *et al.* 2006, 2007). In particular, the C-terminal domain of TRPM8 and TRPV1 is modular and confers the channel phenotype regarding temperature sensitivity, channel gating kinetics, and PIP_2 sensitivity. A chimera in which the proximal part of the C terminus of TRPV1 replaces the equivalent section of TRPM8 is heat- and PIP_2-activated. In addition, PIP_2 activation, but not temperature sensitivity, disappears when positively charged amino acid residues contained in the exchanged region are neutralized. These findings and those described above strongly suggest that temperature, voltage, and agonists interact allosterically, and only the proximal region of the C terminus is essential for conferring channel phenotype regarding temperature sensitivity. These results also agree with the TRPV1 C terminus truncation experiments of Vlachova *et al.* (2003) and Liu *et al.* (2004). In particular, Liu *et al.* (2004) reported that a mutant with a deletion encompassing the last 88 residues of the distal TRPV1 C terminus is fully functional regarding its temperature sensitivity.

The recent results of Grandl *et al.* (2008) show that in the case of TRPV3, mutations in the pore region and the S6 TM domain can render the channel utterly insensitive to temperature.

Allosteric interaction between voltage and temperature sensor, between agonist binding and voltage sensors, and between agonist binding and temperature sensors are determined by allosteric factors E_1, E_2, and E_3 respectively.

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Using the same strategy used to identify residues that are important for menthol binding in the case of TRPM8 (Bandell *et al.* 2006), Grandl *et al.* (2008) screened 14 000 random mutant clones and identified three point mutations – I644 and N647 in pore loop and Y661 in S6 – which render TRPV3 temperature-insensitive. Remarkably, these mutations did not modify channel activation by 2-APB. These observations can be most economically accounted for by assuming that thermoTRP channels behave as polymodal allosterically gated receptors. These are exciting findings, but it is clear that we are far from having unequivocally identified the temperature sensor. As is the case of the C terminus of TRPV1 and TRPM8, the pore loop and S6 in TRPV3 can be essential for the gating transition from an upstream temperature-sensing domain. It is also possible that, as pointed out by Grandl *et al.* (2008), the temperature sensor is different in different thermoTRP channels.

The possibility that membrane lipids act as the temperature-sensing molecular machinery cannot at present be dismissed. The data available until now suggest, however, that the membrane lipid composition affects the temperature threshold but not its temperature sensitivity. Compelled by the question of whether the heat sensitivity of TRPV1 is intrinsic to the lipid bilayer, Liu *et al.* (2003) incubated the cells in a high cholesterol solution or altered the amount of cholesterol in the membrane of TRPV1-expressing HEK cells β cyclodestrin (MeβCD). It is well known that cholesterol can affect the physical properties of lipid bilayers by changing the fluidity of the lipid bilayer and can also modulate the function of membrane proteins (e.g. Ikonen, 2008). In the case of incubating the HEK cells in high cholesterol, Liu *et al.* (2003) found a rightward shift in the TRPV1 channel temperature activation curve toward higher temperatures with no effect on temperature sensitivity. Cholesterol depletion induced by MeβCD, on the other hand, had no effect on the channel heat response.

Morenilla-Palao *et al.* (2009) reported that the TRPM8 channel associates with cholesterol-rich membrane domains known as lipid rafts both in sensory neurons and ion heterologous expression systems. This association is favored by glycosylation of an asparagine residue located in the pore-forming loop of the channel. Lipid raft disruption with MeβCD shifted the threshold for current activation by about 3 °C toward warmer temperatures, with no change in Q_{10}. Thus, in two very different lipid environments, the TRPM8 channel has essentially the same temperature sensitivity albeit different temperature activation thresholds, suggesting that membrane fluidity and/or lipid–protein interaction modify the closed–open temperature-independent equilibrium.

