Transient Receptor Potential Channels and Calcium Signaling

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Transient receptor potential (TRP) cation channels play diverse roles in cellular Ca^{2+} signaling. First, as Ca^{2+} -permeable channels that respond to a variety of stimuli, TRP channels can directly initiate cellular Ca^{2+} signals. Second, as nonselective cation channels, TRP channel activation leads to membrane depolarization, influencing Ca^{2+} influx via voltage-gated and store-operated Ca^{2+} channels. Finally, Ca^{2+} modulates the activity of most TRP channels, allowing them to function as molecular effectors downstream of intracellular Ca^{2+} signals. Whereas the TRP channel field has long been devoid of detailed channel structures, recent advances, particularly in cryo-electron microscopy-based structural approaches, have yielded a flurry of TRP channel structures, including members from all seven subfamilies. These structures, in conjunction with mutagenesis-based functional approaches, provided important new insights into the mechanisms whereby TRP channels permeate and sense Ca^{2+} . These insights will be highly instrumental in the rational design of novel treatments for the multitude of TRP channel-related diseases.

Transient receptor potential (TRP) channels form a large branch within the superfamily of voltage-gated cation channels, which further contains the K^{+} -selective channels, voltage-gated Na^{+} channels, voltage-gated Ca^{2+} channels, cyclic nucleotide-gated channels, and two pore channels (TPCs) (Yu and Catterall 2004). The human genome encodes 27 TRP channel-encoding genes (Gees et al. 2012), and further TRP channel-encoding genes are found in all vertebrates, insects, worms, yeast, and even single-celled algae (Harteneck et al. 2000; Arias-Darraz et al. 2015). The designation TRP originates from the *trp* gene in *Drosophila*, disruption of which leads to a transient receptor potential in the fly

photoreceptors (Minke et al. 1975). As such, the name TRP is a relic from the past rather than an accurate description of the physiological properties of this family of channels, which are involved in diverse biological and pathophysiological processes, mostly completely unrelated to the transient nature of receptor potentials (Clapham 2003; Nilius et al. 2007; Venkatachalam and Montell 2007; Gees et al. 2012).

However, finding a more apt name for this group of channels is not evident: the TRP channel family is really a mixed bag, consisting of cation channels exhibiting a wide range of ion selectivities, gating mechanisms, and biological functions. Arguably, the most common feature

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of TRP channels is that they make a substantial impact on cellular Ca^{2+} signaling. In particular, TRP channels can act as a pathway for Ca^{2+} to enter the cytosol, modulate the activity of other Ca^{2+} -permeable channels, and translate changes in cytosolic Ca^{2+} into cation flux and electrical activity.

In this review, we summarize and discuss recent insights into the role of TRP channels in cellular Ca^{2+} signaling, with particular focus on the structural determinants of TRP channel- Ca^{2+} interactions.

Ca^{2+} ACTION AND REACTION

Ca^{2+} is a ubiquitous intracellular messenger, and Ca^{2+} signaling lies at the basis of innumerable biological processes, including cell adaptation, survival, and cell death (Berridge 2017). Adequate spatiotemporal regulation of intracellular Ca^{2+} therefore represents a central theme in every cell. TRP channels are tightly involved both in shaping Ca^{2+} signals and in responding to physiological changes in intracellular Ca^{2+} in animal cells.

TRP Channel Gating Shaping Cytosolic Ca^{2+} Signals

Based upon their relative permeability to Ca^{2+} and Na^+ ($P_{\text{Ca}}/P_{\text{Na}}$), TRP channels can roughly be divided into three groups (Fig. 1; Owsianik et al. 2006). The majority of TRP channels are Ca^{2+} -permeable nonselective cation channels with $P_{\text{Ca}}/P_{\text{Na}}$ values in the range between 0.1 and 20. In most cell types, opening of these TRP channels will induce an inward current that is carried by a mixture of Na^+ and Ca^{2+} ions (Mulier et al. 2017). The two closely homologous epithelial TRP channels TRPV5 and TRPV6 are exquisitely Ca^{2+} -selective, with estimated $P_{\text{Ca}}/P_{\text{Na}}$ values exceeding 100 (Vennekens et al. 2000; Yue et al. 2001). Under physiological ionic conditions, these channels will generate inward currents that are almost exclusively carried by Ca^{2+} ions, similar to the high Ca^{2+} selectivity of voltage-gated Ca^{2+} channels and store-operated Orai1 channels. TRPM4 and TRPM5 are situated on the other end of the

Figure 1. Ca^{2+} permeability of transient receptor potential (TRP) channels. On a logarithmic scale, these are examples of the indicated mammalian TRP channels illustrating the range of relative Ca^{2+} permeabilities (based on reversal potential measurements). Note that values are indicative, as the obtained values can vary significantly depending on cellular environment and experimental conditions.

selectivity spectrum, showing negligible permeability to Ca^{2+} ions ($P_{\text{Ca}}/P_{\text{Na}} < 0.01$). Inward currents mediated by these channels are mainly carried by Na^+ ions (Launay et al. 2002; Prawitt et al. 2003; Nilius et al. 2005).

Notwithstanding this diversity in the ion selectivity of their pores, it has already been well-established in the prestructural era that TRP channels share a common overall architecture, analogous to that of voltage-gated channels that mediate K^+ , Na^+ , or Ca^{2+} flux (Gaudet 2008). All TRP channel family members are proteins containing six transmembrane (TM) domains (S1 to S6) and relatively long intracellular amino and carboxyl termini. After assembly of four such TRP subunits into one functional tetramer, the domains S5 to S6 and the interconnecting loop complement into one central pore (Hoenderop et al. 2003; Gaudet 2008; Gees et al. 2012). In the last two decades, studies using a combination of site-directed mutagenesis, electrophysiology, and structural modeling have clearly established that amino acids lining this central pore tune the Ca^{2+} selectivity of TRP channel pores, and that variability in this region can explain to a large extent the differences in Ca^{2+} permeability between TRP channels. For instance, mutation of a single aspartate in the pore loop of TRPV5 and TRPV6 to a non-charged residue changes the selectivity of these

channels from Ca^{2+} -selective to nonselective (Nilius et al. 2001). Oppositely, mutation of a glutamine to a glutamate in the pore loop confers detectable Ca^{2+} permeability to TRPM4 (Nilius et al. 2005). Overall, the presence of negatively charged aspartate and glutamate residues in the pore loop was found to be a key determinant of Ca^{2+} permeability and selectivity (García-Martínez et al. 2000; Nilius et al. 2001, 2005; Hoenderop et al. 2003; Voets et al. 2003, 2004; Owsianik et al. 2006).

