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Structure of Thermally Activated TRP Channels

Matthew R. Cohen^{1,2} and Vera Y. Moiseenkova-Bell^{1,2,*}

¹Department of Pharmacology, Case Western Reserve University School of Medicine, Cleveland, OH, USA

²Department of Physiology & Biophysics, Case Western Reserve University School of Medicine, Cleveland, OH, USA

Abstract

Temperature sensation is important for adaptation and survival of organisms. While temperature has the potential to affect all biological macromolecules, organisms have evolved specific thermosensitive molecular detectors that are able to transduce temperature changes into physiologically relevant signals. Among these thermosensors are ion channels from the transient receptor potential (TRP) family. Prime candidates include TRPV1–4, TRPA1, and TRPM8 (the so-called “thermoTRP” channels), which are expressed in sensory neurons and gated at specific temperatures. Electrophysiological and thermodynamic approaches have been employed to determine the nature by which thermoTRPs detect temperature and couple temperature changes to channel gating. To further understand how thermoTRPs sense temperature, high-resolution structures of full-length thermoTRPs channels will be required. Here, we will discuss current progress in unraveling the structures of thermoTRP channels.

1. INTRODUCTION

All living organisms have the ability to detect temperature changes from the outside environment and convert it into specific biological outputs, allowing them to adapt and survive (Sengupta & Garrity, 2013). Cells employ specific biomolecules that undergo temperature-induced conformational changes, initiating signaling cascades that result in these physiological and behavioral responses (Digel, 2011; Digel, Kayser, & Artmann, 2008). It has been suggested that changes in DNA, RNA, and protein conformation or changes in lipid membrane properties initiate temperature-induced signaling cascades (Digel, 2011; Digel et al., 2008).

In higher organisms, skin forms a protective layer that enables the body to detect changes in the physical, chemical, and thermal environment (Schepers & Ringkamp, 2009). A wide array of specialized sensory neurons that specifically detect and transduce thermal changes over a broad range of temperatures innervate skin (McGlone & Reilly, 2010; Schepers & Ringkamp, 2009). These sensory neurons are activated at distinct temperature thresholds and

allow organisms to differentiate between noxious cold ($<15^{\circ}\text{C}$) and heat ($>43^{\circ}\text{C}$), and pleasant cool ($15\text{--}25^{\circ}\text{C}$) and warm ($30\text{--}40^{\circ}\text{C}$) (Figure 7.1) (McKemy, 2013).

The identity of the “molecular devices” that sense and differentiate these temperatures was unraveled by the discovery and characterization of transient receptor potential (TRP) ion channels. The first TRP channel was identified by characterization of a vision-impaired *trp* mutant from *Drosophila* (Cosens & Manning, 1969; Minke, Wu, & Pak, 1975). Presently, the TRP superfamily consists of 28 mammalian members and is subdivided into six major branches: TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPA (ankyrin), TRPP (polycystin), and TRPML (mucolipin). Among them, members of TRPV, TRPA, and TRPM subfamilies have been suggested to play a critical role in temperature sensation (Venkatachalam & Montell, 2007). Specifically, TRPV1 ($>43^{\circ}\text{C}$), TRPV2 ($>52^{\circ}\text{C}$), TRPV3 ($>30\text{--}39^{\circ}\text{C}$), and TRPV4 ($>25\text{--}35^{\circ}\text{C}$) have been implicated in hot and warm sensation, while TRPM8 ($<20\text{--}28^{\circ}\text{C}$) and TRPA1 ($<17^{\circ}\text{C}$) are involved in cool and cold detection, respectively (Belmonte & Viana, 2008).

Thermosensation is likely not limited to TRP channels as the tetrodotoxin-resistant voltage-gated sodium channel Na(v)1.8 has been shown to play a critical role in noxious cold signaling (Abrahamsen et al., 2008; Zimmermann et al., 2007) and two-pore potassium channels TREK-1 and TRAAK have been implicated in cold and warm thermoregulation (Noel et al., 2009). Based on this current knowledge, it is clear that exposure to wide-range temperature changes triggers the generation of Ca^{2+} , K^{+} , and Na^{+} currents, leading to the formation and propagation of action potentials that send signals to the brain (Figure 7.1), thereby modifying behaviors according to the temperature change encountered (Viana, 2011).

Nevertheless, the molecular mechanism of temperature sensation by these ion channels is still unknown. Activity of all proteins is sensitive to the temperature changes; however, only select proteins are considered thermosensors. Temperature sensitivity of proteins is often quantified in terms of Q_{10} , which represents the ratio of a protein property measured at two temperatures 10°C apart (Sengupta & Garrity, 2013). Ion channels that exhibit Q_{10} values of ~ 3 are considered temperature insensitive, while proteins with a Q_{10} value >7 are considered thermosensitive (Sengupta & Garrity, 2013). The Q_{10} of TRPV1 is ~ 40 and of TRPM8 is ~ 28 (Maingret et al., 2000; Sengupta & Garrity, 2013), indicating that these channels are especially sensitive to changes in temperature.

These biophysical properties clearly suggest that TRP channels act as cellular thermosensors; however, the structural features of the channels that determine their thermosensitivity are only recently coming to light. In the following sections, we will focus on the recent progress in structure determination of thermosensitive TRP channels and how these structural details could aid in understanding thermosensation at the molecular level.

2. TRP CHANNELS AS THERMAL SENSORS

The mammalian TRP channel superfamily is one of the largest families of cation channels, consisting of 28 mammalian homologues. Among them, TRPV1–4, TRPA1, and TRPM8 have been shown to play critical roles in thermosensation, detecting temperatures from 4°C

to 52 °C. In addition to temperature, thermoTRP channels are polymodal integrators of multiple types of stimuli (e.g., ligand, voltage, stretch).

All TRP channels form functional tetramers, with each monomer consisting of six transmembrane (TM) segments (Figure 7.2). The primary sequence of the TM region is the most conserved between the thermoTRP channels. Interestingly, the intracellular N- and C-termini of these TRP channels contain divergent structural domains. TRPV1–4 are smaller tetramers (340–390 kDa) with 6 N-terminal ankryin repeats and a conserved C-terminal TRP box sequence per subunit (Figure 7.2). TRPA1 and TRPM8 are larger proteins (~530 kDa). TRPA1 contains 14–19 N-terminal ankyrin repeats and no C-terminal TRP box sequence (Figure 7.2). On the other hand, TRPM8 has no ankyrin repeats but contains a conserved TRPM channel specific amino terminal region and a coiled-coil domain and TRP box sequence on the C-terminus (Figure 7.2).

Despite predicted structural diversity amongst thermoTRP channels, they potentially share the common feature of temperature sensation, which has intrigued the field for many years. Extensive and elegant studies have been employed to determine their “molecular temperature switch” (Brauchi, Orta, Salazar, Rosenmann, & Latorre, 2006; Cordero-Morales, Gracheva, & Julius, 2011; Grandl et al., 2008, 2010; Kim, Patapoutian, & Grandl, 2013; Yang, Cui, Wang, & Zheng, 2010; Yao, Liu, & Qin, 2011); however, a temperature-dependent detector may not exist at a specific site since temperature affects the global conformation of these large membrane protein complexes (Clapham & Miller, 2011; Liu, Hui, & Qin, 2003). High-resolution structural information for these channels will greatly accelerate understanding of how the entire protein operates as a thermosensor.

2.1 TRPV subfamily

2.1.1 Thermosensitivity of TRPV channels—TRPV1 was the first thermoTRP channel to be identified and characterized (Caterina et al., 1997). It has been the most extensively studied TRP channel due to its role in pain and temperature sensation. TRPV1 is activated by chemical compounds including capsaicin, the active ingredient in chili peppers, as well as heat, with an activation threshold of 42 °C (Caterina et al., 1997). TRPV1 shows an intrinsic ability to sense temperature, as purified TRPV1 reconstituted in artificial liposomes shows temperature-sensitive activation (Cao, Cordero-Morales, Liu, Qin, & Julius, 2013). Furthermore, there is evidence that TRPV1 acts as a thermosensor in vivo, as deletion of the TRPV1 gene impairs temperature sensation in mouse and treatment of rodents with TRPV1 antagonists reduces heat sensitivity (Caterina et al., 2000). Other thermoTRPV channels, TRPV2–4, have subsequently been cloned (Caterina, Rosen, Tominaga, Brake, & Julius, 1999; Smith et al., 2002; Strotmann, Harteneck, Nunnenmacher, Schultz, & Plant, 2000; Xu et al., 2002). In heterologous expression systems, these channels show heat sensitivity with different temperature thresholds ranging from 25 to 52 °C (Table 7.1) (Caterina et al., 1999; Guler et al., 2002; Peier, Reeve, et al., 2002). Genetic deletion of these channels in mice has little effect on temperature sensation, leaving their role as in vivo thermosensors unclear (Huang, Li, Yu, Wang, & Caterina, 2011; Park et al., 2011). Therefore, most structure–function studies of temperature sensation by TRPV channels have focused on TRPV1.

2.1.2 In search of the temperature-sensing domain in TRPV channels—TRPV1

functions as a tetramer (Garcia-Sanz et al., 2004; Kedei et al., 2001). The TM region has six α -helical domains. TM5, TM6, and the linker between them form the putative pore of the channel. The cytoplasmic N-terminus of TRPV1 accounts for ~50% of the total molecular weight and contains six ankyrin repeats. Ankyrin repeats consist of 33 amino acids that form an antiparallel helix-turn-helix motif linked by a β -hairpin turn (Gaudet, 2008). Ligands interact with ankyrin repeat domains (ARDs) via the β -turn and the first antiparallel helix from a varying number of repeats (Gaudet, 2008). The C-terminus of TRPV1 contains the conserved TRP box sequence, which may be involved in channel oligomerization (Garcia-Sanz et al., 2004) (Figure 7.3(A)).

In order to identify the temperature-sensitive domains of TRPV1, electrophysiology studies coupled to chimeric and mutagenesis strategies have been employed. Several domains are proposed to act as the temperature sensors of thermoTRPV channels and controversy still exists as to the molecular nature of temperature sensation by thermoTRPVs.

Screens of random mutations in TRPV1 and TRPV3 revealed deficits in temperature sensitivity when residues in the channel pore were mutated, suggesting that the pore domain contributes to thermosensitivity (Grandl et al., 2008, 2010). Later studies showed that the pore turret region of TRPV1, a 24-residue sequence between TM5 and the pore helix, is involved in heat-induced activation of TRPV1 (Yang et al., 2010). Replacement of the pore turret sequence with an artificial linker nearly ablated the response of TRPV1 to heat while maintaining its response to ligands and voltage. However, another group showed that deletion of the pore turret sequence had no effect on temperature-sensitive activation of TRPV1 (Yao, Liu, & Qin, 2010).

