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Sensory TRP Channels in Three Dimensions

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Abstract

Transient receptor potential (TRP) ion channels are sophisticated signaling machines that detect a wide variety of environmental and physiological signals. Every cell in the body expresses one or more members of the extended TRP channel family, which consists of over 30 subtypes, each likely possessing distinct pharmacological, biophysical, and/or structural attributes. While the function of some TRP subtypes remains enigmatic, those involved in sensory signaling are perhaps best characterized and have served as models for understanding how these excitatory ion channels serve as polymodal signal integrators. With the recent resolution revolution in cryo–electron microscopy, these and other TRP channel subtypes are now yielding their secrets to detailed atomic analysis, which is beginning to reveal structural underpinnings of stimulus detection and gating, ion permeation, and allosteric mechanisms governing signal integration. These insights are providing a framework for designing and evaluating modality-specific pharmacological agents for treating sensory and other TRP channel–associated disorders.

Contents

1. INTRODUCTION

Transient receptor potential (TRP) channels were first discovered, functionally characterized, and cloned from fruit flies, in which genetic and electrophysiological studies identified these nonselective cation channels as drivers of photoreceptor cell depolarization in response to light (1–6). TRP channels are now known to exist in all eukaryotes, where they constitute one of the largest and most diverse ion channel families, consisting of over 30 subtypes in mammals (7–12). As in flies, vertebrate TRPs play important roles in sensory transduction, as well as other physiological processes such as calcium uptake, neuronal growth cone guidance, and insulin secretion. Moreover, TRP channelopathies are associated with numerous human disorders (e.g., polycystic kidney disease, skeletal dysplasia, and familial episodic pain syndrome) (13–16), further highlighting biological roles and therapeutic opportunities for members of this extensive ion channel family. At the same time, physiological functions for many TRPs remain unknown, in part reflecting a dearth of pharmacological reagents (natural, endogenous, or synthetic) with which to interrogate these channels in vitro or in vivo.

TRP channels that contribute to our senses of touch and pain (somatosensation) are an exception because they are targeted by natural products from plants or animals that elicit pain for defensive purposes (7, 17). Consequently, these somatosensory channels have led the way in providing insights into functional and structural attributes of TRPs at large, and we have therefore centered this review around three such players: TRPV1, TRPM8, and TRPA1, which are receptors for the plant-derived sensorial agents capsaicin, menthol, and mustard oil, respectively (**Figure 1a**) (7, 18–22). From these channels, we have learned that TRPs are especially intriguing in their capacity to function as polymodal signal integrators, as perhaps best exemplified by the
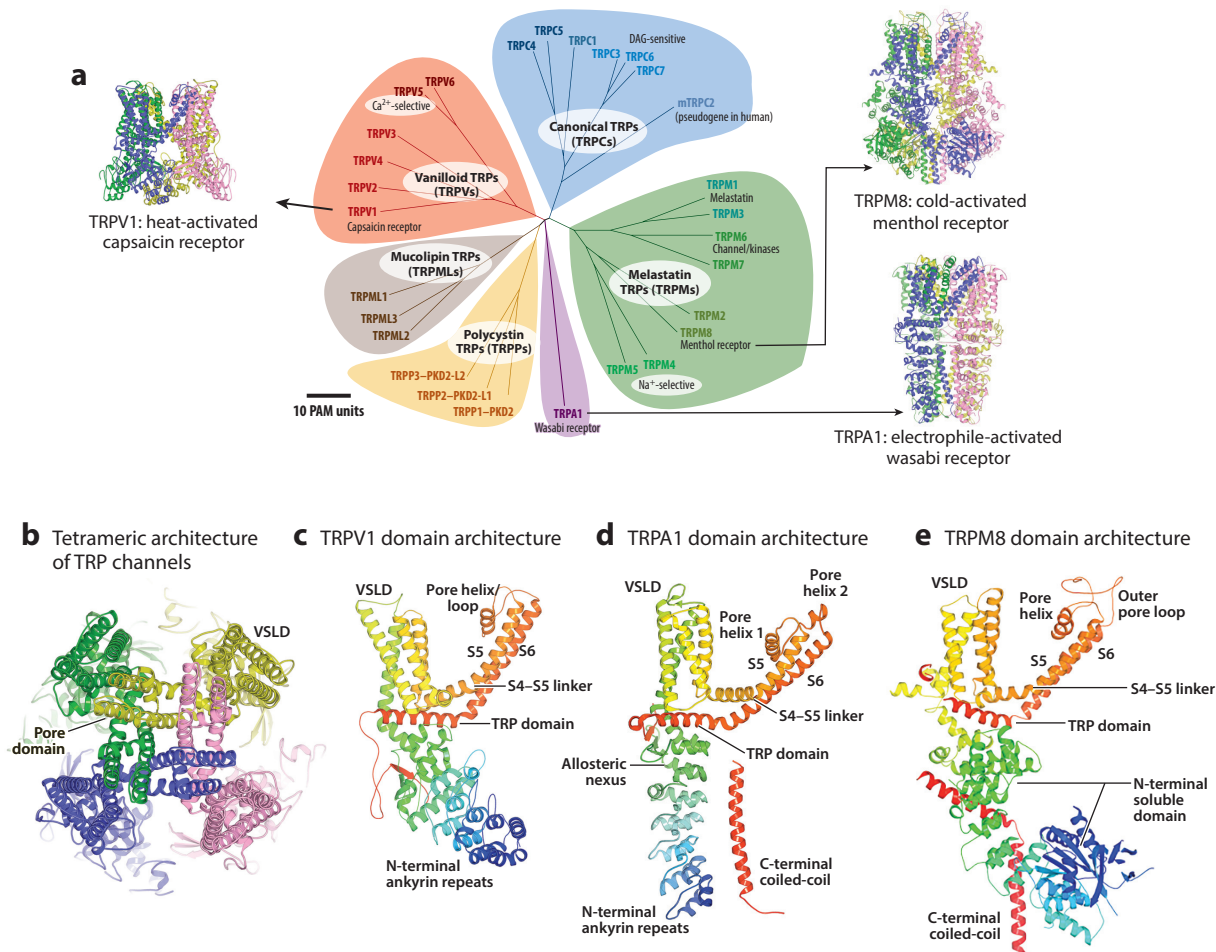
Figure 1

Structural overview of TRPV1, TRPA1, and TRPM8. (*a*) Structures of TRPV1 (PDB ID: 7L2P), TRPA1 (PDB ID: 6V9V), and TRPM8 (PDB ID: 6O77) are illustrated, together with the genetic tree of the TRP channel superfamily. (*b*) Top view of the transmembrane domain of TRPV1, illustrating the domain-swapped tetrameric architecture of TRP channels. Details of the domain architecture are illustrated for a single subunit of (*c*) TRPV1, (*d*) TRPA1, and (*e*) TRPM8. The tree diagram in panel *a* is adapted with permission from The International Union of Basic and Clinical Pharmacology. Panel *c* is adapted with permission from Reference 28. Panel *d* is adapted with permission from Reference 65. Panel *e* is adapted with permission from Reference 60. Abbreviations: DAG, diacylglycerol; PAM, point accepted mutation; PDB ID, Protein Data Bank identifier; S, transmembrane α-helix; TRP, transient receptor potential; VSLD, voltage-sensor-like domain.

capsaicin- and heat-activated receptor, TRPV1, whose ability to detect both physical and chemical stimuli underlies its role in inflammatory and persistent pain (7, 23, 24).

Indeed, an important goal of ongoing and future studies is to understand the biophysical and structural bases of such phenomena, which will provide critical insights into strategies for developing modality-specific drugs to treat pain and other TRP-associated disorders. There are now many excellent and comprehensive reviews covering specific and general aspects of TRP channel history, function, and pharmacology (7, 8, 25, 26). Our goal here is to focus on recent and

exciting advances in TRP channel structural biology that are now revealing detailed mechanisms of ligand binding and stimulus detection, ion permeation, gating, and allosteric regulation. For reasons noted in the introduction, we use somatosensory TRPs as the organizing focal point.

Overall, the transmembrane domain of TRPs resembles tetrameric voltage-gated ion channels (VGICs) in which the subunits are arranged in fourfold symmetry around a central ion permeation pathway (27, 28). Each monomer is composed of six transmembrane α-helices (S1–S6), of which the last two domains (S5–S6) and intervening pore loop form the ion selectivity filter and pore (**Figure 1***c–e*). The S1–S4 domain associates with the pore region of an adjacent subunit in a domain-swap organization characteristic of VGICs (**Figure 1***b*). In VGICs, this extant domain functions as a voltage sensor that undergoes structural movements in response to changes in the membrane electric field. Aside from this general architectural similarity, TRPs differ from other members of the VGIC superfamily in that they exhibit tremendous diversity within their cytoplasmic amino- (N-) and carboxy- (C-)terminal domains (**Figure 1***c–e*). The differences in these domains differentiate one TRP channel subtype from another, but knowledge of their physiological roles in ligand recognition, gating, or modulation by cellular factors remains rudimentary (7, 29, 30). Nevertheless, some elements are common to many TRPs, and mutagenesis and structural studies have begun to shed light on their contributions to subunit interaction and allosteric regulation. Such domains include N-terminal ankyrin repeats, C-terminal coiled coils, calcium-binding sites, and the eponymous C-terminal TRP helix. These and other recent insights are discussed in this review.

2. EXPLOITING NATURAL PRODUCTS TO DELINEATE CHANNEL FUNCTION AND STRUCTURE

Natural products from plants and animals are powerful, evolutionarily honed probes for identifying functionally and conformationally critical control points of proteins and protein complexes. In the context of sensory TRP channels, such agents have presumably evolved to activate nerve fibers that transduce painful signals as a defensive mechanism. Indeed, exploitation of these natural products, both small molecules and peptide toxins, has enabled identification and functional characterization of these biologically important ion channels and their connections to sensory physiology and psychophysics (7, 31, 32).