To determine the contribution of a TRP channel to cellular Ca^{2+} signaling, not only the pore properties but also the gating mechanisms need to be taken into account. TRP channel gating can be induced by a wide variety of physical and chemical stimuli (Table 1), and the relative permeability to Ca^{2+} ions will crucially determine the cell-physiological response to channel opening. Opening of Ca^{2+} -permeable nonselective TRP channels evokes a cytosolic Ca^{2+} rise as well as membrane depolarization, both of which can trigger cellular responses. For instance, several TRP channels are expressed in sensory endings of somatosensory neurons, where they respond to innocuous and noxious stimuli. There, opening of Ca^{2+} -permeable nonselective channels such as TRPA1, TRPV1, and TRPM3 not only causes depolarization that evokes action potential firing and propagation to the central nervous system to signal pain (Vandewauw et al. 2018), but also a local cytosolic Ca^{2+} rise that triggers neuropeptide release and neurogenic inflammation (Julius 2013). In the case of the Ca^{2+} -selective TRPV5 and TRPV6, the entry of Ca^{2+} ions lies at the basis of their role in the selective epithelial uptake, and transepithelial transport, of Ca^{2+} (Hoenderop et al. 2005). Depending on the cellular context, opening of the Ca^{2+} -impermeable TRPM4 and TRPM5 and the ensuing cell depolarization can either evoke, enhance, or dampen cytosolic Ca^{2+} signals. On the one hand, depolarization promotes gating of voltage-gated Ca^{2+} channels present in most excitable cells. On the other hand, TRPM4- and TRPM5-mediated depolarization reduces the driving force for Ca^{2+} influx through both voltage-gated and voltage-independent (e.g., store-operated) Ca^{2+} channels, and can thus act as a

break on Ca^{2+} entry (Liman 2007; Vennekens et al. 2007; Mathar et al. 2014).

To add to the complexity, a subset of TRP channels is functionally expressed at the membranes of intracellular organelles, including lysosomes and the endoplasmic reticulum (ER). Prominent example are the mucolipins (TRPML1, TRPML2, and TRPML3), which are predominantly found in organellar membranes. Mutations in TRPML1 cause the human lysosomal storage disease mucopolipidosis type IV, and evidence is accumulating pointing at a crucial role for this channel in the transport of Ca^{2+} and other divalent cations across the lysosomal membrane as well as in lysosomal biogenesis and autophagy (Dong et al. 2008, 2010; Medina et al. 2015; Zhang et al. 2016). In addition, several TRP channels can be considered as non-committed TRP channels, as they can function in the plasma membrane as well as in intracellular membranes (Zhang et al. 2018a). A prominent example is TRPM2, which is best known as a plasma membrane channel activated by intracellular Ca^{2+} and intracellular ligands such as ADP-ribose (ADPR), and by the recently discovered superagonist 2'-deoxy ADPR (Perraud et al. 2001; Sano et al. 2001; Fliegert et al. 2017), but can also act as a lysosomal Ca^{2+} -release channel (Lange et al. 2009). A more elaborate review on intracellular TRP channels can be found elsewhere (Zhang et al. 2018a).

Cytosolic Ca^{2+} Signals Shaping TRP Channel Activity

Besides TRP channel gating shaping cellular Ca^{2+} signals, cytosolic Ca^{2+} itself also modulates the activity of several TRP channels. The action of Ca^{2+} on TRP channel gating can be both stimulatory and inhibitory, and can either be mediated via direct channel- Ca^{2+} interaction or alternatively involve cytosolic Ca^{2+} -binding proteins and/or Ca^{2+} -dependent signal transduction pathways (Fig. 2).

There are only a few examples in the literature of Ca^{2+} -binding sites in the cytosolic domains of TRP channels that have been proposed to contribute to direct effects of cytosolic Ca^{2+} on channel gating. These include an EF-hand

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Table 1. Activation and regulation mechanisms of TRP channels

	Representative ligand	[Calcium] _i	T°	Voltage dependence
TRPA1	AITC	+: Direct (low Ca ²⁺) -: Direct/CaM (high Ca ²⁺)
TRPC1				
TRPC2	DAG	-:		
TRPC3	DAG	-: CaM		
TRPC4	Gd ³⁺	-: CaM		
TRPC5	Gd ³⁺	+: Ca ²⁺
TRPC6	DAG			
TRPC7	DAG	-: CaM		
TRPM1	PS			
TRPM2	2'-deoxy-ADPR	+: CaM, direct		
TRPM3	PS, CIM0216	-: PIP ₂ depletion
TRPM4	Decavanadate	+: Direct -: PIP ₂ depletion
TRPM5	Ca ²⁺	+: Direct -: PIP ₂ depletion
TRPM6		-: PIP ₂ depletion		
TRPM7	Naltriben	-: PIP ₂ depletion		
TRPM8	Menthol	-: PIP ₂ depletion
TRPML1	PI(3,5)P ₂	-: Direct Ca ²⁺ block		
TRPML2	SF-11, SN-1			
TRPML3	SF-21/41/81			
TRPP1-PKD2		+: Direct
TRPP2-PKD2 L1				
TRPP3-PKD2 L2				
TRPV1	Capsaicin	-: CaM, PIP ₂ depletion
TRPV2	THC	-: PIP ₂ depletion
TRPV3	2-APB	+: CaM + direct
TRPV4	GSK-1016790A	+: CaM -: Desensitization (?)
TRPV5		-: CaM, Calbindin-D28K, PIP ₂ depletion		
TRPV6		-: CaM, PIP ₂ depletion		

❄, cold temperatures; 🔥, warm temperatures; ⚡, voltage-gated.

2-APB, 2-aminoethoxydiphenyl borate; AICT, allyl isothiocyanate; CaM, calmodulin; DAG, diacylglycerol; PIP₂, phosphatidylinositol bisphosphate; PS, pregnenolone sulfate; SF, SN, sulfonamides; THC, tetrahydrocannabinol.

structure in both the amino-terminal domain of TRPA1 and in the cytosolic side of the S1–S4 region in TRPM4 that have been implicated in Ca²⁺-induced channel activation (Doerner et al. 2007; Zurborg et al. 2007; Autzen et al. 2018).