Furthermore, both the N- and C-termini of TRPV1 have been implicated in thermosensation. Chimeras of TRPV1 and TRPM8 in which the C-termini were exchanged conferred heat sensitivity to TRPM8 and cold sensitivity to TRPV1, suggesting that the C-terminus is involved in the directionality of temperature sensation (Brauchi et al., 2006). Another study found that the membrane proximal region linking the N-terminal ARD to the TM1 segment dictates temperature sensation together with the last two ankyrin repeats (Yao et al., 2011).

Based on these chimeric and mutagenesis studies, it appears that the pore domain, C-terminus, and N-terminus of TRPV1 all participate in temperature sensation. Controversy remains as the search for the molecular temperature sensor in TRPV1 and other thermoTRPV channels continues.

2.1.3 Crystal structures of isolated TRPV channel domains—Structure

determination of full-length TRPV1–4 by X-ray crystallography has been hampered by the lack of an expression system to produce sufficient amounts of protein (Moiseenkova-Bell & Wensel, 2009, 2011). Two different approaches have yielded progress in understanding TRP channel structures: a “divide and conquer” strategy in which structures of smaller soluble domains have been determined by X-ray crystallography (Gaudet, 2009) and cryoelectron microscopy (cryo-EM) analysis of the full-length TRP channels, which allowed for determination of TRP channel structures at moderate and recently at side-chain resolution

(Cao, Liao, Cheng, & Julius, 2013; Cvetkov, Huynh, Cohen, & Moiseenkova-Bell, 2011; Huynh et al., 2014; Liao, Cao, Julius, & Cheng, 2013; Moiseenkova-Bell et al., 2008).

The “divide and conquer” approach has yielded structures of the ARDs from TRPV1, TRPV2, TRPV3, TRPV4, and TRPV6 (Inada, Procko, Sotomayor, & Gaudet, 2012; Jin, Touhey, & Gaudet, 2006; Lishko, Procko, Jin, Phelps, & Gaudet, 2007; Phelps, Huang, Lishko, Wang, & Gaudet, 2008; Phelps et al., 2010; Shi, Ye, Cao, Zhang, & Wang, 2013). The ARDs comprise ~60% of the N-terminus and are important in interacting with ligands that regulate channel activity (Binz et al., 2004; Li, Mahajan, & Tsai, 2006; Nakamura et al., 2007). The ARD structures from TRPV channels display overall structural similarity (Figure 7.3(B)). A concave ligand-binding surface is formed by the six antiparallel helices and loops and a large twist is present between repeats 4 and 5 (Gaudet, 2008). Despite the overall similarity of these structures, biochemical studies show that the thermoTRPV ARDs bind different ligands (Phelps et al., 2010). For example, the ARDs of TRPV1, TRPV3, and TRPV4 bind ATP at the concave ligand-binding surface whereas TRPV2, TRPV5, and TRPV6 do not (Phelps et al., 2010). Furthermore, ATP has differential effects on the activity of TRPV1, TRPV3, and TRPV4 (Al-Ansary, Bogeski, Disteldorf, Becherer, & Niemeyer, 2010; Lishko et al., 2007; Phelps et al., 2010). The structures of the thermoTRPV ARDs provided insight into the regulation of these channels by ligands; however, the role of ankyrin repeats in temperature sensation remains unknown.

Recently, the X-ray structure for a 35 amino acid TRPV1 C-terminal peptide (residues 767–801) in complex with calmodulin (CaM) was determined (Figure 7.3(C)) (Lau et al., 2012). TRPV channels do not contain classical CaM-binding motifs, however, it has been proposed that CaM plays a significant role in desensitization of several TRPV channels. In the structure, only 14 out of 35 C-terminal amino acid densities were observed, displaying an overall similarity to previously published structures of CaM-binding peptides (Figure 7.3(C)). Further investigation will be required to gain molecular insights into the role of the C-terminus in temperature sensation and CaM-dependent desensitization of TRPV channels.

2.1.4 EM structures of full-length TRPV channels—Cryo-EM can theoretically provide near atomic resolution structural information for a variety of biological molecules without the need to produce crystals (Henderson, 1995). Recently, this has been confirmed by the determination of near atomic resolution structures for large symmetrical molecules using cryo-EM (Wolf, Garcea, Grigorieff, & Harrison, 2010; Zhang, Jin, Fang, Hui, & Zhou, 2010). A major advantage of cryo-EM versus other structural approaches is the ability to solve protein structures in different functional states, thereby identifying functionally relevant conformational changes (Heymann, Conway, & Steven, 2004). Additionally, cryo-EM requires approximately one hundred times less protein than other structural techniques (Wang & Sigworth, 2006).

Structure determination of full-length eukaryotic ion channels is a challenging task in structural biology. Cryo-EM has provided structures of large ion channels such as the ryanodine receptor and IP₃ receptor at the subnanometer (~10 Å) resolution and revealed precise conformational changes during ligand activation (Ludtke, Serysheva, Hamilton, & Chiu, 2005; Ludtke et al., 2011; Samso, Feng, Pessah, & Allen, 2009; Samso, Wagenknecht,

& Allen, 2005; Serysheva et al., 2008). The same methodology was applied toward structural analysis of full-length TRP channels.

The initial cryo-EM structures of functional TRPV1 and TRPV4 were determined at 19 Å and 35 Å, respectively (Figure 7.3(D)) (Moiseenkova-Bell et al., 2008; Shigematsu et al., 2010). Both cryo-EM maps revealed considerable structural similarities, including fourfold symmetry and the presence of two distinct architectural regions likely corresponding to the TM and cytoplasmic domains of the channels (Figure 7.3(D)).

Preparation of stable samples was a major obstacle in structure determination of TRP channels (Cvetkov et al., 2011; Huynh et al., 2014; Liao et al., 2013). The use of traditional detergents such as decyl-β-D-maltoside and dodecyl-β-D-maltoside led to sample heterogeneity (Huynh et al., 2014; Liao et al., 2013); however, utilization of the newly developed maltoseneopentyl glycol (MNG) class of detergents (Huynh et al., 2014) or A8-35 amphipol molecules (Cvetkov et al., 2011) allowed for stabilization of full-length TRPV2 and TRPA1, respectively. Recently, A8-35 amphipols were also used in stabilizing a truncated TRPV1 construct used for cryo-EM analysis (Liao et al., 2013). Enhanced purification and stabilization methods allowed for the determination of TRPV1 and TRPV2 structures by cryo-EM at higher resolutions.

The recent publication of a high-resolution structure of TRPV1 channel concurrent with the determination of the structure of TRPV2 allows for a comparison of their structural features (Huynh et al., 2014; Liao et al., 2013). Despite nearly 50% sequence identity, TRPV1 and TRPV2 diverge in function (Peralvarez-Marín, Donate-Macian, & Gaudet, 2013). While TRPV1 clearly acts as a thermosensor and receptor for endogenous and exogenous vanilloids (Caterina et al., 1997, 2000), the role of TRPV2 in noxious temperature sensation remains in question (Caterina et al., 1999; Park et al., 2011). Also, TRPV2 is not activated by vanilloids such as capsaicin (Caterina et al., 1999). Comparison between structures of TRPV1 and TRPV2 may provide structural insight into functional divergence between these highly homologous proteins.

Cryo-EM studies were performed on full-length TRPV2 and a stable “minimal” TRPV1 construct in which the N- and C-termini were truncated. The 2D class averages for full-length TRPV2 and minimal TRPV1 are similar (Figure 7.4(A)). A TM domain with an MNG detergent or A8-35 amphipol belt is visible, and a cytoplasmic domain, which consists of the ARD, is also apparent in the 2D class averages (Figure 7.4(A)). The structure of TRPV2 was refined to 13.6 Å (Figure 7.4(B)) (Huynh et al., 2014). A homologous potassium channel structure fits well into the TM domain density of the TRPV2 EM map (Figure 7.4(B) and (C)) and the MNG detergent can be clearly resolved around the TM domain protein density as an uneven belt of ~15–20 Å as previously observed for other membrane proteins (Figure 7.4(C)) (Vahedi-Faridi, Jastrzebska, Palczewski, & Engel, 2013; Westfield et al., 2011).

Remarkably, by employing a new direct electron counting camera and new image processing algorithms, the structure of minimal TRPV1 was refined first to 8.8 Å and then 3.4 Å resolution (Figure 7.4(B)) (Cao, Liao, et al., 2013). The TM helices of minimal

TRPV1 are clearly resolved (Figure 7.4(B) and (C)) and a negligible amount of A8-35 amphipol molecules, which were used to stabilize the TM domain of TRPV1, are apparent at 8.8 Å and 3.4 Å resolution (Figure 7.4(C)). The A8-35 amphipol belt, however, can be visualized at the higher isosurface levels, where it is symmetrically diffuse and not directly interacting with the TM helices of the protein density (Figure 7.4(C)). Intriguingly, the TM region of the truncated TRPV1 displays the highest resolution within the structure (Henderson, 2013), while information for the rest of the molecule is at a lower resolution and not as clearly resolved (Figure 7.4(B)). Therefore, the capability of A8-35 amphipols to stabilize the TM region of membrane proteins makes them a very useful tool for studying the structure of membrane proteins at high resolution by cryo-EM (Popot et al., 2011).

The minimal TRPV1 structure at 3.4 Å resolution revealed that the TM region of TRPV channels is nearly identical to that of Na⁺ and K⁺ channels (Long, Tao, Campbell, & MacKinnon, 2007; Payandeh, Scheuer, Zheng, & Catterall, 2011). Under ligand-free conditions, the TM domain of TRPV1 aligns well with the known structures of Na⁺ and K⁺ channels (Long et al., 2007; Payandeh et al., 2011). Unlike Na⁺ and K⁺ channels, the cryo-EM structures of TRPV1 in the presence of activators show that the TM1–TM4 segment of TRPV1 does not undergo conformational changes during channel activation (Cao, Liao, et al., 2013). Conformational changes upon activation occurred in the pore region (Cao, Liao, et al., 2013). Comparison of TRPV1 structures in the absence and presence of activators revealed that the pore expands at two sites: a selectivity filter in the S5–S6 loop (Gly643) and a lower gate at the intracellular end of S6 (Ile679) (Figure 7.5(A)).