The capsaicin receptor, TRPV1, functions as a heat-activated channel that is also modulated by a range of chemical stimuli, most notably components of the inflammatory soup such as extracellular protons, bioactive lipids, neurotrophins, purines, and neuropeptides (7, 20, 33). TRPM8 is a cold-activated channel that is gated by temperatures below 26°C, as well as by natural and synthetic cooling agents such as menthol, eucalyptol, and icilin (18). TRPM8 channel activity is further modulated by calcium and bioactive lipids [e.g., phosphatidylinositol 4,5-bisphosphate (PIP$_2$)] (34–36). TRPA1 (also known as the wasabi receptor) functions as a low-threshold sensor for reactive electrophiles ranging from small, volatile environmental irritants, like acrolein or allicin, to endogenous reactive lipids, such as 4-hydroxy-2-nonenal and 15-deoxy-$\Delta^{12,14}$-prostaglandin J$_2$ (19, 22, 37, 38).

Sensory TRP channels are also modulated by competitive and noncompetitive antagonists, mainly of synthetic origin. Clinical interest in such compounds originates from the hope that they will be effective for treating chronic inflammatory pain syndromes, acute and chronic itch, or thermal hypersensitivity brought on by nerve injury or treatment with chemotherapeutic agents (39–43). Alongside natural product agonists, these agents have been powerful tools for defining structural mechanisms of activation, inhibition, and allosteric regulation.

The determination of cryo–electron microscopy (cryo-EM) structures of sensory TRP channels bound to these pharmacological probes has shown that these agents target each of the major regions of the channel, including extracellular, intramembrane, and intracellular domains, helping to reveal conformational transitions and functions associated with these sites, as well as interactions among them.

2.1. Extracellular Ligand-Binding Sites

To date, described extracellular ligand–channel interactions pertain largely to TRPV1, whose modulation by extracellular protons underlies its important role as a sensor of local tissue acidosis that accompanies inflammation and drives pain hypersensitivity (23). Thus, delineating the structural basis of proton sensitivity is directly relevant to understanding mechanisms of nociception and analgesia but may also be of general relevance to assessing the contribution of extracellular domains to TRP channel gating.

Protons activate TRPV1 and enhance its sensitivity to other stimuli through their interactions with two main titratable extracellular sites, namely Glu600 and Glu648 (in the rat channel) (44). Protonation of Glu600, which resides in an extracellular loop that undergoes substantial movement upon gating, disrupts its hydrogen bonding network, causing a widening of the selectivity filter (45, 46). These changes alter the local environment around the other critical protonation site, Glu648, likely driving its subsequent protonation, leading to a conformational change to the open state (46).

Given the important role of extracellular domains in TRPV1 physiology, it is perhaps not surprising that this region has been targeted by natural products, in particular peptide toxins that account for the painful actions of spider venom (**Figure 2c,d,e**) (47). Like many arachnid toxins, these so-called vanillotoxins adopt an inhibitor cysteine knot (ICK) structure in which multiple disulfide bonds scaffold the peptide into a rigid, protease-resistant core, thereby facilitating interactions with relevant receptor targets. ICK toxins have been exploited to study a variety of different ion channels. For example, K_v gating modifier toxins such as hanatoxin restrict gating movements through interactions with the voltage-sensing domain (VSD), or more precisely the S3–S4 linker (48, 49). In contrast, the pore-forming domain of TRPV1 specifies sensitivity to vanillotoxins, reflecting the importance of this domain to TRPV1 gating (50–52).

Among the vanillotoxins described to date, one from the Chinese bird spider (*Haplopelma schmidti*) is unique and has been most useful in probing channel structure. This potent TRPV1-activating toxin, termed double-knot toxin (DkTx), consists of two tandemly repeated ICK motifs joined by a short linker (7 amino acids), a bivalent arrangement that confers high avidity to the toxin–channel interaction, resulting in nearly irreversible channel activation (50). Open structures of TRPV1 bound to DkTx indicate that two bivalent DkTx molecules bind to one TRPV1 tetramer in an antiparallel arrangement such that the toxin exerts its action by binding at the top of the pore helix of one subunit and the outer pore loop proximal to S6 from the neighboring subunit, effectively locking the channel in an activated state (**Figure 2e**) (45, 46, 53, 54). In this position, DkTx inserts approximately 9 Å into the bilayer, where it engages in protein–protein interactions with the channel, as well as with membrane phospholipids, thereby forming a tripartite complex that presumably defines the energetics and specificity of toxin–channel engagement (53). Consistent with its role as a gating modifier, DkTx binds preferentially to and stabilizes TRPV1 in its open state by inserting a finger-like structure into the outer pore that prevents the pore loop and helix from returning to the closed-state position. While toxins targeting the extracellular domains of other TRP channels have not yet been characterized in detail, the hunt for such
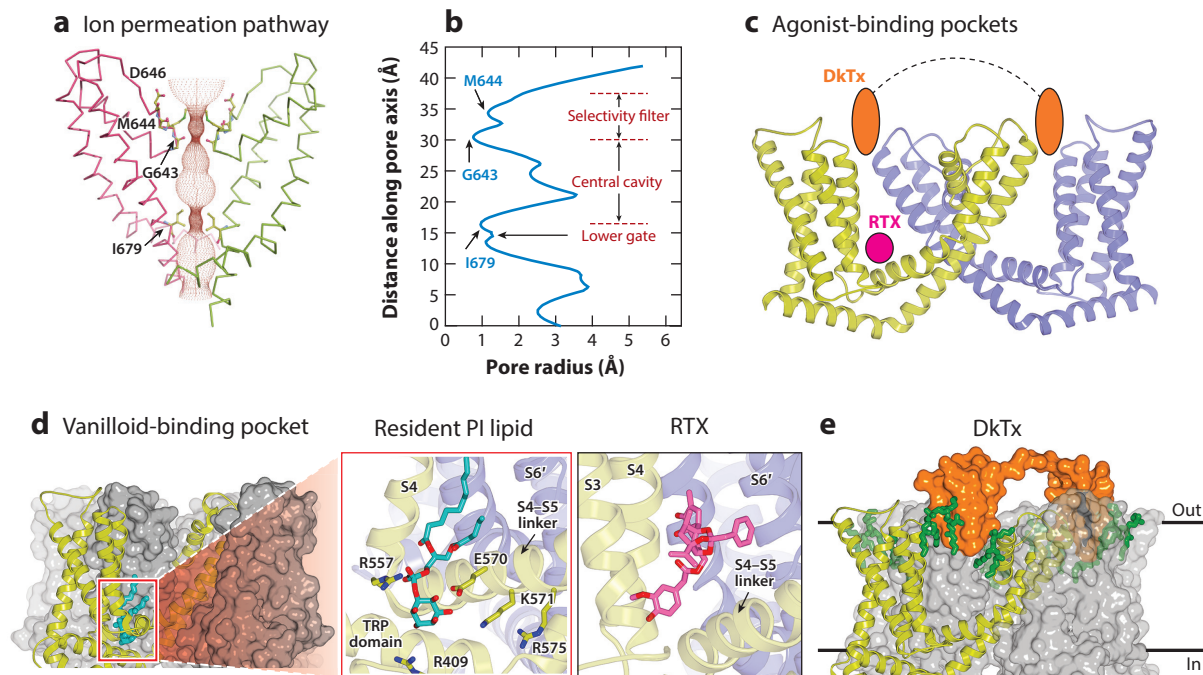
a Ion permeation pathway

b

c Agonist-binding pockets

d Vanilloid-binding pocket   Resident PI lipid   RTX

e DkTx

Figure 2

Structural mechanisms of the heat and capsaicin receptor TRPV1. (*a*) The solvent-accessible pathway and (*b*) pore radius along the ion permeation pathway calculated from the apo structure (PDB ID: 3J5P) show two major restriction sites: the selectivity filter formed by G643 and M644 and the lower gate formed by I679 (rat TRPV1 numbering). (*c*) Agonist-binding pockets are illustrated, including the extracellular ligand-binding site for DkTx and the membrane-embedded vanilloid-binding site where RTX binds (PDB ID: 5IRX). Binding of DkTx pushes the VSLD backward and couples to the opening of the lower gate. Binding of RTX pulls S5/S6 away from the pore to open the lower gate. (*d*) In the absence of ligand, a PI lipid resides in the vanilloid-binding pocket (PDB ID: 5IRZ). Binding of RTX to the vanilloid pocket displaces the resident PI (PDB ID: 5IRX). (*e*) DkTx binding to the extracellular surface of the channel (PDB ID: 5IRX). Panels *a* and *b* are adapted with permission from Reference 28. Panels *d* and *e* are adapted with permission from Reference 53. Abbreviations: DkTx, double-knot toxin; PDB ID, Protein Data Bank identifier; PI, phosphatidylinositol; RTX, resiniferatoxin; S, transmembrane α-helix; TRP, transient receptor potential; VSLD, voltage-sensor-like domain.

reagents is well worthwhile, as they could facilitate both physiological and structural studies for other members of this extended family.

2.2. Intramembrane Binding Sites for Ligands and Regulatory Lipids

Whereas extracellular ligand-binding sites are thus far unique to TRPV1, cryo-EM studies have elucidated the location of agonist and antagonist sites within the transmembrane core of numerous TRP channels. While the relative location of these sites differs among channel subtypes, they tend to accommodate a wide range of endogenous and exogenous agents, including bioactive lipids and small molecule ligands (both natural and synthetic).

Classic natural product agonists [capsaicin and the ultrapotent vanilloid resiniferatoxin (RTX)], synthetic antagonists (capsazepine), and regulatory phosphoinositide lipids bind to the vanilloid pocket in TRPV1, which is located in a crevice formed by the S4–S5 linker (**Figure 2c,d**) (45, 46, 53). Accommodation of these structurally and functionally diverse ligands is facilitated by the cavernous space and rearrangement of key amino acid residues. For example, Tyr511 (in

the rat channel) assumes distinct rotamers in apo versus liganded TRPV1 structures, reminiscent of an induced-fit model for ligand or substrate binding (45). Intriguingly, the vanilloid pocket is not empty in the apo state; rather, a phosphoinositide lipid [likely phosphatidylinositol (PI)] is present when the channel is closed (53). Structural studies suggest a mechanism of vanilloid action whereby RTX or capsaicin displaces the resident PI lipid while facilitating movement of the S4–S5 linker away from the central axis to open the ion gate. While this precise mechanism may pertain specifically to vanilloid modulation of TRPV1, the presence of an endogenous lipid at this key regulatory site has now been observed for many TRP subtypes (55–57). Notably, TRPA1 possesses a pocket analogous to the vanilloid pocket in TRPV1; this pocket is seen to bind a synthetic activator, GNE551, as well as phospholipids (58, 59). As the GNE551-bound TRPA1 structure was captured in a nonconducting state, its mechanism of action is yet to be elucidated. The exact identity of the resident lipid (phosphatidylcholine, PI, PIP_2, etc.) has not been determined for many channels nor have their structural or functional relevance been elucidated.