A more common means whereby Ca²⁺ can affect TRP channel activity involves cytosolic Ca²⁺-binding proteins, with calmodulin (CaM) being the most prominent one. CaM-binding domains (CaMBDs) have been identified in the cytosolic regions of different TRP channels, includ-

ing sites where CaM binds in the apo (Ca²⁺-free) and Ca²⁺-bound (Ca²⁺-CaM) conformations. The effect of Ca²⁺-CaM on TRP channel gating is channel-dependent. For example, binding of Ca²⁺-CaM to one of the CaMBDs located in the cytosolic amino- or carboxyl termini, underlies Ca²⁺-dependent inactivation of TRP channels. This is exemplified by the highly Ca²⁺-selective epithelial channels TRPV5 and TRPV6, where CaM-dependent inactivation functions as a fast feedback mechanism to maintain a tight Ca²⁺

Figure 2. Cartoon illustrating three general mechanisms of Ca^{2+} -dependent regulation of transient receptor potential (TRP) channel activity. (*Left*) Ca^{2+} (red spheres) can cause a decrease in the plasma membrane PIP_2 levels via Ca^{2+} -activated phospholipase C. This decrease is sensed by PIP_2 -binding domains (PBDs, purple), which are found at various locations in the cytosolic part of TRP channels. (*Center*) Ca^{2+} can bind directly to activate TRP channels, for instance, via residues in the S2-S3 region of TRPM channels. (*Right*) Ca^{2+} can influence TRP channel activity via calmodulin (CaM, blue), for example, via Ca^{2+} -CaM binding in the carboxyl terminus of TRPV channels.

homeostasis and prevent excessive Ca^{2+} influx (Kovalevskaya et al. 2012). In TRPV1, Ca^{2+} -CaM exerts a dual effect on channel desensitization, involving both a direct interaction with cytosolic CaMBDs as well as an indirect modulation via Ca^{2+} -CaM-dependent protein kinase II (CaMKII)-dependent channel phosphorylation (Bonnington and McNaughton 2003; Lau et al. 2012). In contrast, binding of Ca^{2+} -CaM to TRPV3 was proposed to sensitize the channel to repeated stimuli, although the underlying mechanism is unclear (Xiao et al. 2008). In addition to CaM, other Ca^{2+} -binding proteins have also been shown to interact with, and regulate, TRP channels. Examples include 80K-H and Calbindin-D28K interacting with TRPV5 (Lambers et al. 2006), and S100A1 interacting with TRPA1 and TRPM3 (Holakovska et al. 2012).

A third and frequently observed mechanism whereby Ca^{2+} affects TRP channel function is through activation of phospholipase C (PLC), which catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) in the plasma membrane into the second messengers inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) (Rohacs 2014). Whereas some PLC isoforms are activated by G-protein coupled recep-

tors (GPCRs), others (PLC δ 1, δ 3, and δ 4) can be activated by a rise in cytosolic Ca^{2+} , for instance upon TRP channel activation. For most TRP channels, PIP_2 acts as a positive regulator of channel activity, and consequently PIP_2 hydrolysis at the plasma membrane, whether through Ca^{2+} - or GPCR-dependent activation of PLC, causes channel desensitization; yet, there are also examples of inhibitory and bimodal effects of PIP_2 on TRP channel gating (Chuang et al. 2001; Lukacs et al. 2007; Zhang et al. 2012; Rohacs 2014).

A more elaborate review on the effect of Ca^{2+} on TRP channels can be found elsewhere (Hasan and Zhang 2018).

TRP CHANNELS ENTERING THE STRUCTURAL AREA

Given the varied and intricate interplay between TRP channels and cellular Ca^{2+} signaling, important questions have been raised regarding the interaction of TRP channels with Ca^{2+} and Ca^{2+} -regulated processes at the submolecular level. Such information is not only of fundamental interest, to elucidate the structural basis of the divergent permeability and gating properties of the different TRP channels, but also highly rele-

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vant to understanding the pathophysiological consequences of disease-causing mutations in TRP channels and to develop specific pharmacological approaches for the treatment of TRP channel-related human diseases (Nilius et al. 2007; Nilius and Voets 2013).

The Resolution Revolution

Although the first TRP gene was cloned in 1989 (Montell and Rubin 1989), it took almost 25 years until the first near-atomic-resolution TRP channel structure was resolved. Before that, high-resolution structural details were only available for a small number of specific intracellular domains of TRP channels (e.g., Yamaguchi et al. 2001), and insights into the 3D structure of entire TRP channels was limited to lower-resolution cryo-electron microscopy (cryo-EM) images, atomic force microscopy (e.g., Barrera et al. 2007; Gaudet 2008; Moiseenkova-Bell and Wensel 2009), and homology modeling using potassium channel structures as templates (e.g., Kalia and Swartz 2013). The lack of progress was partly a result of difficulties in purifying membrane proteins into a stable, native protein conformation after removal from membrane detergents (Whited and Park 2014), as well as to the large cytoplasmic domains of TRP channels displaying significant flexibility and thereby hindering crystallization. Fortunately, recent advantages in cryo-EM, driven by developments in detectors and single-particle analysis (Kühlbrandt 2014), have led to a revolution in TRP channel structural biology (Madej and Ziegler 2018). In 2013, the side-chain resolution barrier was broken for the first time for membrane proteins without crystallization, when structures of TRPV1 were determined at a 3.4 Å resolution (Liao et al. 2013). This was achieved by combining a new, direct electron detector with novel image processing algorithms, thereby greatly improving the signal and correcting for motion-induced blurring. This accomplishment is regarded as a major breakthrough in the world of structural biology and in the TRP channel field. These studies were the start of a resolution revolution, with an exponential increase in the number of structures of ion channels and other membrane proteins re-

solved at atomic detail (Kühlbrandt 2014; Cheng 2015). As a result, detailed structures of at least one member of each TRP subfamily has been unraveled, providing insight into ion permeation and gating with resolution in the 3–4 Å range (Table 2).