6.4 What have we learned from knockout models?

TRPV1. Caterina *et al.* (2000) reported that neurons lacking the TRPV1 channel are severely impaired in their responses to stimuli such as capsaicin, protons, or temperature. *Trpv1*-null mice are also unable to react to injections of capsaicin or resiniferatoxin into the plantar skin of the hind paw and do not avoid solutions containing capsaicin; moreover, subcutaneous injections of capsaicin do not evoke the typical decrease in body temperature that occurs in the $trpv1^{+/+}$ mice (Jancsó-Gábor *et al.* 1970; Caterina *et al.* 2000). Actually, this is the first observation suggesting that TRPV1, in addition to its role in detecting the environmental temperature, was also involved in the regulation of body temperature (see below). Simultaneously, Davis *et al.* (2000) found essentially the same results as those reported by Julius' group in neurons of $trpv1^{-/-}$ mice. It is important to note here that, whereas it is clear that *trpv1*-null mice display deficits in thermally evoked pain, they are not insensitive to noxious heat. Data from

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Caterina *et al.* (2000) clearly suggest that TRPV1 alone cannot account for all the perception of noxious heat. It is possible, however, that some the residual nocifensive response to noxious heat stimuli in *trpv1*-null mice is mediated by the capsaicin-insensitive TRPV2 channel. This hypothesis needs to be tested using TRPV2-deficient mice; unfortunately, such mutant mice have not yet been reported in the literature. Thus, TRPV1 appears to be essential for sensitization to thermal stimulus (e.g. Tominaga, 1998) but not for the normal sensation of noxious heat.

The finding that *trpv1*-null mice have a normal body temperature was taken as evidence that this channel is not involved in determining body temperature. However, Steiner *et al.* (2007) found that TRPV1 has a suppressing effect on body temperature (T_b) (for a review, see Montell & Caterina, 2007). Steiner *et al.* (2007) synthesized and tested a potent ($IC_{50} < 1$ nM) TRPV1 antagonist dubbed AMGO347. Remarkably, this antagonist inhibits TRPV1 regardless of whether channel activation is induced by capsaicin, low pH, or temperature. AMGO347 induces hyperthermia in rat and mice, an effect that, most importantly, is completely abolished when *trpv1*-null mice are used. The antagonist causes hyperthermia through tail-skin vasoconstriction and thermogenesis without affecting behavioral thermoregulation. The main conclusion of this study is that tonic activation of TRPV1 in the abdominal viscera by unknown factors is responsible for the suppressive effect on T_b.

TRPV1 serves as a temperature detector in the CNS in addition to the body surface and gut. TRPV1 channels appear to be involved in the release of vasopressin (VP; antidiuretic hormone) from the neurohypophysis through a temperature-activated depolarization of hypothalamic neurons (Sharif-Naeini *et al.* 2008). In fact, the VP-releasing hypothalamic neurons express a TRPV1 splice variant that lacks part of the N-terminal (Sharif-Naeini *et al.* 2008). The VP release serves as a negative feedback to the evaporative heat lost induced by hyperthermia. The thermal activation threshold for TRPV1 in VP neurons was found to be below that found in recombinant systems (35 *versus* ~43 °C) and it is the TRPV1-induced depolarization evoked by hyperthermia that causes VP release. It is clear that the *trpv1* gene is involved in the temperature-dependent VP release since *trpv1*$^{-/-}$ neurons have an impaired thermosensitivity ($Q_{10} = 8.5$ *versus* $Q_{10} = 19$ in wild-type neurons) and an impaired heat-evoked VP release *in vivo*.

TRPV3. TRPV3 is activated by warm temperatures (>33 °C) and it shows an increased response at higher (noxious) temperatures (reviewed by Dhaka *et al.* 2006). Unlike most thermoTRP channels, this ion channel is expressed in keratinocytes (Peier *et al.* 2002b). *trpv3*-null mice present strong deficits in their response to innocuous and noxious heat as demonstrated by behavioral tests (Moqrich *et al.* 2005), and the activation of the TRPV3 channel by the agonist camphor in utterly lost in *trpv3*$^{-/-}$ keratinocytes. Thus, when animals are made to chose between one plate at room temperature and another heated to 35 °C, wild-type mice spend on average 92% of the time on the side heated at 35 °C, whereas the *trpv3*$^{-/-}$ spent only 64% of the time on the warm side. This difference is highly significant; yet the mutant mice still show a small bias toward the warm side, suggesting that other thermoTRP channels (TRPV4?) may be involved in innocuous thermosensation. On the other hand, mice lacking TRPV3 also show an impaired response to noxious heat as determined by the onset of nociceptive behavior to a tail immersion test or in their avoidance of a hot plate. In the first case, significant differences were observed at temperatures >48 °C and in the second differences between wild-type and mutant animals can be only observed at 55 °C. Together, these observations strongly suggest that TRPV3 in skin cells are essential for mammalian thermosensation.