TRPM8 also binds a bevy of regulatory compounds, including natural and synthetic cooling agents, antagonists, and regulatory lipids. The synthetic antagonists AMTB and TC-I 2014 and the synthetic supercooling agonist icilin also bind within the transmembrane core but at a location distinct from the vanilloid pocket in TRPV1. Instead, these compounds occupy a membrane-embedded cleft formed by the lower half of the S1–S4 domain [i.e., the voltage-sensor-like domain (VSLD)] near the membrane–cytosol interface and just above the TRP domain (**Figure 3a–c**) (60, 61). Although both inhibitory and activating drugs bind to this same flexible pocket, they dock in unique positions, likely locking the channel in a closed or open configuration, respectively. Indeed, shape complementarity is the major determinant of ligand recognition, which in turn is dictated by the absence or presence of the S4–S5 linker and the relative position of the TRP helix, which is markedly different in the closed versus desensitized states (**Figure 3a,d**) (60). Reminiscent of TRPV1, a discrete density can be observed in this pocket when TRPM8 is in its apo state, although the identity of the molecule accounting for this density remains unknown (60). Phosphoinositide lipids also regulate TRPM8 function (as discussed in Section 4.2); however, their location with respect to this malleable ligand-binding site also remains indeterminate.

2.3. Intracellular Ligand-Binding Sites

Perhaps the best understood example of intracellular ligand action relates to the rather unusual mechanism of TRPA1 activation. TRPA1 serves as a detector for a broad range of environmental and endogenous electrophilic irritants of astoundingly diverse structure and size that activate the channel through covalent modification of cytoplasmic cysteine residues (62, 63). Two key cysteines (Cys621 and Cys665 in the human channel) are located within the so-called allosteric nexus, an intricately folded region situated just below the transmembrane domain of the channel (**Figure 4f**) (59, 64–67). Current structural and functional data support a model in which stepwise electrophilic addition to Cys621 then Cys665 promotes rearrangement of a nearby loop, thereby positioning a critical lysine within proximity of the C terminus of the TRP helix, enhancing its dipole moment (65). Enhanced positive electrostatic surface potential of the TRP helix at its N terminus is proposed to promote repulsion between TRP domains of neighboring subunits, thereby driving expansion of the ion gate (**Figure 4f**). This observation provides direct structural evidence for the long-standing notion that the TRP helix is a key element contributing to integration of signals that regulate gating. The two-step mechanism proposed for electrophile-mediated activation may enable TRPA1 to effectively balance sensitivity and fidelity in service of detecting small volatile electrophilic irritants in our environment.
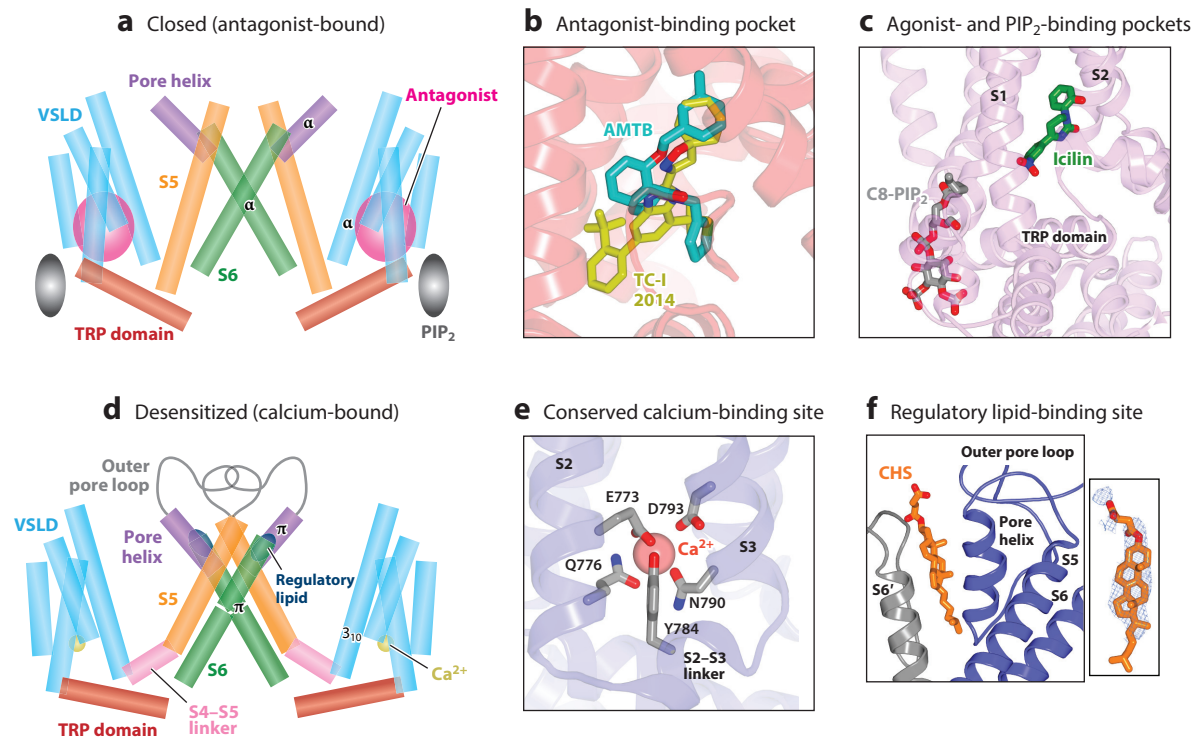
a Closed (antagonist-bound)

b Antagonist-binding pocket

c Agonist- and PIP$_2$-binding pockets

d Desensitized (calcium-bound)

e Conserved calcium-binding site

f Regulatory lipid-binding site

Figure 3

Structural mechanisms of the cold and menthol receptor TRPM8. Structural rearrangements associated with transitioning from the
(*a*) closed to (*d*) desensitized states include a rigid-body tilt of the VSLD; formation of a canonical S4–S5 linker; shifts of S5, the pore
helix, and S6; stabilization of the outer pore loop; and tilting of the TRP domain such that it is parallel to the membrane bilayer. This
transition is accompanied by introduction of a 3_{10}-helix in S4 and a π-helix in both the pore helix and S6. Ligand-binding sites are
indicated. (*b*) The antagonists AMTB and TC-I 2014 adopt distinct poses in the VSLD ligand-binding pocket (PDB IDs: 6O6R and
6O72). (*c*) The agonist icilin also adopts a distinct pose in the VSLD ligand-binding pocket. PIP$_2$ binds nearby in an interfacial cavity
formed by the pre-S1 domain, the S4–S5 linker, the TRP domain, and the cytosolic domain from an adjacent subunit (PDB ID: 6NR3).
(*e*) Interactions with calcium (PDB ID: 6O77). (*f*) In the desensitized state, a stabilizing lipid packs between the pore helix and S6 of
the neighboring subunit (modeled as CHS) (PDB ID: 6O77). The inset shows lipid density (*blue mesh*, 4σ contour). Panels *a*, *b*, *d*, *e*, and
f are adapted with permission from Reference 60. Abbreviations: CHS, cholesteryl hemisuccinate; PDB ID, Protein Data Bank
identifier; PIP$_2$, phosphatidylinositol 4,5-bisphosphate; S, transmembrane α-helix; TRP, transient receptor potential.

The functional importance of the allosteric nexus is further highlighted by a striking example
of convergent evolution in which a scorpion toxin also targets this domain to activate TRPA1. This
so-called wasabi receptor toxin (WaTx) is a disulfide-stabilized helical hairpin that acts as a cell-
penetrating peptide, enabling it to access the allosteric nexus and stabilize the channel's open state
(68). While the allosteric nexus specifies WaTx sensitivity, the precise mechanism of toxin binding
and channel activation remains unknown and awaits structural analysis of a toxin-channel complex.

2.4. Mysterious Intracellular Domains

The allosteric nexus of TRPA1 constitutes a cytoplasmic element for which a now well-defined
function has been elucidated. Yet this region represents but a tiny fraction of the intracellular
mass of the channel. Indeed, this is true for many TRP channels, in which N and C termini
form large intracellular domains that differ extensively between subfamilies and constitute half
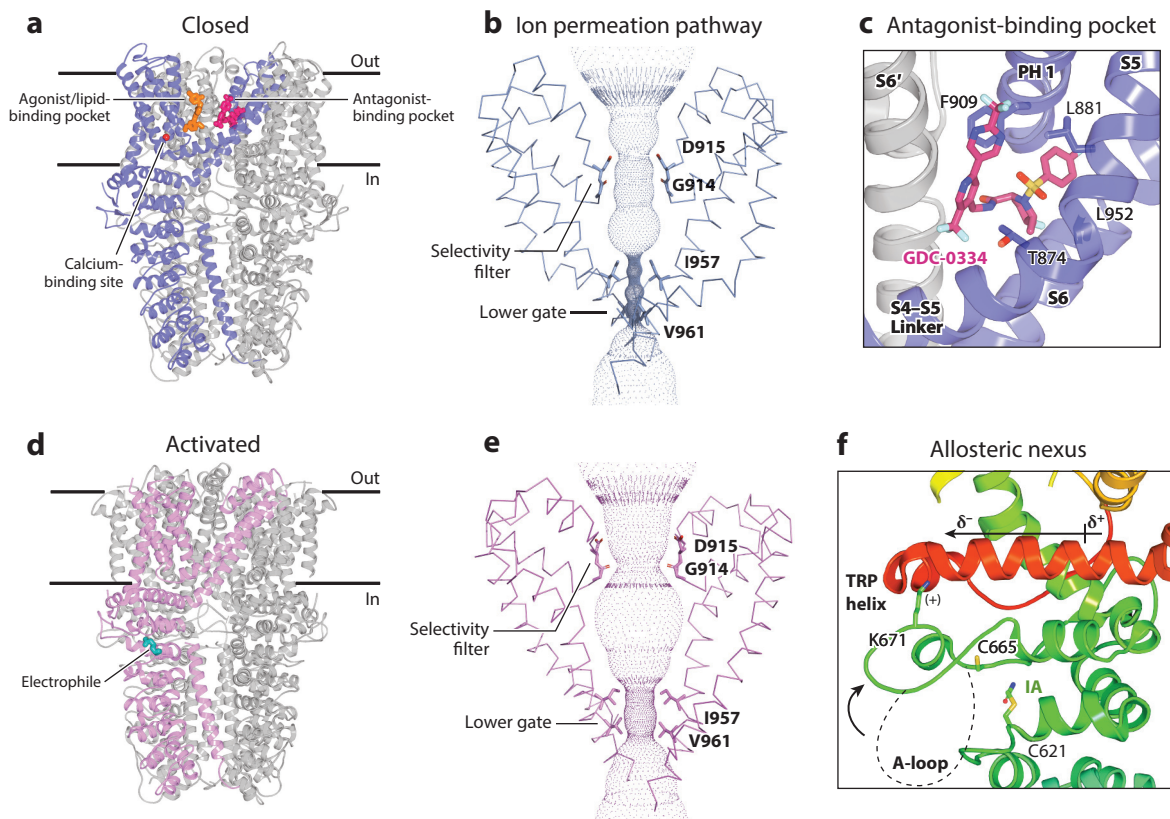
Figure 4

