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Roles of Intramolecular Interactions in the Regulation of TRP Channels

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Contents

Abstract The transient receptor potential (TRP) channels, classified into six (-A, -V, -P, -C, -M, -ML, -N and -Y) subfamilies, are important membrane sensors and mediators of diverse stimuli including pH, light, mechano-force, temperature, pain, taste, and smell. The mammalian TRP superfamily of 28 members share similar membrane topology with six membrane-spanning helices (S1–S6) and cytosolic N-/C-terminus. Abnormal function or expression of TRP channels is associated with cancer, skeletal dysplasia, immunodeficiency, and cardiac, renal, and neuronal diseases. The majority of TRP members share common functional regulators such

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as phospholipid PIP2, 2-aminoethoxydiphenyl borate (2-APB), and cannabinoid, while other ligands are more specific, such as allyl isothiocyanate (TRPA1), vanilloids (TRPV1), menthol (TRPM8), ADP-ribose (TRPM2), and ML-SA1 (TRPML1). The mechanisms underlying the gating and regulation of TRP channels remain largely unclear. Recent advances in cryogenic electron microscopy provided structural insights into 19 different TRP channels which all revealed close proximity of the C-terminus with the N-terminus and intracellular S4–S5 linker. Further studies found that some highly conserved residues in these regions of TRPV, -P, -C and -M members mediate functionally critical intramolecular interactions (i.e., within one subunit) between these regions. This review provides an overview on (1) intramolecular interactions in TRP channels and their effect on channel function; (2) functional roles of interplays between PIP2 (and other ligands) and TRP intramolecular interactions; and (3) relevance of the ligand-induced modulation of intramolecular interaction to diseases.

Keywords 2-APB · Cannabionoid · Channelopathy · Cryo-EM · Intramolecular interaction · PIP2 · TRP channel

Abbreviations

2-APB	2-Aminoethoxydiphenyl borate
ARDs	Ankyrin repeat domains
BTDM	(2-(Benzo[d][1,3]dioxol-5-ylamino)thiazol-4-yl)((3 S,5 R)-3,5-dimethylpiperidin-1-yl)methanone
CBD	Cannabinoid
Cryo-EM	Cryogenic electron microscopy
ER	Endoplasmic reticulum
HEK	Human embryonic kidney
HTH	Helix-turn-helix
MHRs	TRPM homology regions
ML-SA1	Mucolipin synthetic agonist 1
PIP2	Phosphatidylinositol 4,5-bisphosphate
RTx	Resiniferatoxin
TRP	Transient receptor potential
VSLD	Voltage-sensing like domain

1 Introduction

The transient receptor potential (TRP) channels were named after a mutant fruit fly with abnormal phototransduction (Cosens and Manning 1969). Based on amino acid sequence homology, the TRP superfamily is grouped into eight subfamilies,

including polymodal ankyrin (TRPA), calcium-selective vallinoid (TRPV), polycystic kidney disease associated polycystin (TRPP), receptor-operated canonical (TRPC), diverse function melastatin (TRPM), endomembrane mucolipin (TRPML), invertebrates and poikilothermic vertebrates exclusive NOMP (TRPN), and yeast specific (TRPY) (Li 2017). Among different subfamilies, sequence homology is modest and biophysical properties including cation selectivity differ (Li 2017). The 28 mammalian TRP channels function as polymodal cellular sensors that integrate and mediate diverse environmental and physiological stimuli in the processes of vision, somatosensation, gustation, ion equilibrium, olfaction, and audition (Damann et al. 2008). Generally, TRP channels are non-selective cation channels permeable to Ca^{2+}, Na^{+}, and K^{+} with TRPV5 and TRPV6 are the most Ca-selective (Peng et al. 2018). The gatekeepers for human magnesium homeostasis are TRPM6 and TRPM7 (Schlingmann et al. 2007). Defective TRP channels caused by mutations or altered expression result in inherited or acquired human diseases, or channelopathies (Nilius 2007). It is a valuable aim of research on TRP channels to elucidate the mechanisms underlying how stimuli-induced variations in the phospholipid composition, membrane potential, and post-translational modifications as well as natural ligands regulate the function of TRP channels.