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TRPV4. TRPV4 is expressed in the circumventricular organs of the mammalian CNS, a site important for osmotic sensing (Liedtke *et al.* 2000). Using a *trpv4*-null model, Liedtke & Friedman (2003) found that the *trpv4*$^{-/-}$ mice drank less water and became more hyperosmolar than the wild-type controls. Response to both hyper- and hyposmolar stimuli is impaired in *trpv4*$^{-/-}$ mice. Another *trpv4*$^{-/-}$ mouse model (Mizuno *et al.* 2003), however, shows a state akin to diabetes insipidus, making clear the need for more detailed water balance studies to establish where the differences between the two models reside. In transfected cells, TRPV4 is only activated by a hypotononic challenge (Guler *et al.* 2002; Watanabe *et al.* 2002). TRPV4 appears, therefore, to be necessary for the normal response to changes in osmotic pressure, and probably plays the role of an osmotic sensor in the CNS (Strotmann *et al.* 2000, 2003). *trpv4*$^{-/-}$ mice were reported to display no change in escape latency from acute painful stimuli on the hot plate or upon radiant paw heating compared to wild-type (Liedtke & Friedman, 2003; Susuki *et al.* 2003). In contrast to these results, Todaka *et al.* (2004) found that TRPV4 knockout mice displayed longer latencies to escape compared to the wild-type from plates heated 35–45 °C when hyperalgesia was induced, indicating that this channel determines the sensitivity rather than the threshold for painful heat. Moreover, the data of Lee *et al.* (2005) show that on a thermal gradient, TRPV4 knockout mice prefer the warmer temperatures and, in a two temperature choice test, *trpv4*$^{-/-}$ mice show a strong bias for the higher temperature whereas the wild-type mice fail to discriminate between 30 and 34 °C. Lee *et al.* (2005) also found that knockout mice exhibited longer latencies during acute tail heating and that *trpv4*$^{-/-}$ and wild-type mice exhibit similar modifications in behavior on a thermal gradient after paw inflammation. Dhaka *et al.* (2006) have argued that the inconsistencies between the two set of results may be attributable to differences in the sensitivity to hot plate (paws; Todaka *et al.* 2004) *versus* tail flick (Lee *et al.* 2005) assays and/or how these assays are scored. It is also probable that TRPV4 behaves as a mechanosensitive channel in hair cells in the cochlea involved in hearing. *trpv4*$^{-/-}$ mice have a delayed onset hearing loss and an increased susceptibility to acoustic injury (Tabuchi *et al.* 2005). TRPV4 also has a role in peripheral nociceptor sensitization *in vivo* since C-type fibers in TRPV4-deficient mice show a pronounced impairment in their response to an inflammatory cocktail composed of prostaglandin E_2 and serotonin. Compared to wild-type mice, C-type fibers of the TRPV4-null mice present a large decrease in spontaneous activity and mechanical threshold, indicating that TRPV4, as well as TRPV1, is involved in nociceptor sensitization (Cheng *et al.* 2007).