Structural mechanisms of the wasabi/irritant receptor TRPA1. (*a*) Closed structure of TRPA1 (PDB ID: 6V9V) with the conserved calcium-binding site within the VSLD shown. Overlaid are synthetic agonist GNE551 (PDB ID: 6X2J) (58, 59) and synthetic antagonist GDC-0334 (PDB ID: 6WJ5) (88) at their intramembrane binding sites. (*b*) Pore architecture of TRPA1 in a closed (nonconducting) conformation. The ion gate is formed by two hydrophobic residues (Ile957 and Val961), while the selectivity filter is formed by the backbone carbonyl of Gly914 and the side chain carbonyl of Asp915 (human TRPA1 numbering). (*c*) Intramembrane binding site for synthetic antagonists A-967079 (not shown) and GDC-0334 (PDB ID: 6WJ5) (64, 88). This site is comprised largely of hydrophobic residues from S5 helices from adjacent subunits, S6, and the first pore helix. Select residues involved in the binding of both compounds are shown. Binding of antagonists to this site appears to block straightening of the S5 helix and upward translation of S6 during channel opening. (*d*) Activated structure of TRPA1 with irreversible electrophile iodoacetamide attached to Cys621 (PDB ID: 6V9X). (*e*) Pore architecture of TRPA1 in an activated state. In an activated state, the ion gate expands, and the selectivity filter undergoes upward rotation and translation. The latter enhances exposure of Asp915 to the cytoplasm, allowing for enhanced calcium permeability. (*f*) Model for activation of TRPA1 by electrophiles. Electrophilic addition to Cys621 triggers stabilization of the Activation (A-) loop in an up conformation, which repositions Lys671 to the C terminus of the TRP helix, enhancing its N-terminal dipole moment. This promotes repulsion between the symmetrically opposed TRP helices and, ultimately, opening of the ion gate. In the up conformation, Cys665 within the A-loop rotates into the ligand-binding pocket, becoming available for electrophilic modification, which supports stabilization of the A-loop and full channel activation. Panels *b* and *e* are adapted with permission from Reference 65. Abbreviations: cryo-EM, cryo–electron microscopy; PDB ID, Protein Data Bank identifier; S, transmembrane α-helix; TRP, transient receptor potential; VSLD, voltage-sensor-like domain.

or more of the mass of any given channel (29, 30). These intracellular regions often contain a variety of canonical structural motifs, such as ankyrin repeats, Nudix and PDZ domains, kinases, and coiled coils (**Figure 1*c–e***), suggesting that they bind endogenous cellular factors or protein partners that contribute to unique aspects of function, regulation, or pharmacology (8). Some of

the main challenges in this field are to better understand what these and other structured motifs are doing and whether and how they foster interaction of TRP channels with cytoplasmic partners. Indeed, aside from structures of calmodulin (CaM) bound to TRPV5 or TRPV6 (discussed in Section 4.1) and polycystin complexes involving polycystic kidney disease subtypes (69–72), structures of TRPs in complex with other membrane or cytoplasmic partners have not been reported.

2.5. Intracellular Domains Contributing to Subunit Interaction

N-terminal ankyrin repeat domains (ARDs) and C-terminal coiled coil domains are two motifs that have been shown to play critical roles in the assembly and stabilization of TRP channel tetramers (**Figure 1**). For ARDs, the clearest illustration is seen in members of the TRPV subfamily, in which the first two of six repeats engage in interactions with a three-stranded β-sheet from the C terminus of a neighboring subunit (**Figure 1*a***) (28). Interestingly, mutations in this interaction zone of the human TRPV4 channel are associated with a multitude of neural and skeletal developmental disorders, possibly resulting from instability at the lower gate manifesting as a higher spontaneous channel open probability (73).

A well-defined functional role for a given intracellular motif in one TRP channel subtype does not necessarily illuminate its function in other family members, limiting the utility of generalization. For example, while ankyrin repeats engage in subunit–subunit interactions in TRPV channels, they are likely to serve distinct functions in TRPA1 (e.g., specifying chemical versus thermal sensitivity across species) and other channel subtypes (74, 75). TRPA1 contains an exceptionally large array of at least 16 ARDs that accounts for ~80% of the total channel mass and forms an extensive cytoplasmic skirt (64). The majority of TRPA1's ARDs are unresolved in present structures, which suggests that interactions with cofactors or other cytoplasmic ligands are required to scaffold these repeats into a stable configuration.

Coiled coil interactions facilitate assembly of the channel tetramer in several subtypes including TRPA1, TRPM, and TRPC family members (64, 76, 77). These structures show characteristic interlocking of subunits through the formation of four-stranded α-helical coiled coil structures along a fourfold axis. For TRPA1, the coiled coil is noncanonical and apparently requires stabilization through interactions with a polyanion, such as inositol hexakisphosphate (InsP$_6$) (64). This is consistent with physiological studies suggesting that inositol polyphosphates help stabilize channel function (78), but the biological significance of this interaction within the cell remains unknown; in particular, it is not known whether such interactions are only structural in nature or reflect a mechanism of regulation.

3. A REGULATED, DYNAMIC ION PERMEATION PATHWAY

Many TRP channels are nonselective for cations, allowing both mono- and divalent ions to transverse the permeation pathway, although some are highly selective for one or the other. It is also notable that some nonselective TRP channels, like TRPV1, TRPA1, and TRPM8, demonstrate a relatively high permeability for calcium ions (TRPV1: $P_{Ca2+}/P_{Na+}\sim10$, TRPA1: $P_{Ca2+}/P_{Na+}\sim8$, TRPM8: $P_{Ca2+}/P_{Na+}\sim3$), which may be relevant to their involvement in calcium-mediated proinflammatory neuropeptide release (18, 20, 68, 79). Some TRPs can also permeate large organic cations, such as NMDG, YO-PRO-1, or QX-134 and other lidocaine derivatives (80–84). The ion permeation pathway in TRP channels, starting from the extracellular side, consists of an outer pore region that forms the selectivity filter, a central cavity, and a cytoplasmic gate formed by hydrophobic side chains. Structures of TRPV1 and TRPA1 have shown that the cytoplasmic ion gate and selectivity filter constitute two highly dynamic constrictions along

their ion permeation pathway (**Figures 2*a*,*b*** and **4*b*,*e***), which are functionally coupled to regulate channel opening under different physiological conditions (45, 65).

3.1. Dynamic Selectivity Filter

The selectivity filter is composed of elements within the outer pore region of the channel, consisting of one or two short pore helices and an extracellular-facing loop connecting transmembrane helices S5 and S6 (28, 60, 64). In most TRP channels, the filter sits below a shallow, negatively charged bowl that attracts cations without a need for direct coordination. While this can account for cation selectivity, our understanding of how preference for mono- versus divalent cations is specified by structure is limited. In some channels, such as TRPM8, existing structures do not allow for an accurate description of how ions permeate the selectivity filter (60, 61, 76). In contrast, for TRPV6, which is highly selective for calcium, structures show a rigid scaffold of aspartate side chains that coordinate permeating ions, akin to that observed in voltage-gated potassium channels (85).

In between these examples are channels such as TRPV1 and TRPA1, in which multiple conformations of the selectivity filter clearly reveal transitions across a range of widths (45, 46, 53, 65). The dynamic nature of TRPV1's selectivity filter has been a topic of much analysis and discussion, particularly whether it constitutes a true open-and-close gate or otherwise controls ion selectivity or contributes to activation of the cytoplasmic gate (86). In any case, the outer pore domain of TRPV1 is a locus for modulation by pharmacological agents, including spider toxins and extracellular proteins (as discussed in detail in Section 2.1), attesting to the functional importance of this conformationally dynamic region in regulating channel gating. Transitions between apo and liganded states are defined by outward movements of the entire outer pore domain, including a restrictive glycine, as well as associated rotational movements of a hydrophobic methionine side chain, ultimately increasing the diameter of this region of the permeation pathway to allow entry of partially or fully hydrated cations or large organic cations (45, 46).

In TRPA1, upon electrophilic addition, the selectivity filter undergoes an upward rotation and translation, increasing its diameter and negative electrostatic surface potential by repositioning acidic residues that are critical for calcium selectivity (**Figure 4*b*,*e***) (65, 79). Structural and pharmacological evidence supports the notion that this transition underlies the dynamic ion selectivity of TRPA1, whereby the channel displays an enhanced preference for calcium upon activation by electrophilic agonists. Intriguingly, selectivity is dependent on what activates the channel ($P_{Ca2+}/P_{Na+} \sim 10$ for electrophiles versus ~ 3 for WaTx) (68). Taken together, the visualization of distinct selectivity filter states in TRPM8, TRPV1, TRPA1, and structures of other TRP channels suggests that the conformationally dynamic nature of this region is of general functional import and that determining mechanisms of communication between upper and lower restrictions is relevant to understanding the polymodality of numerous TRP subtypes.