Correct folding is critical for a protein to be fully functional (Dill et al. 2008) and requires maintenance of energetically favorable intramolecular interactions, usually through disulfide bridges, hydrogen bonds, ionic bonds, or van der Waals (hydrophobic) forces (Alberts et al. 2002). For the past decade, technical breakthroughs in cryogenic electron microscopy (cryo-EM) and improved image-processing algorithms unprecedentedly revealed the atomic details of most TRP channels (Autzen et al. 2018; Cao et al. 2013; Chen et al. 2017; Dang et al. 2019; Deng et al. 2018; Diver et al. 2019; Dosey et al. 2019; Duan et al. 2018a, b, 2019; Fan et al. 2018; Hirschi et al. 2017; Liao et al. 2013; McGoldrick et al. 2018; Paulsen et al. 2015; Ruan et al. 2021; Shen et al. 2016; Shimada et al. 2020; Song et al. 2021; Su et al. 2018; Tang et al. 2018; Wang et al. 2018). Despite the low amino acid sequence homology found among the TRPs, strikingly they share significant overall structural similarities, such as tetrameric architecture (Fig. 1), six transmembrane spans (S1–S6), and intracellularly localized N- and C-termini (Fig. 2). The S1–S4 helices together resemble the voltage-sensing domain of potassium channels, whereas S5 and S6 helices together with the extracellular loop in between form the pore region (Fig. 1). The S4–S5 linker bridges between the S1–S4 and S5–S6 domains. However, the pore-forming S5-loop-S6 protrudes to the S1–S4 domain of a neighboring subunit, rather than being vicinal to the intramolecular S1–S4 domain (Fig. 1), an arrangement called domain swapping seen in the physiologically relevant structures of TRP channels, which is important for maintaining their channel function. In comparison with TRPPs and TRPMLs, which possess an extended S1–S2 loop and relatively short cytosolic domains, TRPA, TRPVs, TRPCs, and TRPMs are evolutionally closer to each other and contain relatively long cytosolic domains (Fig. 1). Physical proximity among different parts within a TRP subunit is revealed from its structure at atomic resolutions, suggesting the presence of intramolecular interactions.

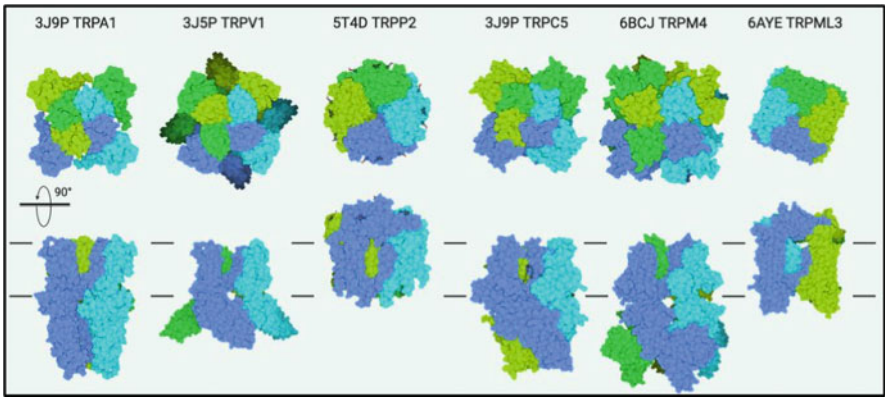

Fig. 1 Tetrameric conformation of six mammalian TRP channels. Four subunits are highlighted in separate colors. PDB numbers of corresponding TRP channels are labeled. Upper and lower panels show the view from the top and side (with 90° rotation), respectively. The lipid bilayer is illustrated by short lines in black

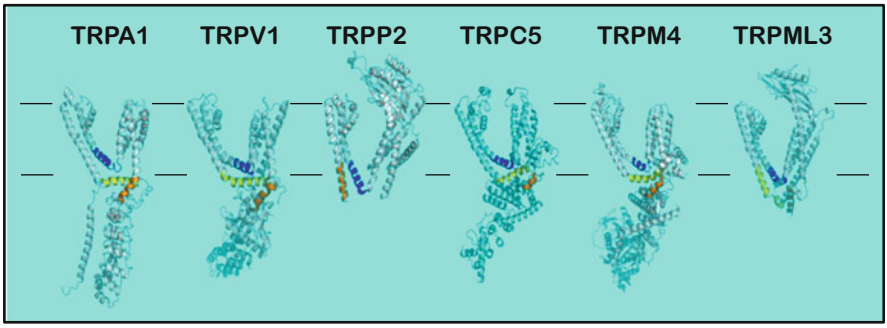

Fig. 2 Monomers of six representative mammalian TRP channels. Pre-S1 helix, S4–S5 linker, and TRP(-like) helix are in orange, purple, and yellow, respectively. "Out" and "In" stand for extracellular/luminal and intracellular side, respectively