TRPV1 Structures: A Paradigm of TRP Channel Architecture and Gating

The cryo-EM structure of TRPV1 revealed an architecture characterized by a fourfold symmetry around a central ion permeation pore formed by TM domains S5 to S6 and the intervening pore loop (S5-P-S6), surrounded by four independently folded modules composed of domains S1 to S4 (Liao et al. 2013). Overall, this architecture is very similar to that of voltage-gated K^+ , Na^+ , and Ca^{2+} channels (Kuang et al. 2015). The TRPV1 channel exhibits a wide extracellular mouth but a short selectivity filter, which sculpts the upper part of the gate (Liao et al. 2013). The conserved TRP domain that follows S6 interacts with the S4–S5 linker, which may facilitate coupling between different channel domains important for allosteric modulation. The assembly into tetramers is facilitated by interactions among cytoplasmic domains of the subunits. The ARD in the amino terminus is followed by a tightly packed linker that makes the connection with the pre-S1 helix.

These initial studies reported not only the structure of TRPV1 in its unliganded state (Liao et al. 2013), but also two structures obtained in the presence of ligand activators (Cao et al. 2013), allowing a first insight into possible structural rearrangements during channel gating. These ligand-bound TRPV1 structures included one obtained in the presence of the canonical vanilloid activator capsaicin, as well as one combining the ultrapotent vanilloid resiniferatoxin (RTX) with the double-knot toxin (DkTx), which in functional assays traps the channel in a fully open state. In these structures, the vanilloid-binding site was found buried in the channel complex in a binding pocket between the S4–S5 linker of one subunit and S6 of a neighboring subunit. In the RTX/DkTx-bound structure, four toxin moieties were ob-

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Table 2. Overview of structures of integral transient receptor potential (TRP) channel proteins

	Species	Method	Resolution	Condition and state	References	PDB
TRPA1	Human	Cryo-EM	4 Å	Agonist AITC Antagonist HC-030031 ± A-967079	Paulsen et al. 2015	3J9P
TRPC1						
TRPC2						
TRPC3	Human	Cryo-EM (lipid nanodisc)	4.4 Å	Lipid-activator OAG pore (closed state)	Tang et al. 2018	5ZBG
	Human	Cryo-EM	3.3 Å	Lipid-occupied (closed state)	Fan et al. 2018	6CUD
	Human	Cryo-EM	5.8 Å	In detergent (GDN)	Sierra-Valdez et al. 2018	6DJS/R
TRPC4	Zebrafish	Cryo-EM	3.6 Å	Apo state (closed)	Vinayagam et al. 2018	6G1K
	Mouse	Cryo-EM	3.3 Å	Apo state (closed/inactivated state)	Duan et al. 2018a	5Z96
TRPC5						
TRPC6	Human	Cryo-EM (lipid nanodisc)	3.8 Å	Complex with inhibitor BTDM (closed)	Tang et al. 2018	5YX9
TRPC7						
TRPM1						
TRPM2	<i>Nematostella vectensis</i>	Cryo-EM	3 Å	Ca ²⁺ -bound (closed state)	Zhang et al. 2018b	6CO7
	Zebrafish	Cryo-EM	3.8 Å	Apo state (closed)	Huang et al. 2018	6DRK 6DRJ
			3.3 Å	ADPR/Ca ²⁺ -bound state (active)		
	Human	Cryo-EM	3.6 Å	Apo state ADPR-bound ADPR/Ca ²⁺ -bound	Wang et al. 2018	6MIX 6MIZ 6MJ2
TRPM3						
TRPM4	Human	Cryo-EM (lipid nanodisc)	3 Å	Ca ²⁺ -free state Ca ²⁺ -bound state	Autzen et al. 2018	6BQR 6BQV
	Human	Cryo-EM	3.7 Å	Closed, Na ⁺ -bound (apo state)	Duan et al. 2018c	6BWI
	Mouse	Cryo-EM	3 Å	Without ATP ATP-bound	Guo et al. 2017	6BCJ 6BCL 6BCO 6BCQ
	Human	Cryo-EM	3.8 Å	Bound to Ca ²⁺ (agonist) and to decavanadate (DVT)	Winkler et al. 2017	5WP6
TRPM5						
TRPM6						
TRPM7	Mouse	Cryo-EM	3.28 Å 3.7 Å 4.1 Å	With EDTA Mg ²⁺ -bound Mg ²⁺ -unbound (divalent free)	Duan et al. 2018b	5ZX5 6BWD 6BWF
TRPM8	<i>Ficedula albicollis</i>	Cryo-EM	4.1 Å	Nonconducting state	Yin et al. 2018	6BPQ

Continued

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Table 2. Continued

	Species	Method	Resolution	Condition and state	References	PDB
TRPML1	Mouse	Cryo-EM in –nanodisc –amphipols	5.4 Å	Closed state	Zhang et al. 2017	5YE5
			5.8 Å	State1 (closed)		5YE2
			7.4 Å	State2 (wide upper gate)		5YDZ
			7.7 Å	State3 (wide lower gate)		5YE1
	Mouse	Cryo-EM (lipid nanodisc)	3.59 Å	Open state	Chen et al. 2017	5WPV
			3.64 Å	Closed 1		5WPQ
	Human	Cryo-EM	3.75 Å	Closed 2	Schmiedege et al. 2017	5WPT
			3.72 Å	Apo-structure (closed)		5WJ5 5WJ9
	Human	Cryo-EM	3.49 Å	Agonist bound (open)	Fine et al. 2018	6E7P
			3.6 Å	PI(3,5)P ₂ -bound PI(4,5)P ₂ -bound PI(3,5)P ₂ /ML-SA1-bound		6E7Y 6E7Z
TRPML2						
TRPML3	<i>Callithrix jacchus</i>	Cryo-EM	2.9 Å	Open state	Hirschi et al. 2017	5W3S
	Human	Cryo-EM	4.06 Å 3.62 Å	Apo channel TRPML3/ML-SA1 complex	Zhou et al. 2017	6AYE 6AYF
TRPP1– PKD2	Human	Cryo-EM (lipid nanodisc)	4.65 Å 3.0 Å	At pH 4.8 Closed/nonconductive state	Shen et al. 2016	6AYG 5T4D
	Human	Cryo-EM	3.5 Å	PKD2-F604P mutant		6D1W
	Human	Cryo-EM	4.2 Å	Both gates closed	Grieben et al. 2017	5K47
TRPP2– PKD2– L1	Human	Cryo-EM	4.3 Å	In complex with cations and lipids (different activation states)	Wilkes et al. 2017	5MKF 5MKE
	Human	Cryo-EM	3.3 Å		Hulse et al. 2018	6DU8
TRPP3– PKD2– L2	Mouse	Cryo-EM	3.38 Å	Open state	Su et al. 2018b	5Z1W
PKD1 + TRPP1	Human	Cryo-EM	3.6 Å	PKD1/PKD2 complex assembled in a 1:3 ratio	Su et al. 2018a	6A70
TRPV1	Rat	Cryo-EM	3.28 Å	Apo state (closed)	Liao et al. 2013	3J5P (3J9J)
			3.8 Å	Resiniferatoxin and capsaicin (activated)	Cao et al. 2013	3J5Q
			4.2 Å	Capsazepine (fully open state)		3J5R
	Rat	Cryo-EM (lipid nanodisc)	3.28 Å		Gao et al. 2016	5IRZ
			3.43 Å	In complex with capsazepine		5IS0