Another notable feature within the outer pore region of TRP channels and other members of the VGIC superfamily is the pore helix or helices. In some TRPs, pore helices undergo substantial movement during gating. In TRPV1, mutations in this region dramatically increase basal channel activity and responsiveness to chemical and thermal stimuli (87), attesting to its functional importance. The pore helix forms a negative dipole at its carboxyl-terminal end, which may facilitate its movement during membrane depolarization, thereby enhancing its open probability at positive membrane potentials (45). It is also an important scaffolding region that may be critical for allosteric coupling between the selectivity filter and cytoplasmic gate. Interestingly, structures of TRPM8 and TRPA1 have shown that regulatory molecules, including drugs and lipids, wedge into this region to stabilize or limit motion (**Figure 3*f***) (60, 64). For example, the TRPA1 antagonists

A-967079 and GDC-0334 bind the channel at a distal membrane-embedded pocket formed by S5, S6, and the first pore helix (64, 65, 88). Presumably, these antagonists hinder channel activity by acting as molecular wedges, thereby impeding pore movements required for opening of the ion gate (**Figure 4c**).

Permeability of TRP channels to large organic cations has been proposed to occur through a controversial process known as pore dilation that is initiated by repetitive or prolonged channel activation (89, 90). Irrespective of whether this involves a temporal mechanism, recent high resolution TRPV1 structures show that dynamic changes in the selectivity filter do occur and accommodate permeation by large organic cations, thus supporting at least one tenet of the pore dilation hypothesis: that pore size is not limited to one fixed open state (46). Clearly an important future goal is to visualize channels in the act of permeating a range of different cation species to understand whether and how these solutes transverse the ion permeation pathway and stabilize the selectivity filter and/or cytoplasmic gate in one or more open states.

3.2. The Cytoplasmic Gate and Its Relationship to the S4–S5 Linker and TRP Domain

The cytoplasmic gate is formed by one or two hydrophobic residues, which represent the narrowest constriction along the ion permeation pathway and the key control point for opening and closing the channel (45, 60, 64). A relatively short constriction allows for diverse structural solutions to achieve channel gating, but the hydrophobic nature of the gate likely specifies permeation by either fully or partially hydrated ions. Reminiscent of VGICs, TRP channel gating involves movement of the S4–S5 linker, a short amphipathic helix that runs almost parallel to the inner membrane and connects the S1–S4 region to the pore domain (S5–S6). The TRP domain, a conserved α-helical motif of ~25 residues that also lies parallel to the inner membrane leaflet just below the S4–S5 linker and cytoplasmic gate, has been implicated in subunit assembly, interaction with membrane phospholipids or scaffolding proteins, and/or regulation of the channel gate (7, 8, 25). For most channel subtypes, the TRP domain is readily recognizable from its primary sequence, whereas for others, such as TRPA1, the existence of this structurally conserved motif became apparent only once their 3D structures were elucidated (64). While mechanisms impinging on the TRP helix are surprisingly distinct for different channel subtypes, the main conclusion from mutagenesis and more recent structural studies is that this domain modulates gating. This is further supported by the discovery of a novel TRPA1 antagonist that lodges into a crevice formed at the interface of S4, the S4–S5 linker, and the TRP helix, where it presumably inhibits conformational transitions in this region that accompany gating (91).

Broadly speaking, one can divide TRP channels into two classes: those exemplified by TRPV channels, in which rather subtle movements lead to gating, and those exemplified by TRPA1 and TRPM channels, in which large, concerted movements throughout the protein occur during the gating cycle. For TRPV1, in which ligands bind adjacent to the cytoplasmic gate, observed movements of the TRP helix are relatively subtle; it resides more-or-less parallel to the inner leaflet when the channel is either open or closed (45, 46). Rigid-body outward rotation of the S1–S4 domain and/or local movement of the S4–S5 linker and S6 are accompanied by lateral displacement of the TRP domain and sequential channel opening. In contrast, for TRPM8, in which ligands bind to the S1–S4 domain, substantial concerted movements in the transmembrane domain result in a more significant movement of the TRP domain, namely tilting that reorients it from an angled position in the closed state to a position parallel to the membrane bilayer in its desensitized state (**Figure 3a,d**) (60). Ultimately, this motion alters the status of the lower gate. In TRPA1, the role of the TRP helix is especially interesting and perhaps mechanistically best understood, owing

to its role in coupling ligand binding at the intracellular allosteric nexus to the overlying gate, as described in Section 2.3 (65).

3.3. Role of Helical Transitions in Gating

Recent structural studies have revealed that helical transitions within the transmembrane domain, including S4, S6, and pore helices, are integral to TRP gating mechanisms. One prominent example, which pertains to numerous TRP channel subgroups, concerns a π-helix in the middle of S6, which introduces a bulge that shifts the register of the helix downstream by one residue (92). In many cases, alternative conformations containing an α-helix in this position are also seen in the same channel, sometimes under distinct pharmacological conditions. Transition between the two helical forms has direct effects on the configuration of the cytoplasmic gate by changing the residue(s) that form the most restrictive part of the ion permeation pathway. To date, the meaning of this transition is unclear because the helical state does not strictly align with a particular functional state. Is the π-to-α transition reversible? Is it a postopening state that leads to a preactivated or desensitized state?

In TRPV1, S6 contains a π-helix when the ion conduction pathway is either closed or opened by simultaneous binding of two ligands, one that modulates the selectivity filter and one that modulates the cytoplasmic gate (e.g., DkTx plus RTX) (46). Intriguingly, a transition from the π- to an α-helix is observed when the channel is activated by only one ligand that stabilizes either the cytoplasmic gate or selectivity filter (e.g., DkTx or RTX). In TRPM8, S6 contains an α-helix when the ion conduction pathway is closed but a π-helix in the desensitized state (**Figure 3a,d**) (60). Thus, one cogent model is that transition between π- and α-helices is associated with desensitization or accounts for the characteristic (but enigmatic) flickery nature of single-channel openings observed for numerous TRP channels.

In TRPA1, π-to-α transitions have not been observed, but the π-helix in S6 shifts its position by ∼1 helical turn in the activated channel. Now located closer to the selectivity filter, the π-helix supports a pronounced bending of S6 that repositions the outer pore loop, including the pore helices and selectivity filter (65). As discussed in Section 3.1, these structural rearrangements may help tune cation selectivity.

TRPM8 structures also reveal that π-to-α transitions can occur in other locations, such as the pore helix, where the α configuration is associated with the closed state and π with the desensitized state (**Figure 3a,d**) (60). The functional significance of this transition to ion permeation and/or gating is not yet understood; however, given the importance of the pore helix, the transition is intriguing.

Other types of helical transitions also play a role in TRP channel gating. In comparing apo to agonist-bound or desensitized TRPM8 structures, an α-to-3_{10} helical transition is seen in S4 that is associated with a dramatic restructuring of two elements, the S4–S5 linker and the TRP domain, that are key to gating, as discussed further in Section 3.5 (**Figure 3a,d**) (60, 61).

3.4. Structural Insights into Temperature-Induced Gating

Mutagenesis studies have identified numerous residues and regions that contribute to the heat or cold sensitivity of thermosensitive TRP channels, including the outer pore region and cytoplasmic N or C termini (75, 93–95). Failure to collectively pinpoint one specific domain suggests that thermal gating reflects a distributed mechanism in which temperature alters channel structure globally, involving conformational transitions affecting numerous domains (96). As such, elucidating mechanisms of thermal gating will likely require structural methods that can visualize a trajectory of conformational substates, which may now be possible using cryo-EM.

A first step in this direction has recently been reported for TRPV1 and TRPV3, in which structures of these channels are described at elevated temperatures (97, 98). Consistent with the notion of a distributed activation mechanism, these studies suggest that heat-evoked channel activation promotes movements throughout the channel, including within the cytoplasmic domains, which generally remain static following activation by chemical ligands. Moreover, analysis of TRPV3 shows that the resident lipid in the vanilloid pocket-like region is absent in the heated sample (97), consistent with the hypothesis that ejection of this regulatory lipid is a key step in channel activation by either chemical or thermal stimuli (53).

3.5. Repurposing the Voltage Sensor–Like Domain

VGICs and TRPs consist of two topologically distinct transmembrane domains; namely, the VSD or voltage sensor–like domain (VSLD) and the central ion permeation pathway (27, 28). For VGICs, structural rearrangement of the VSD in response to changes in membrane potential results in closing or opening of the ion gate. This response originates from the charge transfer center within the VSD, which facilitates movement of positively charged amino acids (an arginine or lysine at every third position in S4) across the membrane field (99).

Some TRP channel subtypes, including TRPV1, TRPM8, and TRPA1, exhibit a degree of voltage dependence characterized by outward rectification (100). However, they are only weakly voltage sensitive, and the critical basic residues in S4 that constitute the charge transfer center of VGICs are not present in TRPs. If this domain is not responsible for voltage sensing, then what is its physiological role? Structures of several sensory TRP channels, most notably TRPM8, illustrate that the S1–S4 domain has been repurposed to bind a variety of ligands of physiological import, as noted in Section 2.2, and that these binding events are coupled to channel gating (60, 61). Specifically, upon agonist binding, the VSLD undergoes a rigid-body tilt away from the central axis that is accompanied by transition from an α-helical turn to a 3_{10} helix in S4 (Thr830 to Arg832 in *Parus major*). This leads to an unusual rearrangement in which the distal regions of S4 and S5 take on the configuration of a canonical S4–S5 linker (**Figure 3a,d**). Though an open conformation of TRPM8 has yet to be captured, from the desensitized state, it is apparent that these structural rearrangements are accompanied by large shifts in S5, the pore helix, the outer pore domain, and S6, which alter the configuration of the lower gate (60). Unique features of the closed state, including absence of the S4–S5 linker and the relative position of the TRP domain, create an antagonist-selective binding pocket that, when occupied, locks the channel in a closed conformation, further attesting to the importance of conformational changes within the VSLD.

In the case of TRPV1, we see a different situation wherein the VSLD has a rigid structure supported by a hydrophobic core and is not noticeably different when comparing distinct channel states (45). Instead, the VSLD undergoes subtle rigid body tilting that transduces stimulus-evoked conformational changes in the outer pore to the cytoplasmic gate via the S4–S5 linker. For TRPA1, rigid body movement of the VSLD upon electrophile-evoked activation is somewhat more exuberant, although the mechanistic link to gating is currently less clear (65). While the specific structural movements for TRPs versus VGICs are quite different, the common connection to gating perhaps reflects their shared evolutionary roots and conserved overall architecture.