TRPM8. The ability to sense a decrease in temperature is fundamental for the survival of warm-blooded animals. However, the ionic mechanisms of nonnoxious cold transduction have remained elusive until recently and, in particular, the contribution of TRPM8 to cold detection *in vivo* was unclear (see, e.g., Viana *et al.* 2002; Madrid *et al.* 2006). This situation has recently changed radically with the appearance of three reports in which the ability of TRPM8-deficient mice to detect cold temperatures was studied (Bautista *et al.* 2005; Colburn *et al.* 2007; Dhaka *et al.* 2007). These studies show that mice lacking TRPM8 show severe behavioral deficits in response to cold. The TRPM8-deficient mice show a reduction in their ability to discriminate between cold and warm temperatures, lack behavioral response to cold-induced icilin application, and exhibit a significant reduction in the nocifensive reaction to acetone-evoked evaporating cooling of the hind paw. TRPM8 appears to contribute to the detection of noxious cold, defined as temperatures below 15 °C since even at these low temperatures the mutant mice explored the cold surface of a plate for longer times than did wild-type mice (Bautista *et al.* 2005). However, TRPM8 null mice are still able to react with a normal nocifensive reaction to

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temperatures below 0 °C, suggesting the presence of a different receptor able to sense more extreme noxious cold temperatures (Dhaka *et al.* 2007). It can be also that noxious cold is elicited by inhibition of warm-sensing fibers. In this regard, an interesting observation is reported by Roza *et al.* (2006), who found that cold-insensitive sensory fibers developed sensitivity to cold when exposed to the K^+ channel blocker 4-amino pyridine, an effect that was not modified by menthol. At the cellular level, in the TRPM8 null mice there is a selective loss of TRPM8 channels in dorsal root and trigeminal ganglia, cornea, and spinal cord. On the other hand, sensory nerve fibers from TRPM8-deficient mice show a diminished firing rate and the sensory neurons of these rodents are totally insensitive to menthol and icilin, and have a much diminished response to cold.

TRPA1. TRPA1 channels are expressed in the sensory epithelium of the utricle, a membranous structure located in the vestibule of the inner ear. In addition, antibodies directed against the TRPA1 C-terminal react with mouse vestibular and cochlear hair cells (reviewed by Corey, 2006). Because of its site of expression, the appearance of TRPA1 mRNA in hair cell epithelia simultaneously with the onset of mechanosensitivity, and its biophysical and pharmacological properties prompted Corey *et al.* (2004) and Nagata *et al.* (2005) to propose that this channel plays a central role in hearing by mediating hair cell transduction (but see below). TRPA1 is also expressed in DRG neurons, which behave as polymodal nociceptors since they also express TRPV1 (Story *et al.* 2003; Bandell *et al.* 2004; Jordt *et al.* 2004).

6.4.1 The puzzle of the cold sensitivity of TRPA1 channels

Conflictive results on the exact role of this channel started when this channel was identified in sensory neurons that were also activated by capsaicin. Jordt *et al.* (2004), in contrast to the findings of Story *et al.* (2003), were unable to obtain reliable responses of TRPA1 expressing neurons to noxious cold. The Patapoutian and Julius groups (and everybody else) agreed, however, that TRPA1 is the molecular target of pungent natural compounds as are, for example, the isothiocyanates contained in mustard oil and wasabi (Fig. 13 and see above). In agreement with the results of Jordt *et al.*, Nagata *et al.* (2005) also reported a lack of sensitivity to noxious cold of the TRPA1 channel. Furthermore, two groups have reported that TRPA1 is activated directly by internal Ca^{2+} and that the apparent cold sensitivity occurs indirectly since there is a cytosolic Ca^{2+} increase during cooling in heterologous systems (Doemer *et al.* 2007; Zurborg *et al.* 2007; Fig. 13*f*). Whether TRPA1 is actually gated by noxious cold still leaves a big question mark, but gains support with the results of Sawada *et al.* (2007), which show that TRPA1 can be activated by deep cooling in inside-out patches in Ca^{2+}-free solutions (Fig. 13*c* and *d*). The results should, nevertheless, be contrasted with those of Zurborg *et al.* (2007), who were unable to activate TRPA1 in the absence of internal Ca^{2+}. Moreover, they found that in the presence of Ca^{2+}, cold inhibited the TRPA1 activation induced by the divalent cation. We note here that the experiments carried out by Zurborg *et al.* (2007) were performed in the absence of Ca^{2+} but used whole-cell patch clamping, whereas Sawada *et al.* (2007) conducted their single-channel current recordings in inside-out patches. Sawada *et al.* (2007) argued that the differences between their observations and those of Jordt *et al.* (2004) and Nagata *et al.* (2005) could be attributable to the length of the cooling stimuli (activation of TRPA1 by cooling is slow) or the transfection procedures (cell expressing tetracycline-inducible mTRPA1 *versus* transient transfected cells). More recently, Karashima *et al.* (2009) have confirmed and extended Sawada *et al.*'s (2007) findings. In particular, they showed that in Ca^{2+}-free solutions, increasing temperature has the effect of