Previous electrophysiological studies using a scanning mutagenesis strategy also revealed insight into the pore architecture of TRPV1 (Islas et al., 2009; Salazar et al., 2009; Susankova, Ettrich, Vyklicky, Teisinger, & Vlachova, 2007). These studies showed a dual gate sensitive to capsaicin and heat (Figure 7.5(B)) (Salazar et al., 2009). Consistent with the cryo-EM structure of TRPV1, a gate for larger cations was proposed at the intracellular end of S6 (Leu681) (Figure 7.5(B)). However, these studies also proposed a gate for smaller cations at a more proximal region of the S6 segment (Leu 671) between the selectivity filter and lower gate (Figure 7.5(B)) (Salazar et al., 2009), which was not apparent in the cryo-EM structure (Figure 7.5(A)) (Cao, Liao, et al., 2013). Further high-resolution structural studies of TRPV channels in the presence of activators are needed to fully understand the conformational changes and pore dynamics upon channel activation.

The cryo-EM structures of the minimal TRPV1 and full-length TRPV2 diverge most in the cytoplasmic region (Figure 7.4(D)). The ARDs, which comprise ~50% of the total mass of the protein, are involved in protein–ligand and possibly protein–protein interactions (Gaudet, 2008). The crystal structure of the TRPV2 ARDs fit unambiguously into the cytoplasmic density of the TRPV2 EM map (Figure 7.4(B) and (D)). The ARDs were fit into the TRPV2 EM map using computational methods (Chacon & Wriggers, 2002) and were oriented such that the concave ligand-binding surface faces outward toward the cytoplasm. This would provide an accessible binding surface for proteins and ligands to interact with the ARDs without clashing with other portions of the channel protein (Figure 7.4(D)).

The ARDs of the minimal TRPV1 structure were not fully resolved; the density for the first two repeats is absent in the structure (Figure 7.4(B) and (D)). The authors explain that the ARDs of TRPV1 may be especially flexible, which may have prevented them from resolving the first two repeats (Liao et al., 2013). Additionally, the ARDs of the minimal TRPV1 are arranged such that the concave ligand-binding surface, important for protein–protein interactions, is facing inward toward the TM domain and C-terminus of the channel (Figure 7.4(D)). Moreover, a β -strand from the N-terminal membrane proximal linker region is observed interacting with the concave surface of the ARDs, which according to the authors is involved in the stabilization of the channel assembly (Figure 7.4(D)). The authors propose that the rest of the ARD surface is available for interactions between TRPV1 and intracellular partners.

Nevertheless, the concave region of the ARDs is the essential interaction surface for a diverse range of binding partners (Gaudet, 2008). A crystal structure of the complex between Gankryin and the C-terminal portion of the S6 ATPase of the 26S proteasome shows that the Gankryin interacts with the S6 ATPase via its concave surface (Nakamura et al., 2007). We performed an analysis where the model of the Gankryin-S6 ATPase complex was superimposed with the TRPV channel ARDs as they are arranged in the TRPV cryo-EM maps. The superimposition of the complex is displayed as the S6-ATPase interacting with the TRPV ARDs as it would with Gankryin (Figure 7.6). It is clear that the β -strand from the N-terminal membrane proximal linker of TRPV1 interferes with the interaction of the S6-ATPase protein and the ARD concave ligand-binding surface (Figure 7.6(A)). A similar analysis performed for the TRPV2 ARDs as they are fitted into the TRPV2 cryo-EM map showed the ARDs have an extensive binding surface with which the S6-ATPase protein and other potential binding proteins can interact (Figure 7.6(B)). This is consistent with the observation from the GIRK2 channel structure in complex with a G protein (Whorton & MacKinnon, 2013), where the channel shows an extensive binding surface exposed to the cytoplasm (Figure 7.6(C)). Divergence in how the ARDs are arranged in the minimal TRPV1 and full-length TRPV2 structures may be due to intrinsic differences in channel architecture, differences in the accuracy with which particle orientation was determined in cryo-EM analysis, resolution limits, or different strategies by which the initial models were generated for each structure (Henderson, 2013). More biochemical and structural studies of TRPV channels are needed to further dissect the different arrangements of the ARDs within the structures of minimal TRPV1 and full-length TRPV2.

A recent FRET-based study provided insight into the domain organization and conformational changes of thermoTRPV channels, specifically TRPV1 (Dela-Rosa, Rangel-Yescas, Ladron-de-Guevara, Rosenbaum, & Islas, 2013). This showed that the C-terminus of TRPV1 is surrounded by the N-terminus, and that the C-terminus is closer to the membrane than the N-terminus. Based on fitting of known structures into the TRPV2 EM map (Figure 7.4(B)), we proposed that the TRPV2 TM domain (Figure 7.4(B)) and the N-terminal ARD (Figure 7.4(B)) are separated by a density that contains C-terminus of the channel (Figure 7.4(B)). Furthermore, this arrangement also suggests that the TRPV2 C-terminus is surrounded by the N-terminal domain (Figure 7.4(B)). A similar overall domain arrangement is also present in the structure of minimal TRPV1 (Figure 7.4(B)). Therefore, the overall architectures of the minimal TRPV1 and full-length TRPV2 EM maps matches

well with the in situ FRET data and lends further evidence that these structures represent the general architecture of TRPV channels.

Based on these structural studies, however, it remains difficult to predict how heat activates TRPV1. Utilization of cryo-EM to generate a high-resolution structure of TRPV1 opens new avenues to determine how heat gates thermoTRPV channels (Cao, Liao, et al., 2013).

2.2 TRPA subfamily

2.2.1 Is TRPA1 a cold sensor?—TRPA1 is the only mammalian member of the TRPA subfamily of proteins. It was first discovered as p120 protein in cultured fibroblasts (Jaquemar, Schenker, & Trueb, 1999) and later identified as ANKTM1 in a bioinformatics screen as a novel noxious cold-sensing channel (Story et al., 2003). TRPA1 is a 525 kDa homotetrameric channel with cytoplasmic N- and C-termini. The N-terminus of each monomer is predicted to contain 14–19 ankyrin repeats followed by a linker region connected to the first TM segment (Figure 7.7(A)).

Evidence that TRPA1 is activated by cold came from patch-clamp experiments demonstrating that temperatures <17 °C and the cooling agent icilin activate currents in Chinese hamster ovary cells heterologously expressing TRPA1 (Story et al., 2003). Since temperatures <17 °C evoke pain in humans, it was concluded that TRPA1 was a sensor for noxious cold; however, this conclusion is controversial (Caspani & Heppenstall, 2009) as it is still unclear if the channel is directly gated by cold or whether such activation is an indirect effect.

Two lines of TRPA1 knockout mice were generated but did not conclusively show that TRPA1 functions as a cold sensor in vivo (Bautista et al., 2006; Kwan et al., 2006). To follow-up on those original experiments, some laboratories were able to demonstrate that TRPA1 deletion disrupted noxious cold sensation (del Camino et al., 2010; Karashima et al., 2009; Sawada, Hosokawa, Hori, Matsumura, & Kobayashi, 2007), while others found that TRPA1 deletion had little effect on cold sensation (Jordt et al., 2004; Zurborg, Yurgionas, Jira, Caspani, & Heppenstall, 2007). This discrepancy could be due to the different strategies in creating these mice (Bautista et al., 2006; Kwan et al., 2006).

The cold sensitivity of the TRPA1 was also shown to be species dependent. While mouse and rat TRPA1 are cold sensitive, human and monkey TRPA1 are insensitive to cold (Chen et al., 2013). A single glycine residue on the mouse and rat TM segment 5 (Gly878) was suggested to be responsible for cold sensing by the channel. Mutation of Gly878 to valine, the equivalent residue in human and monkey TRPA1 (Val875), abolished cold sensitivity of rat TRPA1 (Chen et al., 2013).

Intriguingly, TRPA1 was identified as a sensor responsible for the infrared heat detection in snakes (Gracheva et al., 2010; Moiseenkova, Bell, Motamedi, Wozniak, & Christensen, 2003; Panzano, Kang, & Garrity, 2010; Pappas, Motamedi, & Christensen, 2004). Moreover, TRPA1 also acts as a heat sensor in fly and mosquitos (Kang et al., 2012; Viswanath et al., 2003), suggesting that TRPA1 evolutionally changed from heat- to cold-sensing channel in vertebrates, and became insensitive to temperature in humans (Chen et

al., 2013). The molecular mechanisms by which TRPA1 detects temperature from warm to cold remain to be determined. However, a recent chimeric analysis showed that the TRPA1 ankyrin repeats and N-terminal flexible linker are important for detecting and transducing different stimuli (Cordero-Morales et al., 2011).

In contrast to sensing cold, the role of TRPA1 in pain transduction and inflammatory sensitization is well established (Jordt & Ehrlich, 2007). TRPA1 is best characterized as a chemical nociceptor (Stucky et al., 2009). TRPA1 is activated by numerous exogenous electrophilic compounds and by endogenous reactive species generated during oxidative stress. Recent mutagenesis and chimeric studies have suggested that N-terminal cysteine residues (C622, C642, C666) located on the flexible linker region of the channel (N616-A720) are involved in channel activation and desensitization (Hinman, Chuang, Bautista, & Julius, 2006; Kang et al., 2010; Macpherson et al., 2007; Takahashi et al., 2008, 2011). In addition, non-electrophilic agonists such as 2-aminophenyl borate (2-APB), δ -9-tetrahydrocannabinol (THC), nicotine, and propofol can activate the channel (Talavera et al., 2009).

2.2.2 EM structures of full-length TRPA1 channel—Structural analysis of TRPA1 can provide a valuable starting point for understanding how TRPA1 homologs respond to such a wide range of temperatures and chemical compounds. Our laboratory determined the structure of mouse TRPA1 at 16 Å resolution using negatively stained, A8-35 amphipol stabilized TRPA1 complexes by EM (Figure 7.7(B)).

The structure revealed a tetrameric assembly with a compact TM domain and a basket-like cytoplasmic domain structure (Cvetkov et al., 2011). The size of our TRPA1 EM map is in agreement with its molecular mass. However, some additional volumetric mass was observed in the TRPA1 EM density due to the presence of the A8-35 amphipol molecules that were used for stabilization of the protein.