4. STRUCTURES REVEAL CONSERVED SITES FOR SECOND MESSENGERS

TRP channels in the fly eye function as receptor-operated channels that depolarize the photoreceptor cell following light activation of G protein–coupled rhodopsin (2–4, 6). Genetic and biophysical studies have shown that rhodopsin-dependent activation of phospholipase C (PLC) is

required to activate *Drosophila* TRP. Although the underlying mechanism remains unclear, both PIP_2 depletion and generation of PIP_2-derived second messengers are likely important for channel activation, attributes that have been retained by many members of the vertebrate TRP channel family (101). Indeed, some vertebrate TRPs likewise serve as receptor-operated channels that are activated or sensitized downstream of PLC-coupled metabotropic receptors. Recent structural studies provide new insights into how second messengers, namely calcium and lipids, interact with sensory TRP channels to mediate receptor operation or other aspects of cellular regulation.

4.1. Structural Mechanisms for Modulation by Cytoplasmic Calcium

Some TRP channels are modulated by calcium (via intracellular store release or plasma membrane entry) through CaM-dependent pathways. Indeed, TRPV5 and TRPV6 have been shown to form a tight complex with CaM, which binds to noncanonical binding sites within channel cytosolic domains, leading to calcium-dependent channel inactivation (70–72). In contrast, recent structural studies of TRPM8 and TRPA1 (as well as a subset of other TRPM and TRPC channels) have shown that calcium can also interact directly with TRP channels independently of CaM or other calcium sensors (61, 65, 102, 103). Taken together, these studies have identified a conserved calcium-binding site within the VSLD, where the calcium ion is coordinated by four negatively charged residues (glutamate, glutamine, asparagine, and either aspartate or glutamate) from the S2 and S3 helices, as well as a tyrosine from the S2–S3 linker (**Figures 3e** and **4a**).

TRPM8 shows pronounced calcium-dependent desensitization during continuous agonist application, and the synthetic supercooling agent icilin activates the channel in a calcium-dependent manner (35). Indeed, calcium binding to this single site in TRPM8 accounts for all of these calcium-mediated actions (60). TRPA1 functions as a receptor-operated channel downstream of PLC-coupled G protein–coupled receptors that detect inflammatory and pruritic (itch-inducing) signals, such as bradykinin and chloroquine (38). Electrophysiological studies have shown that elevated intracellular calcium first activates and/or potentiates TRPA1 currents, followed by desensitization (19, 79). Here, again, the conserved calcium binding site accounts for each of these actions (65). While identification of this key site represents an important step forward in elucidating the mechanisms of calcium modulation, we still do not understand the structural mechanisms whereby calcium binding affects gating. Interestingly, calcium-mediated desensitization of TRPV1 was described many years ago, but the mechanism remains elusive, suggesting that other partners or calcium sites remain to be identified in this and other TRP channels, as recently shown for TRPM5 (104).

4.2. Structural Mechanisms for Modulation by Lipids

Many TRP channels require PI lipids at some point in their gating cycle (105–107). The structural basis and physiological consequences of these important regulatory mechanisms remain controversial and incompletely understood, but there has been some recent progress toward visualizing PI lipid–binding sites in several TRP subtypes, which is now critical for bringing mechanistic clarity to these important questions.

Studies of TRPM8 provide insight into the class of channels for which PIP_2 binding contributes to channel opening. Zheng et al. were the first to suggest involvement of the pre-S1 domain in the binding of PIP_2 (108). This has now been corroborated by PIP_2-bound structures of TRPM8, which show that the inositol 1,4,5-trisphosphate head group of this lipid is positioned in an interfacial cavity where it forms direct interactions with basic amino acid residues contributed by the pre-S1 domain, the S4–S5 linker, the TRP domain, and the cytosolic domain from an adjacent subunit and has been proposed to act by stabilizing the 3_{10} configuration of S4

associated with the open state (**Figure 3c**) (61). Presumably, the lipid acyl chains extend upward into the transmembrane region, although these tails are not sufficiently resolved to enable their placement within the protein. In the absence of PIP_2, a mixture of lipids packs into the pre-S1 lipid-binding domain, whose size changes dramatically in different conformational states (60).

Studies of TRPV channels provide insight into the class of channels for which PI hydrolysis or displacement facilitates gating (**Figure 2d**) (109, 110). Physiological experiments showed that activation of PLC-coupled receptors enhances the sensitivity of TRPV1 to capsaicin or heat (111–114). Consistent with this, reconstitution of purified TRPV1 into proteoliposomes showed that inclusion of numerous PI lipid species diminishes channel sensitivity to chemical and thermal stimuli (115). However, other findings have suggested that PI lipids are required for TRPV1 activation (116). As described in Section 2.2, a binding site for phosphoinositides has been identified in TRPV1's vanilloid-binding pocket. Displacement of this lipid by vanilloid agonists, such as capsaicin or RTX, promotes conformational changes that lead to the opening of the cytoplasmic gate (46, 53). Some classic competitive antagonists, most notably capsazepine, also bind to this site, displacing the resident PI lipid while blocking the conformational changes that promote channel gating (53). These observations provide an explanation for how PI lipids may serve as both positive and negative regulators of TRPV1 function (117, 118): Occupancy of this site in the closed state may be required for stability or to prime the channel for activation, which is followed by lipid displacement during gating in response to chemical or thermal stimuli. Mutagenesis studies of TRPV1 suggest that additional sites may contribute to PLC/PI regulation, but this has not yet been confirmed by structural studies (111, 119).

An alternate PLC-derived second messenger, diacylglycerol (DAG), is also capable of directly activating several TRP channel subtypes, including TRPC3 and TRPC6 (101, 120). A recent structure revealed that in TRPC6, DAG binds within an extracellular membrane-facing cavity formed by S6 and the pore helix of an adjacent subunit (121). The analogous site in TRPM8 was occupied by a cholesteryl hemisuccinate molecule in the desensitized structure (the only structure in which the outer pore loop is visible), suggesting that occupancy of lipids at this site may modulate channel activity in sensory TRP channels as well (**Figure 3f**) (60).

5. CONCLUDING REMARKS

The last few years have seen incredible progress in the structural analysis of TRP channels, including not only sensory TRPs but also those of other physiological significance. These studies have revealed both common and unique elements of channel architecture that are providing fresh insight into what makes these channels different, and why. Still, further advances in pharmacological, biochemical, and structural tools are needed to fully bridge gaps between our physiological and biophysical understandings of channel function.

For example, a molecular understanding of gating mechanisms requires high-resolution structure determination, but to confidently assign functional states (e.g., open, closed, or inactivated/desensitized pore configurations) to structural snapshots, it is also essential to capture multiple conformational states, ideally encompassing a trajectory. This is still a challenge for many TRP channels, particularly those that lack adequate pharmacological reagents with which to stabilize discrete physiological states. In all cases, the ability to visualize transient states will improve with advances in cryo-EM technology that facilitate the acquisition and analysis of large data sets of heterogenous protein populations. Also critical will be the continued development of biochemical methods and reagents for solubilizing and stabilizing membrane proteins while maintaining or recapitulating interactions with key structural or regulatory lipids. The identification of lipids and other nonprotein components has greatly improved with increased resolution and computational

methods, but the development of other approaches facilitating unambiguous identification and modeling of such factors represents another important goal for the field.

The cytoplasmic domains of TRP channels are large and complex, and yet, we still know relatively little about whether or with whom these channels interact. Thus, another important future goal is to identify such interactions physiologically and biochemically and ultimately to use structural methods to define the nature of such interactions and their effects on stimulus detection, gating, subcellular localization, or turnover. Such complexes may also reveal additional druggable sites for modulating TRP function outside of those that directly target the channel per se.

DISCLOSURE STATEMENT

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LITERATURE CITED

1. Cosens DJ, Manning A. 1969. Abnormal electroretinogram from a *Drosophila* mutant. *Nature* 224:285–87

2. Hardie RC, Minke B. 1992. The *trp* gene is essential for a light-activated Ca^{2+} channel in Drosophila photoreceptors. *Neuron* 8:643–51

3. Montell C, Jones K, Hafen E, Rubin G. 1985. Rescue of the *Drosophila* phototransduction mutation *trp* by germline transformation. *Science* 230:1040–43

4. Montell C, Rubin GM. 1989. Molecular characterization of the Drosophila trp locus: a putative integral membrane protein required for phototransduction. *Neuron* 2:1313–23

5. Hardie RC. 2001. Phototransduction in *Drosophila melanogaster*. *J. Exp. Biol.* 204:3403–9

6. Smith DP, Stamnes MA, Zucker CS. 1991. Signal transduction in the visual system of *Drosophila*. *Annu. Rev. Cell Biol.* 7:161–90

7. Julius D. 2013. TRP channels and pain. *Annu. Rev. Cell Dev. Biol.* 29:355–84

8. Clapham DE. 2003. TRP channels as cellular sensors. *Nature* 426:517–24

9. Ramsey IS, Delling M, Clapham DE. 2006. An introduction to TRP channels. *Annu. Rev. Physiol.* 68:619–47

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

11. Venkatachalam K, Montell C. 2007. TRP Channels. *Annu. Rev. Biochem.* 76:387–417

12. Vay L, Gu C, McNaughton PA. 2012. The thermo-TRP ion channel family: properties and therapeutic implications. *Br. J. Pharmacol.* 165:787–801

13. Mochizuki T, Wu G, Hayashi T, Xenophontos SL, Veldhuisen B, et al. 1996. PKD2, a gene for polycystic kidney disease that encodes an integral membrane protein. *Science* 272:1339–42

14. Nilius B, Owsianik G, Voets T, Peters JA. 2007. Transient receptor potential cation channels in disease. *Physiol. Rev.* 87:165–217

15. Nilius B, Voets T. 2013. The puzzle of TRPV4 channelopathies. *EMBO Rep.* 14:152–63

16. Kremeyer B, Lopera F, Cox JJ, Momin A, Rugiero F, et al. 2010. A gain-of-function mutation in TRPA1 causes familial episodic pain syndrome. *Neuron* 66:671–80

17. Sexton JE, Vernon J, Wood JN. 2014. TRPs and pain. In *Mammalian Transient Receptor Potential (TRP) Cation Channels: Volume II*, ed. B Nilius, V Flockerzi, pp. 873–97. Cham, Switz.: Springer Int. Publ.