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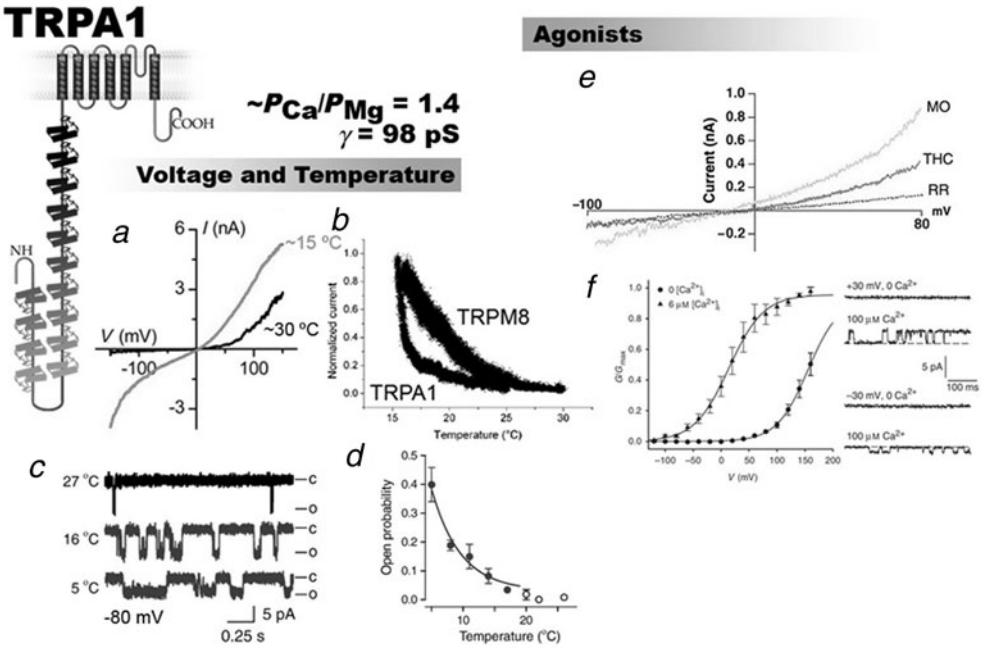


Fig. 13. Polymodal nature of TRPA1. (*a*) Current–voltage relations obtained at ∼30 °C (black trace) ∼15 °C (grey trace) using Ca^{2+}-free intracellular and extracellular solutions (Karashima *et al.* 2009). (*b*) Comparison of the temperature activation curves for TRPA1 and TRPM8 (Story *et al.* 2003). (*c*) Single-channel recordings show that TRPA1 is activated by deep cooling. Inside-out patch recordings (−80 mV) in membrane excised from TRPA1-expressing HEK293 cells. (c, closed state; o, open state). Downward deflections show inward current. (*d*) Open probabilities (P_o) of the deep cooling-induced currents for 5–30 s, data as shown in (*c*) are plotted against temperature (Sawada *et al.* 2007). (*e*) THC and mustard oil activate a subset of sensory neurons. Electrophysiological response of a representative neuron to 20 mM THC or 20 mM mustard oil (MO) in the absence (MO) or presence (RR) of 5 mM ruthenium red. Voltage ramps from −100 to +80 mV (200 ms) were applied every 2 s. THC-evoked currents display slight outward rectification. Mustard oil activates identical currents of larger amplitude in the same cell. Ruthenium red (10 mM) blocks THC- and mustard oil-evoked currents (Jordt *et al.* 2004). (*f*) Left panel. TRPA1 is activated by intracellular Ca^{2+}. Voltage activation curves are shown in the absence and presence of Ca^{2+}. Right panel. Single-channel recordings from inside-out patches at the indicated potentials and internal Ca^{2+} concentrations (Zurborg *et al.* 2007).

shifting the conductance–voltage relation leftward, a behavior reminiscent to that of TRPM8 (Fig. 13*a*).