Continued

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Table 2. Continued

	Species	Method	Resolution	Condition and state	References	PDB
			2.95 Å	In complex with DkTx and RTX		5IRX
TRPV2	Rabbit	Cryo-EM	4 Å	Nonconductive (desensitized state)	Zubcevic et al. 2016	5AN8
	Rat	Cryo-EM	~5 Å	Apo state (constitutive activity)	Huynh et al. 2016	5HI9
	Rabbit	Cryo-EM	3.5 Å	Resiniferatoxin (RTx) and/or Ca ²⁺ -bound (open state)	Zubcevic et al. 2018b	6BWM 6BWJ
	Rat	Cryo-EM	3.6 Å	In partially closed state	Dosey et al. 2018	6BO5
			4 Å	Open state (resolved pore turret domain)		6BO4
TRPV3	Mouse	Cryo-EM	4.3 Å	Closed apo	Singh et al. 2018a	6DVW
			4 Å	Agonist (2-APB)-bound open states (+mutant)		6DXY/ Z
	Human	Cryo-EM	3.4 Å	Apo state	Zubcevic et al. 2018a	6MHO
			3.2 Å	Sensitized conformation		6MHS
			<3.5 Å	In the presence of 2-APB		6MHV/ W/X
TRPV4	<i>Xenopus laevis</i>	Cryo-EM + crystal structure	3.8 Å	Apo state (closed state)	Deng et al. 2018	6BBJ
				In the presence of cesium		6C8F 6C8G 6C8H
TRPV5	Rabbit	Cryo-EM	3.5–4 Å	Barium, gadolinium In complex with its inhibitor econazole (closed state)	Hughes et al. 2018a	6B5V
	Rabbit	Cryo-EM		Lipid-bound in detergent	Hughes et al. 2018b	6DMR
				PI(4,5)P ₂ -bound in nanodisc		6DMU
				CaM-bound in detergent		6DMW
TRPV6	Rat	Crystal structure	3.25 Å	TRPV6	Saotome et al. 2016	5IWK
				In the presence of Ca ²⁺ (open state)		5IWP
				Barium		5IWR
				Gadolinium		5IWT
	Human	Cryo-EM	3.6 Å	In nanodiscs	McGoldrick et al. 2018	6BO8
			4.0 Å	In amphipols		6BO9
				TRPV6–R470E in amphipols		6BOA
	Rat			TRPV6 in nanodisc		6BOB
	Rat	Crystal	3.45 Å	In complex with 2-AP	Singh et al. 2018c	6DO7

Continued

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Table 2. *Continued*

Species	Method	Resolution	Condition and state	References	PDB
			In complex with 2-APB-Br		6D7V
			Y466A		6D7P
			Y466A + in complex with 2-APB		6D7Q/X
			Y467A		6D7S
			Y467A + in complex with 2-APB		6D7T
Human	Cryo-EM	3.9 Å	TRPV6 in complex with calmodulin	Singh et al. 2018b	6E2F
Rat		3.6 Å	TRPV6 in complex with calmodulin		6E2G

PDB, Protein Data Bank; 2-APB, 2-aminoethoxydiphenyl borate; AICT, allyl isothiocyanate; CaM, calmodulin; PIP2, phosphatidylinositol biphosphate; cryo-EM, cryo-electron microscopy; RTX, ultrapotent vanilloid resiniferatoxin; DkTx, double-knot toxin.

served atop each tetrameric channel complex. Each toxin binds at the extracellular loop of the outer pore region of one subunit and connects to the pore helix of a neighboring subunit (Cao et al. 2013).

By comparing the pore structures of TRPV1 in these three different conformational states, significant plasticity was noticed in the central pore, both at the level of the selectivity filter and at a lower region of the pore formed by S6. The funnel-like structure that comprises the pore starts with a rather wide outer pore, followed by a short selectivity filter. When the channel is in the unliganded state, the narrowest point of the filter, 4.6 Å, is created by two opposed carbonyl oxygens at position G643 (Liao et al. 2013). Further down the pore, the most constricted site is located at I679 (S6), where the distance between side chains measures 5.3 Å in the unliganded state (Liao et al. 2013). Upon vanilloid binding, I679 rotates away from the central axis causing an expansion to 7.6 Å (Cao et al. 2013). In addition, in the RTX/DkTx structure, the pore helix tilts away from the central axis, thereby increasing the distance between the G643 carbonyls to 7.6 Å (Cao et al. 2013). Overall, these findings have led to the notion of a dual gating mechanism: in the unliganded conformation, both the upper and lower gate are constricted; the lower gate opens following

vanilloid ligand binding; and binding of DkTx, and possibly allosteric coupling with the lower gate, leads to additional opening of the upper gate.

A Flurry of TRP Channel Structures

Following the determination of TRPV1 structures using cryo-EM, similar approaches were successfully applied to many other members of the TRP channel superfamily. Moreover, by purifying TRP channels into lipid nanodiscs structural analysis of the channels in a more native, membrane-like environment has been achieved. Table 2 provides a summary of the published structures of TRP channels, with the cautionary note that this list is growing steadily.

At this point, structures of at least one member of every TRP channel subfamily are available, and representatives of each subfamily are represented in Figure 3. In general, these different TRP channels structures have several features, including a homotetrameric organization with fourfold symmetry, and a structurally conserved TM organization that resembles that of other members of the superfamily of voltage-gated cation channels. One particular exception is the recent structure of a heterotetramer formed between the polycystic kidney disease-related

PKD1 and TRPP1–PKD2 (Su et al. 2018a). Whereas TRPP1–PKD2 has all the features of a 6-TM TRP subunit and can form a homotetrameric channel, PKD1 is a much larger protein with an exoplasmic amino terminus and 11 TM helices. Of these 11 TM domains, the last six show homology to the TM segments of TRP channels, and one such subunit can apparently engage with three TRPP1–PKD2 subunits to form an asymmetric PKD1–PKD2 complex. However, the natural occurrence and functional properties of this complex remain to be established.