Interpretation of the TRPA1 EM map was achieved through molecular modeling of the TRPA1 N- and C-terminal domains using the I-TASSER protein structure prediction server (Roy, Kucukural, & Zhang, 2010) and manually docking these models into the EM density (Cvetkov et al., 2011). The model of the N-terminus (residues 1–721) predicted an ordered region of 12 ankyrin repeats (residues 31–437) and a more flexible downstream region (residues 438–721). This model was placed within the cytoplasmic domain of the density map (Figure 7.7(B), blue). The putative TM domain was fitted with a previously published molecular model of the TRPV1 TM domain (Figure 7.7(B), purple) (Fernandez-Ballester & Ferrer-Montiel, 2008). The model of the TRPA1 C-terminus (residues 964–1125) was placed into the 3D density map immediately below the predicted TM domain within the region linking the TM domain to the cytoplasmic domain (Figure 7.7(B), light purple).

This TRPA1 map reconstruction together with protein modeling provided the first insight into the overall organization of TRPA1 and allowed us to propose a mechanism of TRPA1 activation by electrophilic compounds. In the TRPA1 model fitted into the EM map, we observed that cysteines important for electrophilic activation structurally cluster in the pocket on the flexible region between TM domain and the ankyrin repeats (Figure 7.7(B),

yellow spheres). The proximity of these cysteines to one another led us to hypothesize that crosstalk or disulfide interactions could be occurring as a part of the channel activation mechanism (Cvetkov et al., 2011).

To test our hypothesis, we performed a mass spectrometry analysis of the *in vivo* thiol status of TRPA1 and identified that when treated with a thiol-reactive agonist, TRPA1 cysteine residues involved in channel activation form disulfide bonds with each other (Wang, Cvetkov, Chance, & Moiseenkova-Bell, 2012). This disulfide rearrangement could lead to channel activation and the rapid desensitization observed in electrophysiological studies.

We intend to use cryo-EM to achieve a higher resolution structure of fly, mouse, and human TRPA1, which will allow us to resolve structural elements in the channel and identify the root of divergence in temperature sensation between species.

2.3 TRPM subfamily

2.3.1 Thermosensitivity of TRPM channels—The TRPM subfamily of ion channels has eight members, which are subdivided based on their structural similarity into four groups: TRPM1/3, TRPM6/7, TRPM4/5, and TRPM2/8 (Zholos, Johnson, Burdya, & Melanaphy, 2011). In the TRPM subfamily, TRPM6/7 are Mg^{2+} -permeable and important regulators of intracellular Mg^{2+} homeostasis, while other TRPM channels are nonselective cation channels (Farooqi et al., 2011). Furthermore, TRPM6/7 contain an atypical kinase domain, allowing them to function as “chanzymes,” capable of phosphorylating and activating the channel (Montell, 2003). TRPM2 also has catalytic ADP-ribose hydrolase activity (Farooqi et al., 2011). TRPM channels are regulated by oxidative stress, Ca^{2+} , ATP, stretch, and temperature (Zholos et al., 2011).

TRPM8 is the only temperature-sensitive channel of this subfamily (Liu & Qin, 2011). TRPM8 was originally cloned from prostate cancer cells (Tsavaler, Shapero, Morkowski, & Laus, 2001) but was later identified in dorsal root ganglion (DRG) sensory neurons and trigeminal neurons, where it was shown to act as a cold sensor. TRPM8 is activated by cool temperatures (<20–28 °C) as well as cooling agents menthol and icilin (Chuang, Neuhausser, & Julius, 2004; McKemy, Neuhausser, & Julius, 2002; Peier, Moqrich, et al., 2002). TRPM8 acts as a cold sensor *in vivo*, as deletion of TRPM8 from mice disrupts responses to non-noxious cold temperatures (Bautista et al., 2007; Colburn et al., 2007; Dhaka et al., 2007). Additionally, dissociated sensory neurons from TRPM8 null mice show decreased responses to cold and menthol (Liu & Qin, 2011).

TRPM8 is a nonselective, Ca^{2+} -permeable cation channel that functions as a homotetramer (Latorre, Brauchi, Madrid, & Orio, 2011). Its molecular mass is ~510 kDa and it possesses long cytoplasmic N- and C-terminal domains that are important for cold- and ligand-induced channel activity (Figure 7.8(A)) (Latorre et al., 2011). The N-terminus contains the TRPM homology domain, which is conserved among all TRPM family members (Figure 7.8(A)). Its role in channel function remains unclear; however, deletion of this domain disrupts channel activity. The C-terminus contains the conserved TRP box sequence, which is important for activation and trafficking of TRPM8 to the plasma membrane as well as modulating its response to phosphoinositides (Latorre et al., 2011). The C-terminus also

harbors a conserved coiled-coil domain, which may be required for TRPM8 channel tetramerization and temperature- and ligand-induced activation (Tsuruda, Julius, & Minor, 2006). The C-terminus is thought to be directly involved in cold sensation by TRPM8, as TRPV1 chimeras with the TRPM8 C-terminus become cold sensitive, while TRPM8 chimeras with the TRPV1 C-terminus are sensitive to heat (Brauchi et al., 2006).

2.3.2 Structural analysis of TRPM channels—TRPM channels are large membrane proteins, ranging from 500 to 950 kDa in molecular size, with dynamic intracellular regions, which may hamper structural analyses of full-length TRPM channels. To date, the structural and biochemical information for the TRPM subfamily comes from studies of the coiled-coil domain. A crystal structure of the 5.5 kDa TRPM7 coiled-coil complex, which represents ~2.5% of the 850 kDa tetramer, was solved recently and provided insight into its role in tetramer assembly (Fujiwara & Minor, 2008). C-terminal coiled-coil domains for several ion channels, including Kv7, Eag, CNG, TRPC, and TRPV channels have been identified as oligomerization domains (Fujiwara & Minor, 2008; Howard, Clark, Holton, & Minor, 2007).

The isolated coiled-coil domain of TRPM8 self-assembles in solution, and point mutations to key residues involved in coiled-coil domain assembly in full-length TRPM8 disrupted oligomerization and translocation of TRPM8 in cells (Tsuruda et al., 2006). Crystallization of the TRPM7 coiled-coil complex further revealed the diversity among the TRPM subfamily members (Fujiwara & Minor, 2008). The TRPM7 coiled-coil consists of antiparallel helices that assemble by core packing and interactions between polar and charged residues across strands (Figure 7.8(B)). Based on sequence homology, the coiled-coil domains of TRPM channels can be categorized into two groups: those like TRPM7 (TRPM1, TRPM3, and TRPM6) and those unlike TRPM7 (TRPM2, TRPM4, TRPM5, and TRPM8) (Fujiwara & Minor, 2008). Those from the TRPM8 group show poor sequence conservation with the TRPM7 coiled-coil domain. They are shorter, and it is unclear whether they form parallel or antiparallel interactions. TRPM8-like coiled-coil domains would likely form incompatible electrostatic interactions with domains from the TRPM7 group. This provides an explanation as to why TRPM8 functions as a homotetramer.

Since 100 residues separate the coiled-coil domain from the TM6 segment, it has been proposed that the coiled-coil domain of TRPM7 fits beneath the TM domain and arranges in an antiparallel manner parallel to the plasma membrane (Figure 7.8(B)). This would allow for the coiled-coil domain to act as a spacer, allowing symmetrical assembly of kinase domain dimers (Fujiwara & Minor, 2008).

This crystal structure provided a detailed analysis of TRPM channel oligomerization. However, analysis of the structure of full-length TRPM8 will likely be necessary to determine the conformational changes and molecular mechanisms underlying cold sensitivity.

3. OUTLOOK AND PROSPECTIVE

Higher organisms have developed specific mechanisms to detect changes in temperature. Their sensory neurons express an array of polymodal receptors that are activated over a wide range of temperatures from painful cold to noxious heat. Most of these receptors come from the TRP channel family, which when activated, conduct cations that activate the sensory neurons and relay a signal to the brain. Since the discovery of thermosensitive TRP channels, the search has been on for the molecular nature of the temperature sensor within these channels; however, the structural features that convey temperature sensitivity to thermoTRP channels remain elusive.

Structure–function analysis of thermoTRP channels has lent insight into the thermodynamic and molecular mechanisms by which these polymodal receptors sense temperature changes. While mutagenesis and chimeric approaches have identified several channel domains responsible for thermosensation, controversy still remains as to how these channels are gated by temperature. It is likely that the domains identified by structure–function studies are all involved in thermosensation. Higher resolution structural data for all full-length thermoTRP channels will be necessary for understanding how these protein complexes respond to a wide range of temperatures and the conformational changes these channels undergo during activation by temperature changes at atomic levels.

The TRPV subfamily is the most extensively characterized of all TRP channels due to the role of its members in pain and heat sensation. TRPV1–4 are heat sensitive in heterologous expression systems while TRPV1 clearly acts as an intrinsic heat sensor in vivo. Moderate resolution structures of TRPV1, TRPV2, and TRPV4 have revealed the general architecture of thermoTRPV channels. Together with high-resolution structures for the soluble ARDs for thermoTRPV channels, we can start to understand how the domains are organized and how the channels are regulated. The recent high-resolution structure of minimal TRPV1 lends the potential to understand the conformation changes of thermoTRPV channels upon activation by heat at a new detailed level.

Both TRPA1 and TRPM8 are cold sensors that form large dynamic complexes, making structural studies more difficult. Progress is being made in studying TRPA1, as a moderate resolution structure allowed us to understand how TRPA1 is modulated by electrophilic ligands. Obtaining higher resolution information for TRPA1 will allow us to understand how cold temperature gates the channel. Structural understanding of TRPM8 is in its infancy. Better methodologies for stabilizing and purifying TRPM8 will allow for more biophysical biochemical and structural characterization of the channel.