Because crystallography and cryo-EM provide high-resolution snapshots of structures under experimental conditions they may not be able to resemble dynamics of TRP and other proteins investigated in living cells. In particular, physiological TRP intramolecular interactions should be investigated using living cells under physiological conditions. We recently characterized intramolecular interactions, functional implication and regulation by phosphatidylinositol 4,5-bisphosphate (PIP2) in several TRPs from different subfamilies including TRPV6, TRPV1, TRPP3, TRPP2, TRPC4, and TRPM8 channels (Cai et al. 2020; Zheng et al. 2018), in addition to previous studies (Nilius et al. 2008). Other research groups also reported intramolecular interactions and their functional importance in thermosensitive TRPVs (Boukalova et al. 2010; Liao et al. 2013), TRPP3 (Ng et al. 2019), TRPP2 (Vien et al. 2020), and TRPM4 (Xian et al. 2018, 2020).

The present review will summarize and provide insights into TRP intramolecular interactions and their involvements in the regulation of channel function.

2 Intramolecular Interactions in TRP Channels

Thanks to the recent cryo-EM revolution, structural details on TRP proteins at atomic levels were revealed, which otherwise are difficult to be crystalized, except for cytosolic domains (Li et al. 2011). Since the resolution of rat TRPV1 structure by cryo-EM (Liao et al. 2013), those of nearly 20 different TRPs have been mapped at near-atomic resolutions (Cao 2020), which suggested the presence of both shared and distinct intramolecular interactions.

2.1 Shared Intramolecular Interactions Mediated by Conserved Residues Across TRP Members and Species

The very first high-resolution structure resolved by cryo-EM was a truncated but functional rat TRPV1 at 3.4 Å, missing part of the N- and C-termini and S5–S6 loop (Liao et al. 2013). The structure revealed physical proximity of the TRP helix (Fig. 2, yellow helix) to the S4–S5 linker (purple helix) and pre-S1 helix (orange helix) and of the S4–S5 linker to S5 (Liao et al. 2013). Based on the structure, it was proposed that a hydrogen bonding between the side chain of W697 (in the TRP helix) and back bone of F559 (S4–S5 linker), and between R701 (TRP helix) and Q423 (pre-S1 helix) mediates the interaction of the TRP helix with the S4–S5 linker and pre-S1 helix, respectively. A subsequent functional study using over-expressed *Xenopus laevis* oocytes finds that R701 pairs with W426, rather than Q432, to mediate the TRP helix/pre-S1 helix interaction, presumably through a cation–π bonding, that is required for the channel function. The cation–π bonding is a type of non-covalent molecular interaction within an electron abundant benzene ring and positively charged side chain of two amino acids. The electronic force of cation–π bonding would stabilize the protein structure regionally. Importantly, in the capsaicin-activated state, the distance between the two residues in the W426:R701 pair is not much affected whereas Q432 moves away from R701 (Liao et al. 2013; Zheng et al. 2018). Thus, the TRP helix/pre-S1 helix interaction in TRPV1 is well-supported by both functional studies in living cells and structural studies using cryo-EM (Cao et al. 2013; Liao et al. 2013; Zheng et al. 2018).

The structures of over 20 different TRP members have now been resolved and all show close proximity of the TRP (TRP-like) helix to pre-S1 helix and S4–S5 linker (Cao 2020) (Fig. 2). By means of electrophysiology and molecular biology, the functionally important TRP/pre-S1 helix interaction has been validated in TRPV6, TRPP3, TRPP2, TRPC4, and TRPM8, and the TRP helix/S4–S5 linker interaction in