Despite common global architectures, there are several subfamily-specific features. In the exoplasmic region, TRPML and TRPP members exhibit a large structured domain formed by the S1–S2 linkers, known as the luminal domain (TRPML) and the polycystin domain (TRPP),

which forms a fenestrated canopy-like structure atop the channel (Shen et al. 2016; Chen et al. 2017; Grieben et al. 2017; Hirschi et al. 2017; Schmiede et al. 2017). In contrast, members of the other subfamilies have only short and largely unstructured exoplasmic loops. In TRPA, TRPC, and TRPV channels, the amino terminus contains multiple ARDs, which are implicated in the protein–protein interaction, trafficking, and gating. The number of ARDs ranges from three ARDs in TRPCs to 24 repeats in TRPA1. TRPM channels have relatively long amino termini but lack ARDs, as do the short amino-terminal tails of TRPP and TRPML channels. In their proximal carboxyl terminus following the S6 helix, TRPC, TRPV, and TRPM channels contain a highly conserved TRP domain, implicated in channel gating, which is not found in TRPA, TRPP, and

Figure 3. Cladogram of mammalian transient receptor potential (TRP) channel subfamilies and their structures. A representative structure of a member of each subfamily is illustrated: hTRML1 (PDB ID: 5WJ9), hTRPP2 (5K47), hTRPA1 (3J9P), rTRPV1 (3J5P), hTRPM4 (6BQR), and hTRPC3 (6CUD). Specific domains are indicated with the following color code: pink = transmembrane domains (S1–S6), light blue = pore region, dark blue = selectivity filter, orange = ankyrin repeat domain, dark green = TRP domain, yellow = TRP box, and lime = S1–S2 extracellular domain (luminal domain [TRPML] and polycystin domain [TRPP]).

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TRPML channels. At their distal carboxy terminal, three TRPM channels exhibit an integral enzyme domain: an atypical α kinase in TRPM6 and TRPM7, and a NUDIX domain in TRPM2. Below, we highlight the structural features that specifically relate to Ca^{2+} permeation and TRP channel regulation by Ca^{2+} .

STRUCTURAL INSIGHTS INTO Ca^{2+} PERMEATION

The central ion-conducting pore of TRP channels can be divided into an upper part, which forms the narrow selectivity filter lined by residues from the S5–S6 linker, and a lower part lined by residues from S6 that includes the lower gate. The lower gate is formed by hydrophobic residues that seal the lower part of the permeation pathway in the closed conformation, and rotate away to allow ion permeation upon channel activation (Cao et al. 2013; Gao et al. 2016). This movement may be dependent on a short π -helical segment observed in the middle of S6, which has been suggested to facilitate helix bending during gating (Hughes et al. 2018b; Singh et al. 2018a). In addition to the lower gate, structural evidence has been provided for the existence of a second, upper gate at the level of the selectivity filter in some but not all TRP channels (Deng et al. 2018). However, the exact relevance of this upper gate to TRP channel gating and ion permeation is currently unclear.

A key question is whether the different TRP channel structures provide a framework to explain the large diversity in Ca^{2+} permeability properties of TRP channel pores. To address this, we provide here a comparison between the pores of TRP channels that reflect the entire range of Ca^{2+} permeability observed within the TRP superfamily (Fig. 1): the highly Ca^{2+} -selective TRPV6, the nonselective, Ca^{2+} -permeable TRPV1, and the Ca^{2+} -impermeable TRPM4 (Fig. 4).

The Ca^{2+} -Selective Pore of TRPV6

TRPV6 and its close homolog TRPV5 are the only two highly calcium-selective TRP channels, with estimated $P_{\text{Ca}^{2+}}/P_{\text{Na}^{+}}$ values >100 (Gunthorpe et al. 2002). Under normal ionic condi-

tions, the inward current through TRPV6 is almost fully carried by Ca^{2+} ions (Nijenhuis et al. 2005). Earlier work combining structural modeling, the substituted cysteine accessibility method, and patch-clamp recordings, had already established that a short stretch of amino acids in the S5–S6 linker, TIIDG, forms the selectivity filter (Nilius et al. 2001), with the aspartate residue (D541) forming the narrowest part of the open pore, with an estimated diameter of 5.4 Å. In addition, it was shown that substituting D541 (and the corresponding D542 in TRPV5) by a noncharged residue abolishes calcium selectivity and blocks by other divalent cations (Voets et al. 2003). Many of these prestructural findings were corroborated by recent structures obtained using both X-ray crystallography and cryo-EM. Indeed, crystal structures of TRPV6 obtained in the absence and presence of Ca^{2+} confirm that the narrowest part of the pore is formed by the four side chains of D541, with a minimum interatomic distance of 4.6 Å, and that these aspartate side chains form a high-affinity site coordinating a single dehydrated Ca^{2+} ion (Saitome et al. 2016). Side-chain hydroxyls of T838 (also part of the TIIDG motif) participate in a second, lower-affinity binding site for a dehydrated Ca^{2+} ion 6–8 Å below the ring of aspartates, whereas a third binding site in the vestibule below the selectivity filter may accommodate (partly) hydrated Ca^{2+} . Note that, similar to other highly Ca^{2+} -selective channels, TRPV6 permeates large currents carried by Na^{+} , K^{+} , and other monovalent cations in the absence of extracellular Ca^{2+} ions. In the wild-type channel, these monovalent currents are blocked by low micromolar concentrations of Ca^{2+} or Cd^{2+} , and this inhibition is abolished when mutating D541 to a noncharged residue (Nilius et al. 2001).

Based on these results, a “knock-off” mechanism of Ca^{2+} permeation was proposed (Saitome et al. 2016), where the binding of Ca^{2+} in an electronegative ring formed by D541 side chains is highly energetically favorable compared to the charge repulsion between the acidic side chains in the unbound state. This implicates that removal of Ca^{2+} from this site leads to immediate replacement by another Ca^{2+} ion, a process that

Figure 4. Structure of transient receptor potential (TRP) channel pores. Comparison of the pore domains of TRPM4 (*left*, Ca^{2+} -impermeable), TRPV1 (*middle*, Ca^{2+} -permeable, nonselective), and TRPV6 (*right*, Ca^{2+} -selective). Structures are created from data in Winkler et al. (2017) (TRPM4), Liao et al. (2013) (TRPV1-apo state), and Hughes et al. (2018a) (TRPV6) (see text for more details).

may be facilitated by recruitment of Ca^{2+} ions from the external solution by the highly electro-negative extracellular vestibule of TRPV6. In the absence of extracellular Ca^{2+} ions, the ring of aspartates is no longer occupied by tightly bound Ca^{2+} ions, thus allowing large fluxes of monovalent cations.