References

- Abrahamsen B, Zhao J, Asante CO, Cendan CM, Marsh S, Martinez-Barbera JP, et al. The cell and molecular basis of mechanical, cold, and inflammatory pain. *Science*. 2008; 321(5889):702–705. <http://dx.doi.org/10.1126/science.1156916>. [PubMed: 18669863]
- Al-Ansary D, Bogeski I, Disteldorf BM, Becherer U, Niemeyer BA. ATP modulates Ca^{2+} uptake by TRPV6 and is counteracted by isoform-specific phosphorylation. *FASEB Journal*. 2010; 24(2):425–435. [fj.09-141481](https://doi.org/10.1096/fj.09-141481), pii. 10.1096/fj.09-141481 [PubMed: 19805577]

- Bautista DM, Jordt SE, Nikai T, Tsuruda PR, Read AJ, Poblete J, et al. TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. *Cell*. 2006; 124(6):1269–1282. S0092-8674(06)00240-6, pii. 10.1016/j.cell.2006.02.023 [PubMed: 16564016]
- Bautista DM, Siemens J, Glazer JM, Tsuruda PR, Basbaum AI, Stucky CL, et al. The menthol receptor TRPM8 is the principal detector of environmental cold. *Nature*. 2007; 448(7150):204–208. <http://dx.doi.org/10.1038/nature05910>. [PubMed: 17538622]
- Belmonte C, Viana F. Molecular and cellular limits to somatosensory specificity. *Molecular Pain*. 2008; 4:14. <http://dx.doi.org/10.1186/1744-8069-4-14>. [PubMed: 18419827]
- Binz HK, Amstutz P, Kohl A, Stumpp MT, Briand C, Forrer P, et al. High-affinity binders selected from designed ankyrin repeat protein libraries. *Nature Biotechnology*. 2004; 22(5):575–582. 10.1038/nbt962,pii:nbt962
- Brauchi S, Orta G, Salazar M, Rosenmann E, Latorre R. A hot-sensing cold receptor: C-terminal domain determines thermosensation in transient receptor potential channels. *The Journal of Neuroscience*. 2006; 26(18):4835–4840. <http://dx.doi.org/10.1523/JNEUROSCI.5080-05.2006>. [PubMed: 16672657]
- del Camino D, Murphy S, Heiry M, Barrett LB, Earley TJ, Cook CA, et al. TRPA1 contributes to cold hypersensitivity. *The Journal of Neuroscience*. 2010; 30(45):15165–15174. <http://dx.doi.org/10.1523/JNEUROSCI.2580-10.2010>. [PubMed: 21068322]
- Cao E, Cordero-Morales JF, Liu B, Qin F, Julius D. TRPV1 channels are intrinsically heat sensitive and negatively regulated by phosphoinositide lipids. *Neuron*. 2013; 77(4):667–679. <http://dx.doi.org/10.1016/j.neuron.2012.12.016>. [PubMed: 23439120]
- Cao E, Liao M, Cheng Y, Julius D. TRPV1 structures in distinct conformations reveal activation mechanisms. *Nature*. 2013; 504(7478):113–118. <http://dx.doi.org/10.1038/nature12823>. [PubMed: 24305161]
- Caspani O, Heppenstall PA. TRPA1 and cold transduction: an unresolved issue? *The Journal of General Physiology*. 2009; 133(3):245–249. jgp.200810136, pii. 10.1085/jgp.200810136 [PubMed: 19237589]
- Caterina MJ, Leffler A, Malmberg AB, Martin WJ, Trafton J, Petersen-Zeit KR, et al. Impaired nociception and pain sensation in mice lacking the capsaicin receptor. *Science*. 2000; 288(5464):306–313. [PubMed: 10764638]
- Caterina MJ, Rosen TA, Tominaga M, Brake AJ, Julius D. A capsaicin-receptor homologue with a high threshold for noxious heat. *Nature*. 1999; 398(6726):436–441. <http://dx.doi.org/10.1038/18906>. [PubMed: 10201375]
- Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature*. 1997; 389(6653):816–824. <http://dx.doi.org/10.1038/39807>. [PubMed: 9349813]
- Chacon P, Wriggers W. Multi-resolution contour-based fitting of macromolecular structures. *Journal of Molecular Biology*. 2002; 317(3):375–384. <http://dx.doi.org/10.1006/jmbi.2002.5438>. [PubMed: 11922671]
- Chen J, Kang D, Xu J, Lake M, Hogan JO, Sun C, et al. Species differences and molecular determinant of TRPA1 cold sensitivity. *Nature Communications*. 2013; 4:2501. <http://dx.doi.org/10.1038/ncomms3501>.
- Chuang HH, Neuhauser WM, Julius D. The super-cooling agent icilin reveals a mechanism of coincidence detection by a temperature-sensitive TRP channel. *Neuron*. 2004; 43(6):859–869. <http://dx.doi.org/10.1016/j.neuron.2004.08.038>. [PubMed: 15363396]
- Clapham DE, Miller C. A thermodynamic framework for understanding temperature sensing by transient receptor potential (TRP) channels. *Proceedings of the National Academy of Sciences USA*. 2011; 108(49):19492–19497. <http://dx.doi.org/10.1073/pnas.1117485108>.
- Colburn RW, Lubin ML, Stone DJ Jr, Wang Y, Lawrence D, D'Andrea MR, et al. Attenuated cold sensitivity in TRPM8 null mice. *Neuron*. 2007; 54(3):379–386. <http://dx.doi.org/10.1016/j.neuron.2007.04.017>. [PubMed: 17481392]
- Cordero-Morales JF, Gracheva EO, Julius D. Cytoplasmic ankyrin repeats of transient receptor potential A1 (TRPA1) dictate sensitivity to thermal and chemical stimuli. *Proceedings of the*

- National Academy of Sciences USA. 2011; 108(46):E1184–E1191. <http://dx.doi.org/10.1073/pnas.1114124108>.
- Cosens DJ, Manning A. Abnormal electroretinogram from a *Drosophila* mutant. *Nature*. 1969; 224(5216):285–287. [PubMed: 5344615]
- Cvetkov TL, Huynh KW, Cohen MR, Moiseenkova-Bell VY. Molecular architecture and subunit organization of TRPA1 ion channel revealed by electron microscopy. *The Journal of Biological Chemistry*. 2011; 286(44):38168–38176. <http://dx.doi.org/10.1074/jbc.M111.288993>. [PubMed: 21908607]
- De-la-Rosa V, Rangel-Yescas GE, Ladron-de-Guevara E, Rosenbaum T, Islas LD. Coarse architecture of the transient receptor potential vanilloid 1 (TRPV1) ion channel determined by fluorescence resonance energy transfer. *The Journal of Biological Chemistry*. 2013; 288(41):29506–29517. <http://dx.doi.org/10.1074/jbc.M113.479618>. [PubMed: 23965996]
- Dhaka A, Murray AN, Mathur J, Earley TJ, Petrus MJ, Patapoutian A. TRPM8 is required for cold sensation in mice. *Neuron*. 2007; 54(3):371–378. <http://dx.doi.org/10.1016/j.neuron.2007.02.024>. [PubMed: 17481391]
- Digel I. Primary thermosensory events in cells. *Advances in Experimental Medicine and Biology*. 2011; 704:451–468. http://dx.doi.org/10.1007/978-94-007-0265-3_25. [PubMed: 21290311]
- Digel I, Kayser P, Artmann GM. Molecular processes in biological thermosensation. *Journal of Biophysics*. 2008; 2008:602870. <http://dx.doi.org/10.1155/2008/602870>. [PubMed: 20130806]
- Farooqi AA, Javeed MK, Javed Z, Riaz AM, Mukhtar S, Minhaj S, et al. TRPM channels: same ballpark, different players, and different rules in immunogenetics. *Immunogenetics*. 2011; 63(12):773–787. <http://dx.doi.org/10.1007/s00251-011-0570-4>. [PubMed: 21932052]
- Fernandez-Ballester G, Ferrer-Montiel A. Molecular modeling of the full-length human TRPV1 channel in closed and desensitized states. *The Journal of Membrane Biology*. 2008; 223(3):161–172. <http://dx.doi.org/10.1007/s00232-008-9123-7>. [PubMed: 18791833]
- Fujiwara Y, Minor DL Jr. X-ray crystal structure of a TRPM assembly domain reveals an antiparallel four-stranded coiled-coil. *Journal of Molecular Biology*. 2008; 383(4):854–870. <http://dx.doi.org/10.1016/j.jmb.2008.08.059>. [PubMed: 18782578]
- Garcia-Sanz N, Fernandez-Carvajal A, Morenilla-Palao C, Planells-Cases R, Fajardo-Sanchez E, Fernandez-Ballester G, et al. Identification of a tetramerization domain in the C terminus of the vanilloid receptor. *The Journal of Neuroscience*. 2004; 24(23):5307–5314. <http://dx.doi.org/10.1523/JNEUROSCI.0202-04.2004>. [PubMed: 15190102]
- Gaudet R. A primer on ankyrin repeat function in TRP channels and beyond. *Molecular BioSystems*. 2008; 4(5):372–379. <http://dx.doi.org/10.1039/b801481g>. [PubMed: 18414734]
- Gaudet R. Divide and conquer: high resolution structural information on TRP channel fragments. *The Journal of General Physiology*. 2009; 133(3):231–237. <http://dx.doi.org/10.1085/jgp.200810137>. [PubMed: 19237587]
- Gracheva EO, Ingolia NT, Kelly YM, Cordero-Morales JF, Hollopeter G, Chesler AT, et al. Molecular basis of infrared detection by snakes. *Nature*. 2010; 464(7291):1006–1011. <http://dx.doi.org/10.1038/nature08943>. [PubMed: 20228791]
- Grandl J, Hu H, Bandell M, Bursulaya B, Schmidt M, Petrus M, et al. Pore region of TRPV3 ion channel is specifically required for heat activation. *Nature Neuroscience*. 2008; 11(9):1007–1013. <http://dx.doi.org/10.1038/nn.2169>.
- Grandl J, Kim SE, Uzzell V, Bursulaya B, Petrus M, Bandell M, et al. Temperature-induced opening of TRPV1 ion channel is stabilized by the pore domain. *Nature Neuroscience*. 2010; 13(6):708–714. <http://dx.doi.org/10.1038/nn.2552>.
- Guler AD, Lee H, Iida T, Shimizu I, Tominaga M, Caterina M. Heat-evoked activation of the ion channel, TRPV4. *The Journal of Neuroscience*. 2002; 22(15):6408–6414. [PubMed: 12151520]
- Henderson R. The potential and limitations of neutrons, electrons and X-rays for atomic resolution microscopy of unstained biological molecules. *Quarterly Reviews of Biophysics*. 1995; 28(2):171–193. [PubMed: 7568675]
- Henderson R. Structural biology: Ion channel seen by electron microscopy. *Nature*. 2013; 504(7478):93–94. <http://dx.doi.org/10.1038/504093a>. [PubMed: 24305155]