TRPV4, TRPV6, and TRPM4 (Cai et al. 2020; Xian et al. 2020; Zheng et al. 2018). Briefly, currents mediated by these TRP channels are functionally examined by two-electrode voltage clamp and patch clamp using *Xenopus* oocytes and mammalian cells, respectively. In addition, the physical association of the TRP helix with pre-S1 or S4–S5 linker has been verified by co-immunoprecipitation, co-immunostaining, and purified peptide in vitro binding. It remains to be determined whether these functionally important intramolecular interactions are shared by other TRP channels. S5, S6, and S5–S6 loop are in close vicinity and together form the so-called pore region. While the S5–S6 loop contains the selectivity filter (or upper pore gate) and the S6 helix contains the lower pore gate (or pore gate) the exact role of S5 is unclear. Our recent study showed that the TRPV6 S5 interacts with S6 through a pair of residues R532:D620 that should form a salt bridge and is critical for maintaining the basal channel function (Cai et al. 2021). In a subunit of a tetrameric TRP channel, the S1–S4 helices stay together while the S5–S6 helices extend to be adjacent to the S1–S4 helices of a neighboring subunit. This interesting arrangement is called domain swapping and may be a requirement under physiological conditions (Singh et al. 2017), but whether and how it affects channel gating remains unclear. The potential importance of the S5/S6 helix interaction in other TRP channels has not been reported yet.

Although the overall sequence similarities among the TRP channels are pretty low (~16%) (Palovcak et al. 2015) the residues that were shown by functional studies to be involved in the interactions of the TRP helix with the pre-S1 helix and S4–S5 linker are conserved among different TRP subfamilies (Fig. 3) (Cai et al. 2020; Zheng et al. 2018). For example, an aromatic residue (W or F) in pre-S1 is conserved in all TRP members (Fig. 3) and mediates interactions with the TRP helix. A cationic (K or R) or hydrophobic residue in the S4–S5 linker is also conserved in all TRP members except for TRPC1 (Fig. 3) and mediates interactions with the TRP helix. The residue in the TRP (-like) helix involved in these interactions is located within a shared motif **W/Y**XXX**Φ**, where X indicates any amino acid and Φ indicates K/R except for TRPV4 (W) and TRPV5/TRPV6 (I/V) (Fig. 3) (Cai et al. 2020; Montell 2001; Zheng et al. 2018). The functional roles of the corresponding conserved residues in other TRP channels remain to be determined. Of note, these conserved residues that were shown to mediate the interactions within TRP helix/pre-S1 or TRP helix/S4–S5 linker by functional studies are not fully consistent with structures resolved by means of cryo-EM or crystallography, possibly due to different study models and experimental conditions (Deng et al. 2018; Saotome et al. 2016). Live cells, which would preserve the native conditions for TRP channels, are used as models to examine the channel function, while structures captured by cryo-EM are snapshots of dynamic TRP channels under non-physiological conditions supplemented by artificial components. Also, in contrast to full-length constructs encoding TRP channels studied by electrophysiology, structural studies frequently utilize truncation(s) or mutations to increase the protein yield or stability and are carried out under unnatural conditions. These facts may explain the inconsistencies between the functional and structural studies.