Interestingly, comparison of the structures of the closed and open TRPV6 pore did not reveal any significant conformational changes at the level of the selectivity filter. In contrast, there was significant widening of the lower part of the pore lined by S6 residues, apparently induced by an α - to π -helical transition in the middle of S6 (McGoldrick et al. 2018). Therefore, in the case of TRPV6, the selectivity filter is static and solely involved in ion selectivity, whereas channel opening is controlled by a single (lower) gate.

The Ca^{2+} -Permeable Nonselective Pore of TRPV1

TRPV1, like the related TRPV2–TRPV4 and the majority of other TRP channels (Owsianik et al. 2006), encompasses a pore that is nonselective for cations and shows significant Ca^{2+} permeability. Several studies report $P_{\text{Ca}^{2+}}/P_{\text{Na}^+}$ values for TRPV1 of approximately 10 (Caterina et al. 1997) and, under physiological ionic conditions,

approximately 10% of the inward current is carried by Ca^{2+} ions. Very similar permeability properties have been described for TRPV2–TRPV4.

The selectivity filter of these four Ca^{2+} -permeable nonselective TRPV channels contains the motif TIGMGD/E (Deng et al. 2018). Mutating the aspartate in TRPV1 to a non-charged residue significantly reduces $P_{\text{Ca}^{2+}}/P_{\text{Na}^+}$, indicative of the essential role of negative charges in the selectivity filter for Ca^{2+} permeation (García-Martínez et al. 2000). However, in contrast to TRPV5 and TRPV6, the negatively charged side chain is not located at the narrowest part of the pore, which instead is lined by neutral methionine side chains and backbone carbonyls (Liao et al. 2013). As a result, TRPV1–TRPV4 lack a high-affinity Ca^{2+} -binding site in the selectivity filter, thus explaining the poor discrimination between mono- and divalent cations. Note that in the unliganded state this narrowest constriction has a minimum diameter of 4.6 Å (Liao et al. 2013), which increases to 7.6 Å in the structure with RTX/DkTx (Cao et al. 2013). These dynamics at the level of the selectivity filter may contribute to the reported alterations in pore permeability properties upon strong stimulation of TRPV1, although further research is required to establish this.

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The Ca^{2+} -Impermeable Pore of TRPM4

TRPM4 and the related TRPM5 are unique within the TRP superfamily in that they only conduct monovalent cations and are impermeable to Ca^{2+} (Liman 2007). Recent structures of TRPM4 reveal a normal ion-conducting pore with two constriction sites, similar to many other TRP channel structures. Although these structures do not clearly establish why TRPM4 fails to allow detectable permeation of Ca^{2+} ions, different mechanisms may contribute. First, the short but wide selectivity filter of TRPM4 consists of the motif FGQ, and thus does not contain any negatively charged amino acid, unlike other TRP channels (Winkler et al. 2017). Importantly, mutating the glutamine Q in the selectivity filter to a glutamate indeed resulted in detectable Ca^{2+} permeation through TRPM4 (Nilius et al. 2005), establishing the importance of negative charge. Nevertheless, TRPM2 and TRPM8 contain the same FGQ motif in their pore region, but are nonetheless able to permeate Ca^{2+} . A different selectivity filter configuration, as their pore loop is one amino acid shorter compared to TRPM4 and TRPM5, might explain this conundrum. Second, residues in the lower gate may also play a role in the monovalent ion selectivity. Interestingly, a serine that is shown to interact with permeating Na^+ ions in the lower pore region is only conserved in TRPM4 and TRPM5, while other TRPM subfamily members contain an isoleucine at this position (Duan et al. 2018c). Note that TRPM4 contains a negative charge at the top of the ion permeation pathway, just outside the selectivity filter. This electronegative mouth may serve to attract cations, but the local negative charge is likely not strong enough to promote dehydration of divalent cations, which is necessary to permeate the pore (Duan et al. 2018c).

STRUCTURAL INSIGHTS INTO Ca^{2+} REGULATION

In addition to these insights into the ion permeation pathway, recent TRP channel structures also provide interesting novel structural clues toward understanding Ca^{2+} -dependent processes that regulate channel gating (Figs. 2 and 5).

Intracellular Ca^{2+} -Binding Site in TRP Channels

The activity of several members of the TRPM subfamily is enhanced by a direct binding of Ca^{2+} ions to the channel protein. In particular, the Ca^{2+} -impermeable TRPM4 and TRPM5 are activated by a cytosolic Ca^{2+} rise in a voltage-dependent manner. Cryo-EM structures of TRPM4 were obtained in both Ca^{2+} -bound and -unbound conformations, revealing a Ca^{2+} -binding site located at, and coordinated by, side chains of TM domains S2 and S3 (Fig. 5; Autzen et al. 2018). Based on these structures, Ca^{2+} binding to TRPM4 triggers a cascade of small conformational effects throughout the whole channel, to prime the channel for voltage-dependent opening.

Interestingly, several of the residues implicated in Ca^{2+} binding in TRPM4 are conserved only in other members of the TRPM family that show Ca^{2+} -dependent gating, namely, TRPM2, TRPM5, and TRPM8. Moreover a high-resolution structure of the ADPR-activated TRPM2 channel in the presence of Ca^{2+} (Huang et al. 2018; Wang et al. 2018) revealed, similar to TRPM4, a Ca^{2+} -binding site at the cytosolic side of S2 and S3 of both the human and the zebrafish TRPM2 orthologs (Fliegert et al. 2018). Structural comparison with the apo structure indicates a repositioning of S3 upon Ca^{2+} binding, enabling movement of the S4–S5 linker to facilitate activation. At the functional level, earlier studies showed that mutation of residues in this region indeed impairs activation of TRPM2 in the presence of Ca^{2+} (Winking et al. 2012). Although structural data to confirm this are not yet available, is likely that this cytosolic Ca^{2+} -binding site is also present in TRPM5 and TRPM8. In support of this notion, mutations of the corresponding amino acids in TRPM8 abolish the Ca^{2+} -dependent activation by icilin, while leaving responses to cold and menthol intact (Chuang et al. 2004).