- Heymann JB, Conway JF, Steven AC. Molecular dynamics of protein complexes from four-dimensional cryoelectron microscopy. *Journal of Structural Biology*. 2004; 147(3):291–301. pii:S1047847704000425. 10.1016/j.jsb.2004.02.006 [PubMed: 15450298]
- Hinman A, Chuang HH, Bautista DM, Julius D. TRP channel activation by reversible covalent modification. *Proceedings of the National Academy of Sciences USA*. 2006; 103(51):19564–19568. 0609598103, pii. 10.1073/pnas.0609598103
- Howard RJ, Clark KA, Holton JM, Minor DL Jr. Structural insight into KCNQ (Kv7) channel assembly and channelopathy. *Neuron*. 2007; 53(5):663–675. <http://dx.doi.org/10.1016/j.neuron.2007.02.010>. [PubMed: 17329207]
- Huang SM, Li X, Yu Y, Wang J, Caterina MJ. TRPV3 and TRPV4 ion channels are not major contributors to mouse heat sensation. *Molecular Pain*. 2011; 7:37. <http://dx.doi.org/10.1186/1744-8069-7-37>. [PubMed: 21586160]
- Huynh KW, Cohen MR, Chakrapani S, Holdaway HA, Stewart PL, Moiseenkova-Bell VY. Structural insight into the assembly of TRPV channels. *Structure*. 2014; 22(2):260–268. <http://dx.doi.org/10.1016/j.str.2013.11.008>. [PubMed: 24373766]
- Inada H, Procko E, Sotomayor M, Gaudet R. Structural and biochemical consequences of disease-causing mutations in the ankyrin repeat domain of the human TRPV4 channel. *Biochemistry*. 2012; 51(31):6195–6206. <http://dx.doi.org/10.1021/bi300279b>. [PubMed: 22702953]
- Islas LD, Salazar H, Jara-Oseguera A, Nieto-Posadas A, Llorente I, Rangel-Yescas G, et al. The helical character of the S6 segment of TRPV1 channels. *Channels (Austin, Tex)*. 2009; 3(5):311–313.
- Jaquemar D, Schenker T, Trueb B. An ankyrin-like protein with transmembrane domains is specifically lost after oncogenic transformation of human fibroblasts. *The Journal of Biological Chemistry*. 1999; 274(11):7325–7333. [PubMed: 10066796]
- Jin X, Touhey J, Gaudet R. Structure of the N-terminal ankyrin repeat domain of the TRPV2 ion channel. *The Journal of Biological Chemistry*. 2006; 281(35):25006–25010. <http://dx.doi.org/10.1074/jbc.C600153200>. [PubMed: 16809337]
- Jordt SE, Bautista DM, Chuang HH, McKemy DD, Zygmunt PM, Hogestatt ED, et al. Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. *Nature*. 2004; 427(6971):260–265. <http://dx.doi.org/10.1038/nature02282>. [PubMed: 14712238]
- Jordt SE, Ehrlich BE. TRP channels in disease. *Subcellular Biochemistry*. 2007; 45:253–271. [PubMed: 18193640]
- Kang K, Panzano VC, Chang EC, Ni L, Dainis AM, Jenkins AM, et al. Modulation of TRPA1 thermal sensitivity enables sensory discrimination in *Drosophila*. *Nature*. 2012; 481(7379):76–80. <http://dx.doi.org/10.1038/nature10715>. [PubMed: 22139422]
- Kang K, Pulver SR, Panzano VC, Chang EC, Griffith LC, Theobald DL, et al. Analysis of *Drosophila* TRPA1 reveals an ancient origin for human chemical nociception. *Nature*. 2010; 464(7288):597–600. nature08848, pii. 10.1038/nature08848 [PubMed: 20237474]
- Karashima Y, Talavera K, Everaerts W, Janssens A, Kwan KY, Vennekens R, et al. TRPA1 acts as a cold sensor in vitro and in vivo. *Proceedings of the National Academy of Sciences USA*. 2009; 106(4):1273–1278. <http://dx.doi.org/10.1073/pnas.0808487106>.
- Kedei N, Szabo T, Lile JD, Treanor JJ, Olah Z, Iadarola MJ, et al. Analysis of the native quaternary structure of vanilloid receptor 1. *The Journal of Biological Chemistry*. 2001; 276(30):28613–28619. <http://dx.doi.org/10.1074/jbc.M103272200>. [PubMed: 11358970]
- Kim SE, Patapoutian A, Grandl J. Single residues in the outer pore of TRPV1 and TRPV3 have temperature-dependent conformations. *PLoS One*. 2013; 8(3):e59593. <http://dx.doi.org/10.1371/journal.pone.0059593>. [PubMed: 23555720]
- Kwan KY, Allchorne AJ, Vollrath MA, Christensen AP, Zhang DS, Woolf CJ, et al. TRPA1 contributes to cold, mechanical, and chemical nociception but is not essential for hair-cell transduction. *Neuron*. 2006; 50(2):277–289. S0896-6273(06)00269-8, pii. 10.1016/j.neuron.2006.03.042 [PubMed: 16630838]
- Latorre R, Brauchi S, Madrid R, Orio P. A cool channel in cold transduction. *Physiology (Bethesda, Md)*. 2011; 26(4):273–285. <http://dx.doi.org/10.1152/physiol.00004.2011>.

- Lau SY, Procko E, Gaudet R. Distinct properties of Ca^{2+} -calmodulin binding to N- and C-terminal regulatory regions of the TRPV1 channel. *The Journal of General Physiology*. 2012; 140(5):541–555. <http://dx.doi.org/10.1085/jgp.201210810>. [PubMed: 23109716]
- Liao M, Cao E, Julius D, Cheng Y. Structure of the TRPV1 ion channel determined by electron cryomicroscopy. *Nature*. 2013; 504(7478):107–112. <http://dx.doi.org/10.1038/nature12822>. [PubMed: 24305160]
- Li J, Mahajan A, Tsai MD. Ankyrin repeat: a unique motif mediating protein-protein interactions. *Biochemistry*. 2006; 45(51):15168–15178. <http://dx.doi.org/10.1021/bi062188q>. [PubMed: 17176038]
- Lishko PV, Procko E, Jin X, Phelps CB, Gaudet R. The ankyrin repeats of TRPV1 bind multiple ligands and modulate channel sensitivity. *Neuron*. 2007; 54(6):905–918. <http://dx.doi.org/10.1016/j.neuron.2007.05.027>. [PubMed: 17582331]
- Liu B, Hui K, Qin F. Thermodynamics of heat activation of single capsaicin ion channels VR1. *Biophysical Journal*. 2003; 85(5):2988–3006. [http://dx.doi.org/10.1016/S0006-3495\(03\)74719-5](http://dx.doi.org/10.1016/S0006-3495(03)74719-5). [PubMed: 14581201]
- Liu Y, Qin N. TRPM8 in health and disease: cold sensing and beyond. *Advances in Experimental Medicine and Biology*. 2011; 704:185–208. http://dx.doi.org/10.1007/978-94-007-0265-3_10. [PubMed: 21290296]
- Long SB, Tao X, Campbell EB, MacKinnon R. Atomic structure of a voltage-dependent K^+ channel in a lipid membrane-like environment. *Nature*. 2007; 450(7168):376–382. <http://dx.doi.org/10.1038/nature06265>. [PubMed: 18004376]
- Ludtke SJ, Serysheva II, Hamilton SL, Chiu W. The pore structure of the closed RyR1 channel. *Structure*. 2005; 13(8):1203–1211. S0969-2126(05)00215-7, pii. 10.1016/j.str.2005.06.005 [PubMed: 16084392]
- Ludtke SJ, Tran TP, Ngo QT, Moiseenkova-Bell VY, Chiu W, Serysheva II. Flexible architecture of IP3R1 by Cryo-EM. *Structure*. 2011; 19(8):1192–1199. S0969-2126(11)00180-8, pii. 10.1016/j.str.2011.05.003 [PubMed: 21827954]
- Macpherson LJ, Dubin AE, Evans MJ, Marr F, Schultz PG, Cravatt BF, et al. Noxious compounds activate TRPA1 ion channels through covalent modification of cysteines. *Nature*. 2007; 445(7127):541–545. nature05544, pii. 10.1038/nature05544 [PubMed: 17237762]
- Maingret F, Lauritzen I, Patel AJ, Heurteaux C, Reyes R, Lesage F, et al. TREK-1 is a heat-activated background K^+ channel. *EMBO Journal*. 2000; 19(11):2483–2491. <http://dx.doi.org/10.1093/emboj/19.11.2483>. [PubMed: 10835347]
- McGlone F, Reilly D. The cutaneous sensory system. *Neuroscience and Biobehavioral Reviews*. 2010; 34(2):148–159. <http://dx.doi.org/10.1016/j.neubiorev.2009.08.004>. [PubMed: 19712693]
- McKemy DD. The molecular and cellular basis of cold sensation. *ACS Chemical Neuroscience*. 2013; 4(2):238–247. <http://dx.doi.org/10.1021/cn300193h>. [PubMed: 23421674]
- McKemy DD, Neuhauser WM, Julius D. Identification of a cold receptor reveals a general role for TRP channels in thermosensation. *Nature*. 2002; 416(6876):52–58. <http://dx.doi.org/10.1038/nature719>. [PubMed: 11882888]
- Minke B, Wu C, Pak WL. Induction of photoreceptor voltage noise in the dark in *Drosophila mutant*. *Nature*. 1975; 258(5530):84–87. [PubMed: 810728]
- Moiseenkova-Bell VY, Stanciu LA, Serysheva II, Tobe BJ, Wensel TG. Structure of TRPV1 channel revealed by electron cryomicroscopy. *Proceedings of the National Academy of Sciences USA*. 2008; 105(21):7451–7455. <http://dx.doi.org/10.1073/pnas.0711835105>.
- Moiseenkova-Bell VY, Wensel TG. Hot on the trail of TRP channel structure. *The Journal of General Physiology*. 2009; 133(3):239–244. <http://dx.doi.org/10.1085/jgp.200810123>. [PubMed: 19237588]
- Moiseenkova-Bell V, Wensel TG. Functional and structural studies of TRP channels heterologously expressed in budding yeast. *Advances in Experimental Medicine and Biology*. 2011; 704:25–40. http://dx.doi.org/10.1007/978-94-007-0265-3_2. [PubMed: 21290288]
- Moiseenkova V, Bell B, Motamedi M, Wozniak E, Christensen B. Wideband spectral tuning of heat receptors in the pit organ of the copperhead snake (Crotalinae). *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*. 2003; 284(2):R598–R606.