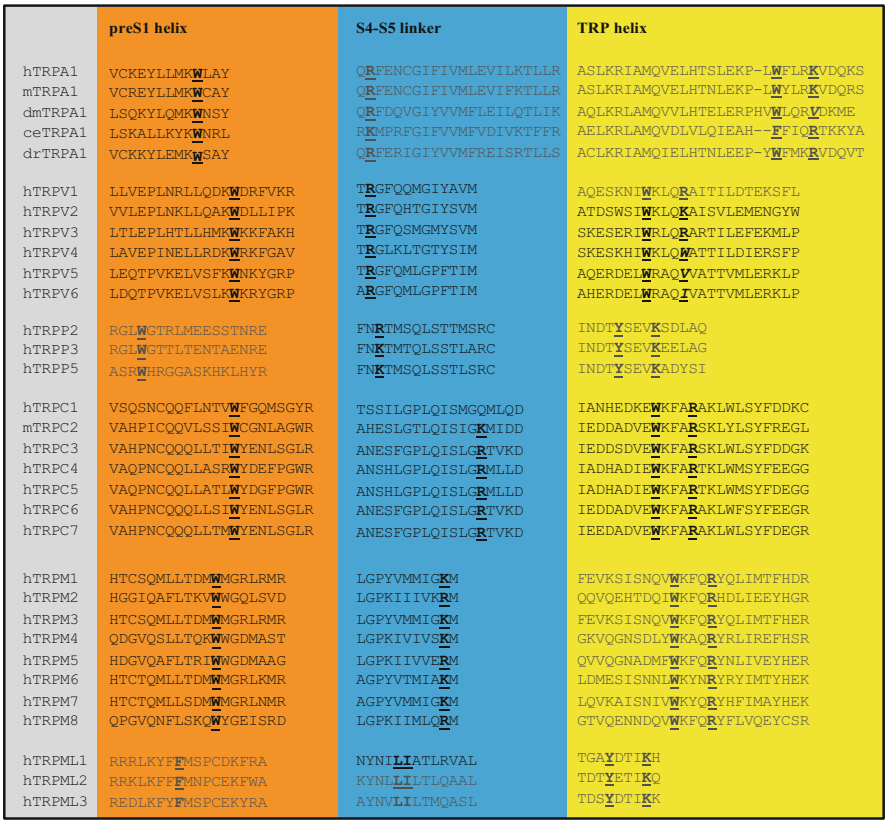

Fig. 3 Sequence alignments of pre-S1, S4–S5 linker and TRP(-like) helix across mammalian TRP channels. Conserved amino acid residues suggested to be involved in interactions are bolded and underlined. The species for TRP channels are listed as "h" for human, "m" for mouse, "dm" for *drosophila melanogaster* (fruit fly), "ce" for *Caenorhabditis elegans*, and "dr" for *Danio rerio* (zebrafish)

While similar physical interactions of the TRP helix with the pre-S1 helix and S4–S5 linker and similar residues mediating the corresponding interactions are shared among different TRP channels, their impacts on the channel function can be distinct (Fig. 4). For instance, in TRPV1, TRPP3, TRPP2, TRPC4, and TRPM8 expressed in oocytes or cultured human embryonic kidney (HEK) 293 cells, the TRP helix to pre-S1 interaction is required for the channel function or activation (Zheng et al. 2018), and the TRP helix to the S4–S5 linker interaction in TRPM4 positively correlates with the function in HEK293 cells (Xian et al. 2020). In contrast, in TRPV6, these interactions were found to be autoinhibitory, i.e. disruption of either of these interactions resulted in significant gain-of-function in *Xenopus* oocytes, cultured HEK293 cells, and zebrafish embryos (Cai et al. 2020). Similar to TRPV6, mutations in the TRPV4 S4–S5 linker that disrupts the presumable S4–S5 linker/TRP helix interaction retard budding yeast growth (Teng et al. 2015), by inducing

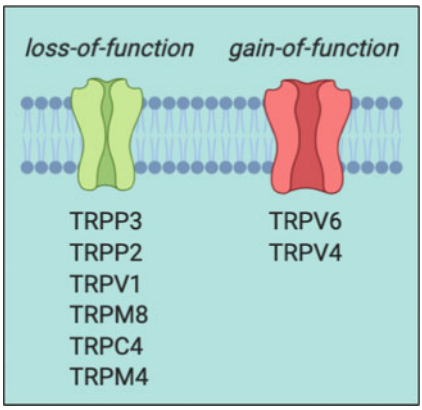

Fig. 4 Functional effects of disrupted intramolecular interactions. TRP channels are classified, respectively, based on functional studies. The TRP channels are presented as membrane-embedded with closed (left, loss-of-function) or enlarged (right, gain-of-function) channel pore

excessive ion influx (Loukin et al. 2010). Thus, whether disruption of these interactions inhibits or activates the channel function would depend on the specific conformational changes induced by a mutation.

2.2 *Subfamily- or Member-Specific Intramolecular Interactions*

Besides the structural arrangements that are shared across TRP subfamilies, there are other intramolecular interactions within transmembrane, extracellular or cytosolic domains that were reported for an individual TRP(s) or for members within a TRP subfamily (Fig. 5). Since all TRP channels are transmembrane proteins, the lipid bilayer naturally divided a TRP channel protein into three parts, namely extracellular/luminal domains, transmembrane domains, and intracellular domains based on which we here group intramolecular interactions.

2.2.1 Interactions Within Extracellular/Luminal Domains

The TRPPs are featured with the presence of a polycystin domain, a large extracellular loop between the S1 and S2 helices (Figs. 1 and 2). Several intramolecular interactions, including cation–π interaction (R322:F423), disulfide bonding (C331:C344), and hydrogen bonding (R325:T419), have been revealed by cryo-EM in an engineered human TRPP2 with N- and C-terminal truncations (198–703 vs. 1–968 for full-length protein) (Shen et al. 2016). Interestingly, mutation C331S, which presumably breaks down the C331:C344 disulfide bond, destabilizes the polycystin domain and overall protein architecture (Vien et al. 2020). Mutations disrupting the potential disulfide bonding abolish the ciliary TRPP2 function through impairment in voltage gating (Vien et al. 2020). Note that these residue pairs are conserved in the