In addition to these TRPM channels, structural evidence has also been provided for intracellular Ca^{2+} -binding sites in other TRP channels, including a well-defined EF hand in the proximal carboxyl terminus of TRPP1–PKD2, implicated in Ca^{2+} -dependent activation

Figure 5. Structural elements underlying Ca^{2+} -dependent regulation of transient receptor potential (TRP) channels. (*Left*) Interaction between phosphatidylinositol biphosphate (PIP_2) and the PIP_2 -binding pocket in TRPV5, which is formed by residues from the amino terminus (Arg302, Arg305), the S4–S5 linker (Lys484), and the S6-helix (Arg584). PIP_2 is shown in red, interacting amino acids in salmon. (*Center*) A Ca^{2+} ion (red) interacting with TRPM4 via residues Glu828, Gln831 (from S2), and Asn865 and Asp868 (from S3). (*Right*) Ca^{2+} ions (red) bound to calmodulin (pink), interacting with the carboxyl terminus of TRPV5 via an interaction with His699, Trp702, and Thr709 (salmon).

of the channel (Yang et al. 2016; Grieben et al. 2017; Wilkes et al. 2017).

Cytosolic Interactions between TRPV Channels and Ca^{2+} -CaM

CaM consists of two globular domains, connected by a flexible linker. Two Ca^{2+} ions can bind to the EF-hand motifs of every domain, resulting in a maximum of four Ca^{2+} ions bound to a single CaM. Ca^{2+} binding induces a conformational change in the backbone of CaM, allowing interaction with a wide range of target proteins, including TRP channels. The number of TRP channels interacting with CaM is large (see trpchannel.org/proteins/show?id=calmodulin), and the effects it can trigger are equally diverse. Furthermore, there can be multiple CaM interaction sites within one TRP channel, where binding can mediate divergent effects on channel function (Zhu 2005).

The first high-resolution insights into CaM–TRP channel interactions were provided by a crystal structure of Ca^{2+} -bound CaM in complex with a 35-residue carboxy-terminal segment of TRPV1 (Lau et al. 2012). This study revealed that CaM clasps around the helical carboxyl terminus of TRPV1 in a Ca^{2+} -dependent manner, and that disruption of the interaction slows Ca^{2+} -dependent channel desensitization,

although a second CaM interaction site in the amino terminus was found that plays a more prominent role in this process.

Recently, structures were determined for the full-length TRPV5 and TRPV6 in a complex with Ca^{2+} -bound CaM (Hughes et al. 2018b; Singh et al. 2018b). In the structure of TRPV5 saturated with Ca^{2+} -CaM, both lobes of CaM interact with different sections of the carboxyl terminus via numerous hydrophobic contacts (Hughes et al. 2018b). This results in an occupation of the tryptophan amino acids in the lower gate of TRPV5, directly obstructing ion permeation (Fig. 5). The critical role of this interaction in Ca^{2+} -CaM-mediated effects was established in functional studies showing that mutating the critical tryptophan residues abolishes Ca^{2+} -CaM-dependent channel inhibition. This mechanism represents an important negative feedback loop that regulates calcium influx in Ca^{2+} -reabsorbing epithelia. A very similar structural mechanism has been put forward for the close homolog TRPV6, as structures showed a comparable binding of CaM to the carboxyl terminus of TRPV6, with the C-lobe of CaM inserted into the pore's intracellular entrance (Singh et al. 2018b). Currently, there is insufficient structural information to establish whether this mechanism is conserved in other TRP channels.

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TRP Channel Interactions with PIP₂

The level of PIP₂, the most abundant phosphoinositide in the inner leaflet of the plasma membrane of mammalian cells, is typically around 1% of total plasma membrane anionic phospholipids (Czech 2000). Despite being a minor constituent of the plasma membrane, PIP₂ has a major impact on the functioning of many, if not all TRP channels (Voets and Nilius 2007). Both a rise in intracellular Ca²⁺ levels, acting via Ca²⁺-dependent PLC (see above), as well as activation of PLC downstream of GPCRs and receptor tyrosine kinases, can result in a rapid decrease in PIP₂ levels, constituting a common and powerful mechanism of TRP channel regulation (Rohacs 2014). Most TRP channels are inhibited when PIP₂ levels decrease, although inhibitory effects of PIP₂ have also been observed in some TRP channels (Qin 2007; Rohacs 2014). Recently, by combining cryo-EM with nanodisc technology, several structures have been obtained of TRP channels in a biological membrane-like environment, allowing structural characterization of PIP₂ interaction sites.

In the case of TRPV1, a PIP₂ molecule was identified in the binding pocket for vanilloids such as capsaicin and RTX, suggesting that these activating ligands must displace PIP₂ to bind and activate the channel. A cryo-EM structure of TRPV5 in the presence of PIP₂ revealed a single PIP₂ molecule interacting with the N-linker, the S4–S5 linker, and the S6 helix of the channel (Hughes et al. 2018b), a finding that correlated with molecular dynamics predictions of a PIP₂-binding site in the close homolog TRPV6 (Fig. 5). Comparing structures of TRPV5 with and without bound PIP₂ revealed important conformational rearrangements in the channel (Hughes et al. 2018b). Upon binding of PIP₂ the aspartate residues in the selectivity filter change orientation, clearing the initial road of the ion-conducting pathway. In addition, the lower gate of the pore extends wide enough to allow the flow of hydrated Ca²⁺ ions. These results form a beautiful example of how high-resolution structures can elucidate previously mysterious mechanisms of TRP channel regulation.

CONCLUSION AND OUTLOOK

TRP channel research benefited greatly from the recent resolution revolution. TRPV1 was the first full-length high-resolution membrane protein structure that was unraveled without the need for crystallizing the protein, representing a milestone for the fields of structural biology and TRP channel biology alike.

Thanks to the growing number of channel structures, including members of every TRP subfamily, subgroup, we are now able to visualize different conformational states leading to channel gating, rationalize ion permeation and define the details of TRP channel interactions with regulatory proteins, lipids, and ions. In this review, we have selectively highlighted novel insights related to Ca²⁺ permeation and Ca²⁺-dependent regulation of these channels. Undoubtedly, these novel insights and further structural work will not only fuel fundamental research into TRP channel function and regulation, but also increase our understanding of the etiology of TRP channel-related human diseases and assist in the rational design of pharmacological therapies for such diseases.

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