- Montell C. Mg^{2+} homeostasis: the Mg^{2+} -sensitive TRPM channels. *Current Biology*. 2003; 13(20):R799–R801. [PubMed: 14561419]
- Nakamura Y, Nakano K, Umehara T, Kimura M, Hayashizaki Y, Tanaka A, et al. Structure of the oncoprotein gankyrin in complex with S6 ATPase of the 26S proteasome. *Structure*. 2007; 15(2): 179–189. <http://dx.doi.org/10.1016/j.str.2006.11.015>. [PubMed: 17292836]
- Noel J, Zimmermann K, Busserolles J, Deval E, Alloui A, Diochot S, et al. The mechano-activated K^+ channels TRAAK and TREK-1 control both warm and cold perception. *EMBO Journal*. 2009; 28(9):1308–1318. <http://dx.doi.org/10.1038/emboj.2009.57>. [PubMed: 19279663]
- Panzano VC, Kang K, Garrity PA. Infrared snake eyes: TRPA1 and the thermal sensitivity of the snake pit organ. *Science Signaling*. 2010; 3(127):pe22. <http://dx.doi.org/10.1126/scisignal.3127pe22>. [PubMed: 20571127]
- Pappas TC, Motamedi M, Christensen BN. Unique temperature-activated neurons from pit viper thermosensors. *American Journal of Physiology Cell Physiology*. 2004; 287(5):C1219–C1228. pii: 00040.2004. 10.1152/ajpcell.00040.2004 [PubMed: 15213055]
- Park U, Vastani N, Guan Y, Raja SN, Koltzenburg M, Caterina MJ. TRP vanilloid 2 knock-out mice are susceptible to perinatal lethality but display normal thermal and mechanical nociception. *The Journal of Neuroscience*. 2011; 31(32):11425–11436. <http://dx.doi.org/10.1523/JNEUROSCI.1384-09.2011>. [PubMed: 21832173]
- Payandeh J, Scheuer T, Zheng N, Catterall WA. The crystal structure of a voltage-gated sodium channel. *Nature*. 2011; 475(7356):353–358. <http://dx.doi.org/10.1038/nature10238>. [PubMed: 21743477]
- Peier AM, Moqrich A, Hergarden AC, Reeve AJ, Andersson DA, Story GM, et al. A TRP channel that senses cold stimuli and menthol. *Cell*. 2002; 108(5):705–715. [PubMed: 11893340]
- Peier AM, Reeve AJ, Andersson DA, Moqrich A, Earley TJ, Hergarden AC, et al. A heat-sensitive TRP channel expressed in keratinocytes. *Science*. 2002; 296(5575):2046–2049. <http://dx.doi.org/10.1126/science.1073140>. [PubMed: 12016205]
- Peralvarez-Marín A, Donate-Macian P, Gaudet R. What do we know about the transient receptor potential vanilloid 2 (TRPV2) ion channel? *FEBS Journal*. 2013; 280(21):5471–5487. <http://dx.doi.org/10.1111/febs.12302>. [PubMed: 23615321]
- Phelps CB, Huang RJ, Lishko PV, Wang RR, Gaudet R. Structural analyses of the ankyrin repeat domain of TRPV6 and related TRPV ion channels. *Biochemistry*. 2008; 47(8):2476–2484. <http://dx.doi.org/10.1021/bi702109w>. [PubMed: 18232717]
- Phelps CB, Wang RR, Choo SS, Gaudet R. Differential regulation of TRPV1, TRPV3, and TRPV4 sensitivity through a conserved binding site on the ankyrin repeat domain. *The Journal of Biological Chemistry*. 2010; 285(1):731–740. M109.052548, pii. 10.1074/jbc.M109.052548 [PubMed: 19864432]
- Popot JL, Althoff T, Bagnard D, Baneres JL, Bazzacco P, Billon-Denis E, et al. Amphipols from A to Z. *Annual Review of Biophysics*. 2011; 40:379–408. <http://dx.doi.org/10.1146/annurev-biophys-042910-155219>.
- Roy A, Kucukural A, Zhang Y. I-TASSER: a unified platform for automated protein structure and function prediction. *Nature Protocols*. 2010; 5(4):725–738. nprot.2010.5, pii. 10.1038/nprot.2010.5
- Salazar H, Jara-Oseguera A, Hernandez-Garcia E, Llorente I, Arias, Olguin II, Soriano-Garcia M, et al. Structural determinants of gating in the TRPV1 channel. *Nature Structural and Molecular Biology*. 2009; 16(7):704–710. <http://dx.doi.org/10.1038/nsmb.1633>.
- Samso M, Feng W, Pessah IN, Allen PD. Coordinated movement of cytoplasmic and transmembrane domains of RyR1 upon gating. *PLoS Biology*. 2009; 7(4):e85. 08-PLBI-RA-5391, pii. 10.1371/journal.pbio.1000085 [PubMed: 19402748]
- Samso M, Wagenknecht T, Allen PD. Internal structure and visualization of transmembrane domains of the RyR1 calcium release channel by cryo-EM. *Nature Structural and Molecular Biology*. 2005; 12(6):539–544. nsmb938, pii. 10.1038/nsmb938
- Sawada Y, Hosokawa H, Hori A, Matsumura K, Kobayashi S. Cold sensitivity of recombinant TRPA1 channels. *Brain Research*. 2007; 1160:39–46. <http://dx.doi.org/10.1016/j.brainres.2007.05.047>. [PubMed: 17588549]

- Schepers RJ, Ringkamp M. Thermoreceptors and thermosensitive afferents. *Neuroscience and Biobehavioral Reviews*. 2009; 33(3):205–212. <http://dx.doi.org/10.1016/j.neubiorev.2008.07.009>. [PubMed: 18761036]
- Sengupta P, Garrity P. Sensing temperature. *Current Biology*. 2013; 23(8):R304–R307. <http://dx.doi.org/10.1016/j.cub.2013.03.009>. [PubMed: 23618661]
- Serysheva II, Ludtke SJ, Baker ML, Cong Y, Topf M, Eramian D, et al. Subnanometer-resolution electron cryomicroscopy-based domain models for the cytoplasmic region of skeletal muscle RyR channel. *Proceedings of the National Academy of Sciences USA*. 2008; 105(28):9610–9615. 0803189105, pii. 10.1073/pnas.0803189105
- Shigematsu H, Sokabe T, Danev R, Tominaga M, Nagayama K. A 3.5-nm structure of rat TRPV4 cation channel revealed by Zernike phase-contrast cryoelectron microscopy. *The Journal of Biological Chemistry*. 2010; 285(15):11210–11218. <http://dx.doi.org/10.1074/jbc.M109.090712>. [PubMed: 20044482]
- Shi DJ, Ye S, Cao X, Zhang R, Wang K. Crystal structure of the N-terminal ankyrin repeat domain of TRPV3 reveals unique conformation of finger 3 loop critical for channel function. *Protein and Cell*. 2013; 4(12):942–950. <http://dx.doi.org/10.1007/s13238-013-3091-0>. [PubMed: 24248473]
- Smith GD, Gunthorpe MJ, Kelsell RE, Hayes PD, Reilly P, Facer P, et al. TRPV3 is a temperature-sensitive vanilloid receptor-like protein. *Nature*. 2002; 418(6894):186–190. <http://dx.doi.org/10.1038/nature00894>. [PubMed: 12077606]
- Story GM, Peier AJ, Reeve AJ, Eid SR, Mosbacher J, Hricik TR, et al. ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. *Cell*. 2003; 112(6):819–829. S0092867403001582. [PubMed: 12654248]
- Strotmann R, Harteneck C, Nunnenmacher K, Schultz G, Plant TD. OTRPC4, a nonselective cation channel that confers sensitivity to extracellular osmolarity. *Nature Cell Biology*. 2000; 2(10):695–702. <http://dx.doi.org/10.1038/35036318>.
- Stucky CL, Dubin AE, Jeske NA, Malin SA, McKemy DD, Story GM. Roles of transient receptor potential channels in pain. *Brain Research Reviews*. 2009; 60(1):2–23. S0165-0173(08)00157-4, pii. 10.1016/j.brainresrev.2008.12.018 [PubMed: 19203589]
- Susankova K, Ettrich R, Vyklicky L, Teisinger J, Vlachova V. Contribution of the putative inner-pore region to the gating of the transient receptor potential vanilloid subtype 1 channel (TRPV1). *The Journal of Neuroscience*. 2007; 27(28):7578–7585. <http://dx.doi.org/10.1523/JNEUROSCI.1956-07.2007>. [PubMed: 17626219]
- Takahashi N, Kuwaki T, Kiyonaka S, Numata T, Kozai D, Mizuno Y, et al. TRPA1 underlies a sensing mechanism for O(2). *Nature Chemical Biology*. 2011; 7(10):701–711. nchembio.640, pii. 10.1038/nchembio.640
- Takahashi N, Mizuno Y, Kozai D, Yamamoto S, Kiyonaka S, Shibata T, et al. Molecular characterization of TRPA1 channel activation by cysteine-reactive inflammatory mediators. *Channels (Austin, Tex)*. 2008; 2(4):287–298. 6745.
- Talavera K, Gees M, Karashima Y, Meseguer VM, Vanoirbeek JA, Damann N, et al. Nicotine activates the chemosensory cation channel TRPA1. *Nature Neuroscience*. 2009; 12(10):1293–1299. <http://dx.doi.org/10.1038/nn.2379>.
- Tsavalier L, Shapero MH, Morkowski S, Laus R. Trp-p8, a novel prostate-specific gene, is up-regulated in prostate cancer and other malignancies and shares high homology with transient receptor potential calcium channel proteins. *Cancer Research*. 2001; 61(9):3760–3769. [PubMed: 11325849]
- Tsuruda PR, Julius D, Minor DL Jr. Coiled coils direct assembly of a cold-activated TRP channel. *Neuron*. 2006; 51(2):201–212. <http://dx.doi.org/10.1016/j.neuron.2006.06.023>. [PubMed: 16846855]
- Vahedi-Faridi A, Jastrzebska B, Palczewski K, Engel A. 3D imaging and quantitative analysis of small solubilized membrane proteins and their complexes by transmission electron microscopy. *Microscopy (Oxford, England)*. 2013; 62(1):95–107. <http://dx.doi.org/10.1093/jmicro/dfs091>.
- Venkatachalam K, Montell C. TRP channels. *Annual Review of Biochemistry*. 2007; 76:387–417. <http://dx.doi.org/10.1146/annurev.biochem.75.103004.142819>.