Unfortunately, the answer to this puzzle did not come from *trpa1* knockout mice because the behavioral responses of *trpa1*$^{-/-}$ knockout mice to noxious cold temperatures are also conflicting (e.g. Kwan & Corey, 2009). Kwan *et al.* (2006) reported that *trpa1*$^{-/-}$ knockout mice are deficient in their behavioral responses to 0 °C and mustard oil, but that their hearing is normal. Although these results clearly support the role of TRPA1 as the noxious cold detector, they are difficult to reconcile with the proposal that this channel is the transduction channel in hair cells (Corey *et al.* 2004). In agreement with Kwan *et al.* (2006), Karashima *et al.* (2009) showed that there is a subset of cold-sensitive trigeminal ganglion neurons that are not present in the *trpa1* knockout mice, and cold plate and tail-flick behavioral experiments revealed TRPA1-dependent, cold-induced, nociceptive behavior in mice.

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However, Bautista *et al.* (2006) found that the cold- or menthol-evoked responses in trigeminal neurons were identical in ganglia of wild-type and *trpa1*$^{-/-}$ mice. Behaviorally, TRPA1-deficient mice showed the same responses as wild-type mice to evaporative cooling and paw withdrawal from a surface cooled from 20 to −10 °C. The knockout TRPA1 model reported by Bautista *et al.* (2006), as is the case of the *trpa1*$^{-/-}$ mice reported by Kwan *et al.* (2006), have normal auditory responses. The use of *trpa1*$^{-/-}$ mice is also important in demonstrating that the target of the active compounds of garlic and mustard oil is the TRPA1 channel, but given the results of Salazar *et al.* (2008) that showed activation of TRPV1 by pungent extracts of onion and garlic, TRPA1 does not appear to be the sole target.

We conclude that the most recent evidence tends to support direct activation of TRPA1 by cold. In particular, the experiments of Sawada *et al.* (2007) and those of Karashima *et al.* (2009) demonstrating that TRP1 can be activated by noxious cold in cell-free patches are very convincing.

7. Conclusion

Despite the considerable advances in the TRP channel field that have occurred during the past few years, we are still missing most of the pieces that will give us the solution to the puzzle of how these molecular machines are able to integrate all the stimuli that modify their electrical behavior. It is clear, as is the case of voltage-dependent K^+ channels, that TRP channel-forming proteins are modular in nature and that the existence of these modules determines, for example, their modulation by agonists and their ion and selectivity properties. This modularity may help provide a structural basis for the notion, which is well supported by mutagenesis and electrophysiological evidence, that TRP channels are allosterically gated polymodal receptors. Mutagenesis experiments have also substantially helped in the localization of the binding sites for several agonists, but how the free energy of ligand binding is converted into mechanical energy (pore opening) remains a mystery. Some data, however, suggest that in the case TRPA1, the pore region is involved in coupling the covalent binding of electrophilic compounds to channel gating. The pore domain has attracted much attention lately because agonists are able to modify channel gating and evoke ionic selectivity changes. In other words, the pore is not a static structure but its properties are dynamically modulated by agonists during channel activation. The crystal structures of the ARD domains of TRPV channels and the coiled coil domain present in the C terminus of TRPM7 have provided important hints about channel assembly and the mechanisms that lead to the modulation of TRPV1 responses to noxious stimuli. The first steps toward the elucidation of the TRP channel structure have been provided using the electron microscopy and the conclusion of these observations is that the different TRP channel subfamilies may not share the same structural design.

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