18. McKemy DD, Neuhausser WM, Julius D. 2002. Identification of a cold receptor reveals a general role for TRP channels in thermosensation. *Nature* 416:52–58

19. Jordt S-E, Bautista DM, Chuang H-H, McKemy DD, Zygmunt PM, et al. 2004. Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. *Nature* 427:260–65

20. Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D. 1997. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature* 389:816–24

21. Peier AM, Moqrich A, Hergarden AC, Reeve AJ, Andersson DA, et al. 2002. A TRP channel that senses cold stimuli and menthol. *Cell* 108:705–15

22. Bandell M, Story GM, Hwang SW, Viswanath V, Eid SR, et al. 2004. Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. *Neuron* 41:849–57

23. Tominaga M, Caterina MJ, Malmberg AB, Rosen TA, Gilbert H, et al. 1998. The cloned capsaicin receptor integrates multiple pain-producing stimuli. *Neuron* 21:531–43

24. Laing RJ, Dhaka A. 2016. ThermoTRPs and pain. *Neuroscientist* 22:171–87

25. Montell C. 2005. The TRP superfamily of cation channels. *Sci. STKE* 2005:re3

26. Zhao Y, McVeigh BM, Moiseenkova-Bell VY. 2021. Structural pharmacology of TRP channels. *J. Mol. Biol.* 433:166914

27. Long SB, Campbell EB, MacKinnon R. 2005. Crystal structure of a mammalian voltage-dependent *Shaker* family K$^+$ channel. *Science* 309:897–903

28. Liao M, Cao E, Julius D, Cheng Y. 2013. Structure of the TRPV1 ion channel determined by electron cryo-microscopy. *Nature* 504:107–12

29. Cao E. 2020. Structural mechanisms of transient receptor potential ion channels. *J. Gen. Physiol.* 152:e201811998

30. Madej MG, Ziegler CM. 2018. Dawning of a new era in TRP channel structural biology by cryo-electron microscopy. *Pflügers Arch.* 470:213–25

31. Herzig V, Cristofori-Armstrong B, Israel MR, Nixon SA, Vetter I, King GF. 2020. Animal toxins—Nature's evolutionary-refined toolkit for basic research and drug discovery. *Biochem. Pharmacol.* 181:114096

32. Vetter I, Lewis RJ. 2011. Natural product ligands of TRP channels. In *Transient Receptor Potential Channels*, ed. MS Islam, pp. 41–85. Dordrecht, Neth.: Springer

33. Geppetti P, Nassini R, Materazzi S, Benemei S. 2008. The concept of neurogenic inflammation. *Br. J. Urol. Int.* 101:2–6

34. Liu B, Qin F. 2005. Functional control of cold- and menthol-sensitive TRPM8 ion channels by phosphatidylinositol 4,5-bisphosphate. *J. Neurosci.* 25:1674–81

35. Chuang H-H, Neuhausser WM, Julius D. 2004. The super-cooling agent icilin reveals a mechanism of coincidence detection by a temperature-sensitive TRP channel. *Neuron* 43:859–69

36. Rohacs T, Lopes CMB, Michailidis I, Logothetis DE. 2005. PI(4,5)P$_2$ regulates the activation and desensitization of TRPM8 channels through the TRP domain. *Nat. Neurosci.* 8:626–34

37. Bautista DM, Jordt S-E, Nikai T, Tsuruda PR, Read AJ, et al. 2006. TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. *Cell* 124:1269–82

38. Bautista DM, Pellegrino M, Tsunozaki M. 2013. TRPA1: a gatekeeper for inflammation. *Annu. Rev. Physiol.* 75:181–200

39. Xing H, Chen M, Ling J, Tan W, Gu JG. 2007. TRPM8 mechanism of cold allodynia after chronic nerve injury. *J. Neurosci.* 27:13680–90

40. Descoeur J, Pereira V, Pizzoccaro A, Francois A, Ling B, et al. 2011. Oxaliplatin-induced cold hypersensitivity is due to remodelling of ion channel expression in nociceptors. *EMBO Mol. Med.* 3:266–78

41. Souza Monteiro de Araujo D, Nassini R, Geppetti P, Logu FD. 2020. TRPA1 as a therapeutic target for nociceptive pain. *Expert Opin. Ther. Targets* 24:997–1008

42. Gunthorpe MJ, Chizh BA. 2009. Clinical development of TRPV1 antagonists: targeting a pivotal point in the pain pathway. *Drug Discov. Today* 14:56–67

43. Moran MM, Szallasi A. 2018. Targeting nociceptive transient receptor potential channels to treat chronic pain: current state of the field. *Br. J. Pharmacol.* 175:2185–203

44. Jord S-E, Tominaga M, Julius D. 2000. Acid potentiation of the capsaicin receptor determined by a key extracellular site. *PNAS* 97:8134–39

45. Cao E, Liao M, Cheng Y, Julius D. 2013. TRPV1 structures in distinct conformations reveal activation mechanisms. *Nature* 504:113–18

46. Zhang K, Julius D, Cheng Y. 2021. Structural snapshots of TRPV1 reveal mechanism of polymodal functionality. *Cell* 184:5138–50

47. Geron M, Hazan A, Priel A. 2017. Animal toxins providing insights into TRPV1 activation mechanism. *Toxins* 9:326

48. Swartz KJ, MacKinnon R. 1997. Mapping the receptor site for hanatoxin, a gating modifier of voltage-dependent K⁺ channels. *Neuron* 18:675–82

49. Swartz KJ. 2007. Tarantula toxins interacting with voltage sensors in potassium channels. *Toxicon* 49:213–30

50. Bohlen CJ, Priel A, Zhou S, King D, Siemens J, Julius D. 2010. A bivalent tarantula toxin activates the capsaicin receptor, TRPV1, by targeting the outer pore domain. *Cell* 141:834–45

51. Siemens J, Zhou S, Piskorowski R, Nikai T, Lumpkin EA, et al. 2006. Spider toxins activate the capsaicin receptor to produce inflammatory pain. *Nature* 444:208–12

52. Bohlen CJ, Julius D. 2012. Receptor-targeting mechanisms of pain-causing toxins: How ow? *Toxicon* 60:254–64

53. Gao Y, Cao E, Julius D, Cheng Y. 2016. TRPV1 structures in nanodiscs reveal mechanisms of ligand and lipid action. *Nature* 534:347–51

54. Bae C, Swartz KJ. 2016. Structural insights into the mechanism of activation of the TRPV1 channel by a membrane-bound tarantula toxin. *eLife* 5:e11273

55. Hughes TET, Lodowski DT, Huynh KW, Yazici A, Rosario JD, et al. 2018. Structural basis of TRPV5 channel inhibition by econazole revealed by cryo-EM. *Nat. Struct. Mol. Biol.* 25:53–60

56. Wang Q, Corey R, Hedger G, Aryal P, Grieben M, et al. 2019. Lipid interactions of a ciliary membrane TRP channel: simulation and structural studies of polycystin-2. *Structure* 28:169–84.e5

57. Zubcevic L, Hsu AL, Borgnia MJ, Lee S-Y. 2019. Symmetry transitions during gating of the TRPV2 ion channel in lipid membranes. *eLife* 8:e45779

58. Liu C, Reese R, Vu S, Rougé L, Shields SD, et al. 2020. A non-covalent ligand reveals biased agonism of the TRPA1 ion channel. *Neuron* 109:273–84.e4

59. Suo Y, Wang Z, Zubcevic L, Hsu AL, He Q, et al. 2020. Structural insights into electrophile irritant sensing by the human TRPA1 channel. *Neuron* 105:882–94

60. Diver MM, Cheng Y, Julius D. 2019. Structural insights into TRPM8 inhibition and desensitization. *Science* 365:1434–40

61. Yin Y, Le SC, Hsu AL, Borgnia MJ, Yang H, Lee SY. 2019. Structural basis of cooling agent and lipid sensing by the cold-activated TRPM8 channel. *Science* 363:eaav9334

62. Hinman A, Chuang H-H, Bautista DM, Julius D. 2006. TRP channel activation by reversible covalent modification. *PNAS* 103:19564–68

63. Macpherson LJ, Dubin AE, Evans MJ, Marr F, Schultz PG, et al. 2007. Noxious compounds activate TRPA1 ion channels through covalent modification of cysteines. *Nature* 445:541–45

64. Paulsen CE, Armache JP, Gao Y, Cheng Y, Julius D. 2015. Structure of the TRPA1 ion channel suggests regulatory mechanisms. *Nature* 520:511–17

65. Zhao J, Lin King JV, Paulsen CE, Cheng Y, Julius D. 2020. Irritant-evoked activation and calcium modulation of the TRPA1 receptor. *Nature* 585:141–45

66. Parks TA, Bahia PK, Taylor-Clark TE. 2021. Functional evidence of distinct electrophile-induced activation states of the ion channel TRPA1. *Biochem. Biophys. Rep.* 27:101044

67. Bahia PK, Parks TA, Stanford KR, Mitchell DA, Varma S, et al. 2016. The exceptionally high reactivity of Cys 621 is critical for electrophilic activation of the sensory nerve ion channel TRPA1. *J. Gen. Physiol.* 147:451–65

68. Lin King JV, Emrick JJ, Kelly MJS, Herzig V, King GF, et al. 2019. A cell-penetrating scorpion toxin enables mode-specific modulation of TRPA1 and pain. *Cell* 178:1362–74

69. Su Q, Hu F, Ge X, Lei J, Yu S, et al. 2018. Structure of the human PKD1-PKD2 complex. *Science* 361:eaat9819

70. Dang S, van Goor MK, Asarnow D, Wang Y, Julius D, et al. 2019. Structural insight into TRPV5 channel function and modulation. *PNAS* 116:8869–78

71. Hughes TET, Pumroy RA, Yazici AT, Kasimova MA, Fluck EC, et al. 2018. Structural insights on TRPV5 gating by endogenous modulators. *Nat. Commun.* 9:4198