- Viana F. Chemosensory properties of the trigeminal system. *ACS Chemical Neuroscience*. 2011; 2(1): 38–50. <http://dx.doi.org/10.1021/cn100102c>. [PubMed: 22778855]
- Viswanath V, Story GM, Peier AM, Petrus MJ, Lee VM, Hwang SW, et al. Opposite thermosensor in fruitfly and mouse. *Nature*. 2003; 423(6942):822–823. <http://dx.doi.org/10.1038/423822a>. [PubMed: 12815418]
- Wang L, Cvetkov TL, Chance MR, Moiseenkova-Bell VY. Identification of in vivo disulfide conformation of TRPA1 ion channel. *The Journal of Biological Chemistry*. 2012; 287(9):6169–6176. <http://dx.doi.org/10.1074/jbc.M111.329748>. [PubMed: 22207754]
- Wang L, Sigworth FJ. Cryo-EM and single particles. *Physiology (Bethesda, Md)*. 2006; 21:13–18. 21/1/13, pii. 10.1152/physiol.00045.2005
- Westfield GH, Rasmussen SG, Su M, Dutta S, DeVree BT, Chung KY, et al. Structural flexibility of the G alpha s alpha-helical domain in the beta2-adrenoceptor Gs complex. *Proceedings of the National Academy of Sciences USA*. 2011; 108(38):16086–16091. <http://dx.doi.org/10.1073/pnas.1113645108>.
- Whorton MR, MacKinnon R. X-ray structure of the mammalian GIRK2-beta gamma G-protein complex. *Nature*. 2013; 498(7453):190–197. <http://dx.doi.org/10.1038/nature12241>. [PubMed: 23739333]
- Wolf M, Garcea RL, Grigorieff N, Harrison SC. Subunit interactions in bovine papillomavirus. *Proceedings of the National Academy of Sciences USA*. 2010; 107(14):6298–6303. <http://dx.doi.org/10.1073/pnas.0914604107>.
- Xu H, Ramsey IS, Kotecha SA, Moran MM, Chong JA, Lawson D, et al. TRPV3 is a calcium-permeable temperature-sensitive cation channel. *Nature*. 2002; 418(6894):181–186. <http://dx.doi.org/10.1038/nature00882>. [PubMed: 12077604]
- Yang F, Cui Y, Wang K, Zheng J. Thermosensitive TRP channel pore turret is part of the temperature activation pathway. *Proceedings of the National Academy of Sciences USA*. 2010; 107(15): 7083–7088. <http://dx.doi.org/10.1073/pnas.1000357107>.
- Yao J, Liu B, Qin F. Pore turret of thermal TRP channels is not essential for temperature sensing. *Proceedings of the National Academy of Sciences USA*. 2010; 107(32):E126–E127. <http://dx.doi.org/10.1073/pnas.1008272107.E125>; author reply.
- Yao J, Liu B, Qin F. Modular thermal sensors in temperature-gated transient receptor potential (TRP) channels. *Proceedings of the National Academy of Sciences USA*. 2011; 108(27):11109–11114. <http://dx.doi.org/10.1073/pnas.1105196108>.
- Zhang X, Jin L, Fang Q, Hui WH, Zhou ZH. 3.3 Å cryo-EM structure of a nonenveloped virus reveals a priming mechanism for cell entry. *Cell*. 2010; 141(3):472–482. <http://dx.doi.org/10.1016/j.cell.2010.03.041>. [PubMed: 20398923]
- Zholos A, Johnson C, Burdya T, Melanaphy D. TRPM channels in the vasculature. *Advances in Experimental Medicine and Biology*. 2011; 704:707–729. http://dx.doi.org/10.1007/978-94-007-0265-3_37. [PubMed: 21290323]
- Zimmermann K, Leffler A, Babes A, Cendan CM, Carr RW, Kobayashi J, et al. Sensory neuron sodium channel Nav1.8 is essential for pain at low temperatures. *Nature*. 2007; 447(7146):855–858. <http://dx.doi.org/10.1038/nature05880>. [PubMed: 17568746]
- Zurborg S, Yurgionas B, Jira JA, Caspani O, Heppenstall PA. Direct activation of the ion channel TRPA1 by Ca²⁺ *Nature Neuroscience*. 2007; 10(3):277–279. <http://dx.doi.org/10.1038/nn1843>.

Figure 7.1.

Thermosensitive ion channels in sensory neurons. Sensory neurons innervate the skin and contain thermosensitive nonselective cation channels in their terminals that sense a wide range of temperatures. Activation of these channels depolarizes the sensory neuron, leading to propagation of action potentials that are relayed to the spinal cord and eventually reach the brain. *Illustration by Kelly Paralis, Penumbra Design. (See the color plate.)*

Figure 7.2.

Schematic of the domain organization for thermo transient receptor potential (TRP) channels. Representation of the domain organization for TRPV1–4, TRPA1, and TRPM8. For clarity, dimers are shown. A monomer for each channel contains 6 transmembrane segments and large N- and C-termini in the cytoplasm. TRPV1–4 have 6 N-terminal ankyrin repeats and a conserved C-terminal TRP box sequence. TRPA1 contains 14–19 N-terminal ankyrin repeats. TRPM8 has an N-terminal conserved region and C-terminal TRP box and coiled-coil domains. *Illustration by Kelly Paralís, Penumbra Design. (See the color plate.)*

Figure 7.3.

Structural analyses of thermoTRPV channels. (A) Domain organization of thermoTRPV channels. (B) Comparison of the crystal structures for the ankyrin repeat domains of TRPV1 (PDB code: 2PNN), TRPV2 (PDB code: 2ETB), and TRPV4 (PDB code: 4DX2) (Phelps, Wang, Choo, & Gaudet, 2010). (C) Crystal structure of a TRPV1 C-terminal peptide (red) in complex with calmodulin (gray) (PDB code: 3SUI) (Lau, Procko, & Gaudet, 2012). (D) Comparison of the cryoelectron microscopy structures for full-length TRPV1 at 19 Å resolution (Moiseenkova-Bell, Stanciu, Serysheva, Tobe, & Wensel, 2008) and TRPV4 at 35 Å resolution (Shigematsu, Sokabe, Danev, Tominaga, & Nagayama, 2010). Scale bar represents 25 Å. (See the color plate.)

Figure 7.4. Comparison of cryoelectron microscopy (EM) structures of full-length TRPV2 and minimal TRPV1. (A) 2D class averages of TRPV2 at 13.6 Å (Huynh et al., 2014) and minimal TRPV1 at 8.8 Å and 3.4 Å resolution (*Adopted and reprinted from Liao et al. (2013)*). (B) 3D reconstruction of TRPV2 at 13.6 Å resolution (Huynh et al., 2014) and minimal TRPV1 at 8.8 Å and 3.4 Å resolution (*Adopted and reprinted from Liao et al. (2013)*). The TRPV2 EM map is fitted with crystal structures of the MlotiK1 transmembrane (TM) domain (PDB code: 3BEH) and the TRPV2 ankyrin repeat domain (PDB code: 2ETB). Dashed lines correspond to cross-sections represented in (C) and (D). (C) Cross-sectional view through the TM domains of the TRPV2 (Huynh et al., 2014) and minimal TRPV1 EM maps (*Adopted and reprinted from Liao et al. (2013)*). The unfilled densities correspond to the maltoseneopentyl glycol (MNG) detergent belt for TRPV2 and the A8-35 amphipol belt for minimal TRPV1. (D) Cross-sectional view through the cytoplasmic density of the TRPV2 (Huynh et al., 2014) and minimal TRPV1 EM maps. Liao et al. (2013). (See the color plate.)

Figure 7.5.

Structural analysis of the TRPV1 pore region. (A) Architecture of the TRPV1 pore as determined by cryoelectron microscopy at 3.4 Å (Liao et al., 2013). Residues important for forming the dual gate are indicated by arrows. (B) Predicted pore architecture based on homology modeling and electrophysiological studies using a cysteine accessibility strategy (*Adopted and reprinted from* Salazar et al. (2009).). A gate for passage of small cations is predicted to begin from the intracellular side at Tyr671. A second gate for larger molecules is predicted to begin from the intracellular side at Leu681 (both residues indicated by arrows). (See the color plate.)

Figure 7.6.

Comparison of the ankyrin repeat domain (ARD) orientation in the TRPV2 and minimal TRPV1 electron microscopy (EM) maps. (A) Model of the TRPV1 ARDs as they are arranged in the minimal TRPV1 cryo-EM map and aligned with the crystal structure of the Gankyrin-S6 ATPase C-terminus complex (PDB code: 2DVW). The TRPV1 ARDs are shown in complex with the S6-ATPase protein. (B) Crystal structures of the TRPV2 ARDs (PDB code: 2ETB) as fitted into the TRPV2 cryo-EM map and aligned with the Gankyrin-S6 ATPase C-terminus complex (PDB code: 2DVW). The TRPV2 ARDs are shown in complex with the S6-ATPase protein. (C) Crystal structure of GIRK2 in complex with Gβγ (PDB code: 4KFM). (See the color plate.)

Figure 7.7.

Topology model of TRPA1 and TRPA1 electron microscopy (EM) structure at 16 Å resolution. (A) Domain organization of TRPA1. (B) Two side views of the TRPA1 EM structure (Cvetkov et al., 2011). The transmembrane (TM) domain was fitted with a molecular model of the TRPV1 TM domain (purple) (Fernandez-Ballester & Ferrer-Montiel, 2008). A model of the TRPA1 N-terminus was fitted within the cytoplasmic domain of the density map (blue). The cysteine residues critical for electrophilic ligand activation are represented as yellow spheres. A model of the TRPA1 C-terminus was fitted into the 3D density map immediately below the predicted TM domain within the region linking the TM domain to the cytoplasmic domain (light purple). (See the color plate.)

Figure 7.8.

Topology model of TRPM channels and crystal structure of TRPM7 coiled-coil domain. (A) Domain organization of TRPM channels. (B) Crystal structure of the TRPM7 coiled-coil domain (PDB code: 3E7K) incorporated into a cartoon model of the TRPM7 transmembrane (TM) domain, showing the antiparallel coiled-coil oriented perpendicular to the TM6 segment. Fujiwara & Minor (2008).

Table 7.1

Biophysical and physiological properties of TRPV1–4 channels

TRPV member	Sequence identity with TRPV1	Temperature threshold (°C)	Q ₁₀	KO mouse phenotype	References
TRPV1	—	>43	40	Impaired temperature sensation	Caterina et al. (1997, 2000)
TRPV2	50%	>52	100	No major deficits in thermosensation	Caterina et al. (1999), Park et al. (2011)
TRPV3	43%	>30	22	No major deficits in thermosensation	Huang et al. (2011), Smith et al. (2002), Xu et al. (2002)
TRPV4	40%	>25	20	No major deficits in thermosensation	Huang et al. (2011), Strotmann et al. (2000)