72. Singh AK, McGoldrick LL, Sobolevsky AI. 2018. Structure and gating mechanism of the transient receptor potential channel TRPV3. *Nat. Struct. Mol. Biol.* 25:805–13

73. Deng Z, Paknejad N, Maksaev G, Sala-Rabanal M, Nichols CG, et al. 2018. Cryo-EM and X-ray structures of TRPV4 reveal insight into ion permeation and gating mechanisms. *Nat. Struct. Mol. Biol.* 25:252–60

74. Gracheva EO, Ingolia NT, Kelly YM, Cordero-Morales JF, Hollopeter G, et al. 2010. Molecular basis of infrared detection by snakes. *Nature* 464:1006–11

75. Cordero-Morales JF, Gracheva EO, Julius D. 2011. Cytoplasmic ankyrin repeats of transient receptor potential A1 (TRPA1) dictate sensitivity to thermal and chemical stimuli. *PNAS* 108:E1184–91

76. Yin Y, Wu M, Zubcevic L, Borschel WF, Lander GC, Lee S-Y. 2018. Structure of the cold- and menthol-sensing ion channel TRPM8. *Science* 359:237–41

77. Tang Q, Guo W, Zheng L, Wu J-X, Liu M, et al. 2018. Structure of the receptor-activated human TRPC6 and TRPC3 ion channels. *Cell Res.* 28:746–55

78. Kim D, Cavanaugh EJ. 2007. Requirement of a soluble intracellular factor for activation of transient receptor potential A1 by pungent chemicals: role of inorganic polyphosphates. *J. Neurosci.* 27:6500–9

79. Wang YY, Chang RB, Waters HN, McKemy DD, Liman ER. 2008. The nociceptor ion channel TRPA1 is potentiated and inactivated by permeating calcium ions. *J. Biol. Chem.* 283:32691–703

80. Chung M-K, Güler AD, Caterina MJ. 2008. TRPV1 shows dynamic ionic selectivity during agonist stimulation. *Nat. Neurosci.* 11:555–64

81. Chen J, Kim D, Bianchi BR, Cavanaugh EJ, Faltynek CR, et al. 2009. Pore dilation occurs in TRPA1 but not in TRPM8 channels. *Mol. Pain* 5:3

82. McCoy DD, Palkar R, Yang Y, Ongun S, McKemy DD. 2017. Cellular permeation of large molecules mediated by TRPM8 channels. *Neurosci. Lett.* 639:59–67

83. Binshtok AM, Bean BP, Woolf CJ. 2007. Inhibition of nociceptors by TRPV1-mediated entry of impermeant sodium channel blockers. *Nature* 449:607–10

84. Puopolo M, Binshtok AM, Yao G-L, Oh SB, Woolf CJ, Bean BP. 2013. Permeation and block of TRPV1 channels by the cationic lidocaine derivative QX-314. *J. Neurophysiol.* 109:1704–12

85. McGoldrick LL, Singh AK, Saotome K, Yelshanskaya MV, Twomey EC, et al. 2017. Opening of the human epithelial calcium channel TRPV6. *Nature* 553:233–37

86. Jara-Oseguera A, Huffer KE, Swartz KJ. 2019. The ion selectivity filter is not an activation gate in TRPV1–3 channels. *eLife* 8:e51212

87. Myers BR, Bohlen CJ, Julius D. 2008. A yeast genetic screen reveals a critical role for the pore helix domain in TRP channel gating. *Neuron* 58:362–73

88. Balestrini A, Joseph V, Dourado M, Reese RM, Shields SD, et al. 2021. A TRPA1 inhibitor suppresses neurogenic inflammation and airway contraction for asthma treatment. *J. Exp. Med.* 218:e20201637

89. Chung MK, Guler AD, Caterina MJ. 2008. TRPV1 shows dynamic ionic selectivity during agonist stimulation. *Nat. Neurosci.* 11:555–64

90. Bean BP. 2015. Pore dilation reconsidered. *Nat. Neurosci.* 18:1534–35

91. Terrett JA, Chen H, Shore DG, Villemure E, Larouche-Gauthier R, et al. 2021. Tetrahydrofuran-based transient receptor potential ankyrin 1 (TRPA1) antagonists: ligand-based discovery, activity in a rodent asthma model, and mechanism-of-action via cryogenic electron microscopy. *J. Med. Chem.* 64:3843–69

92. Zubcevic L, Lee S-Y. 2019. The role of π-helices in TRP channel gating. *Curr. Opin. Struct. Biol.* 58:314–23

93. Matos-Cruz V, Schneider ER, Mastrotto M, Merriman DK, Bagriantsev SN, Gracheva EO. 2017. Molecular prerequisites for diminished cold sensitivity in ground squirrels and hamsters. *Cell Rep.* 21:3329–37

94. Zhang F, Jara-Oseguera A, Chang T-H, Bae C, Hanson SM, Swartz KJ. 2018. Heat activation is intrinsic to the pore domain of TRPV1. *PNAS* 115:E317–24

95. Brauchi S, Orta G, Salazar M, Rosenmann E, Latorre R. 2006. A hot-sensing cold receptor: C-terminal domain determines thermosensation in transient receptor potential channels. *J. Neurosci.* 26:4835–40

96. Clapham DE, Miller C. 2011. A thermodynamic framework for understanding temperature sensing by transient receptor potential (TRP) channels. *PNAS* 108:19492–97

97. Nadezhdin KD, Neuberger A, Trofimov YA, Krylov NA, Sinica V, et al. 2021. Structural mechanism of heat-induced opening of a temperature-sensitive TRP channel. *Nat. Struct. Mol. Biol.* 28:564–72

98. Kwon DH, Zhang F, Suo Y, Bouvette J, Borgnia MJ, Lee S-Y. 2021. Heat-dependent opening of TRPV1 in the presence of capsaicin. *Nat. Struct. Mol. Biol.* 28:554–63

99. Tao X, Lee A, Limapichat W, Dougherty DA, MacKinnon R. 2010. A gating charge transfer center in voltage sensors. *Science* 328:67–73

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

101. Hardie RC. 2007. TRP channels and lipids: from *Drosophila* to mammalian physiology. *J. Physiol.* 578:9–24

102. Autzen HE, Myasnikov AG, Campbell MG, Asarnow D, Julius D, Cheng Y. 2018. Structure of the human TRPM4 ion channel in a lipid nanodisc. *Science* 359:228–32

103. Duan J, Li J, Bo Z, Chen G-L, Peng X, et al. 2018. Structure of the mouse TRPC4 ion channel. *Nat. Commun.* 9:3102

104. Ruan Z, Haley E, Orozco IJ, Sabat M, Myers R, et al. 2021. Structures of the TRPM5 channel elucidate mechanisms of activation and inhibition. *Nat. Struct. Mol. Biol.* 28:604–13

105. Suh B-C, Hille B. 2008. PIP_2 is a necessary cofactor for ion channel function: How and why? *Annu. Rev. Biophys.* 37:175–95

106. Rohacs T. 2014. Phosphoinositide regulation of TRP channels. In *Mammalian Transient Receptor Potential (TRP) Cation Channels: Volume II*, ed. B Nilius, V Flockerzi, pp. 1143–76. Cham, Switz.: Springer Int. Publ.

107. Qin F. 2007. Regulation of TRP ion channels by phosphatidylinositol-4,5-bisphosphate. In *Transient Receptor Potential (TRP) Channels*, ed. V Flockerzi, B Nilius, pp. 509–25. Berlin: Springer

108. Zheng W, Cai R, Hofmann L, Nesin V, Hu Q, et al. 2018. Direct binding between Pre-S1 and TRP-like domains in TRPP channels mediates gating and functional regulation by PIP_2. *Cell Rep.* 22:1560–73

109. Harraz OF, Longden TA, Hill-Eubanks D, Nelson MT. 2018. PIP_2 depletion promotes TRPV4 channel activity in mouse brain capillary endothelial cells. *eLife* 7:e38689

110. Chuang H-h, Prescott ED, Kong H, Shields S, Jordt S-E, et al. 2001. Bradykinin and nerve growth factor release the capsaicin receptor from $PtdIns(4,5)P_2$-mediated inhibition. *Nature* 411:957–62

111. Prescott ED, Julius D. 2003. A modular PIP_2 binding site as a determinant of capsaicin receptor sensitivity. *Science* 300:1284–88

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

113. Shu X-Q, Llinas A, Mendell LM. 1999. Effects of trkB and trkC neurotrophin receptor agonists on thermal nociception: a behavioral and electrophysiological study. *Pain* 80:463–70

114. Bonnington JK, McNaughton PA. 2003. Signalling pathways involved in the sensitisation of mouse nociceptive neurones by nerve growth factor. *J. Physiol.* 551:433–46

115. Cao E, Cordero-Morales JF, Liu B, Qin F, Julius D. 2013. TRPV1 channels are intrinsically heat sensitive and negatively regulated by phosphoinositide lipids. *Neuron* 77:667–79

116. Ufret-Vincenty CA, Klein RM, Collins MD, Rosasco MG, Martinez GQ, Gordon SE. 2015. Mechanism for phosphoinositide selectivity and activation of TRPV1 ion channels. *J. Gen. Physiol.* 145:431–42

117. Liu B, Zhang C, Qin F. 2005. Functional recovery from desensitization of vanilloid receptor TRPV1 requires resynthesis of phosphatidylinositol 4,5-bisphosphate. *J. Neurosci.* 25:4835–43

118. Rohacs T. 2015. Phosphoinositide regulation of TRPV1 revisited. *Pflügers Arch. Eur. J. Physiol.* 467:1851–69

119. Ufret-Vincenty CA, Klein RM, Hua L, Angueyra J, Gordon SE. 2011. Localization of the PIP_2 sensor of TRPV1 ion channels. *J. Biol. Chem.* 286:9688–98

120. Hofmann T, Obukhov AG, Schaefer M, Harteneck C, Gudermann T, Schultz G. 1999. Direct activation of human TRPC6 and TRPC3 channels by diacylglycerol. *Nature* 397:259–63

121. Bai Y, Yu X, Chen H, Horne D, White R, et al. 2020. Structural basis for pharmacological modulation of the TRPC6 channel. *eLife* 9:e53311

Contents

Errata

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