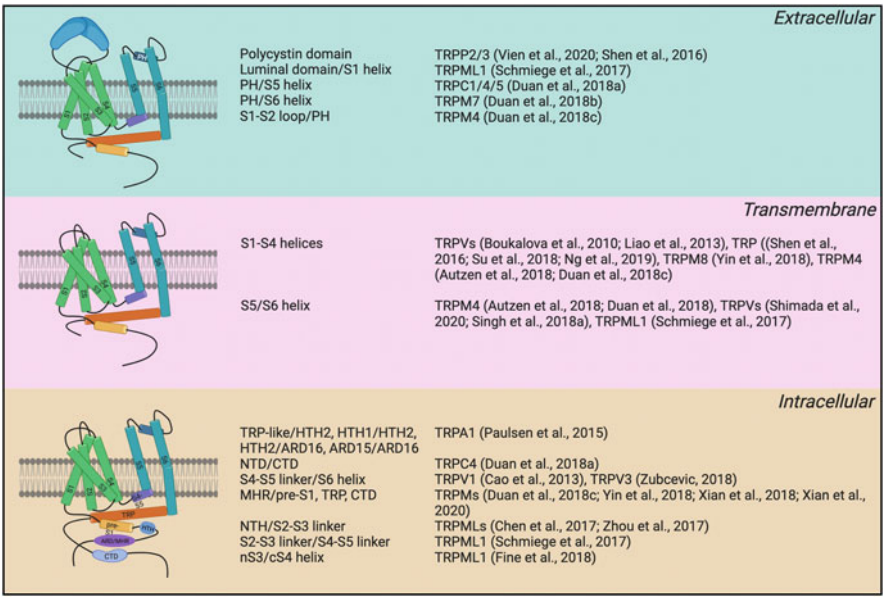

Fig. 5 Unique intramolecular interactions among different layers of TRP channels. Full names of abbreviated domains are pore helix (PH), ankyrin repeat (ARD), helix-turn-helix (HTH), N-terminal domain (NTD), C-terminal domain (CTD), melastatin homology region (MHR), N-terminal helix (NTH). The extracellular, transmembrane, intracellular domains involved in intramolecular interactions and related references are listed

other two TRPP members (TRPP3 and TRPP5) and have similar functional importance for TRPP3 (Vien et al. 2020). The TRPP2 polycystin domain was also found to bind to the extracellular S3–S4 linker and the second pore helix of the S5–S6 loop (F646-A652) through π–cation interaction (Shen et al. 2016).

TRPC4 does not have a large S1–S2 loop but its S5–S6 loop contains a disulfide bond formed by two cysteine residues (Duan et al. 2018a). This covalent interaction presumably maintains the channel in its basal state because disrupting the bond by dithiothreitol or other reducing chemicals activates the channel (and TRPC5 as well). Further, alanine substitution of both cysteines (C549A + C554A) in TRPC4 abolishes redox-related activation while retaining the englerin A-induced activation, whereas single C-to-A mutation (C549A or C554A) abolishes the function (Duan et al. 2018a). In contrast, TRPC5 with alanine substitution(s) of one or both corresponding cysteine(s) (C553A or C558A or C553A + C558A) is insensitive to DTT or englerin A (Duan et al. 2019). The underlying mechanisms are unclear. The two cysteine residues in the TRPC5 pore loop presumably form a disulfide bond and mediate its redox-induced activation, which is, however, inconsistent with a previous report that alanine substitution of either cysteine residue, which breaks the disulfide bond, constitutively opens the channel in HEK cells (Xu et al. 2008). Of note, these two cysteine residues are conserved in TRPC1, TRPC4, and TRPC5 but

not in other redox-insensitive TRPCs, indicating their distinctive regulation by redox status (Duan et al. 2018a). Interestingly, a similar cysteine pair was also found in the pore loop of all TRPMs (Duan et al. 2018c). Serine substitution of either of the two cysteine residues found in the corresponding region of TRPM7 somehow linearizes the I–V relationship from typical outward rectification (Duan et al. 2018b). A number of π–π and π–cation interactions were revealed between the S1–S2 loop and pore-helix of TRPM4 based on structural data (Duan et al. 2018c) but the functional roles of these interactions remain to be determined. The TRPML channels also possess a large luminal S1–S2 loop, consisting of four α helices and seven β strands (Figs. 1 and 2) (Schmiege et al. 2017). It was shown that its fourth α helix in the luminal domain links to the extended S1 helix through salt bridge and hydrogen bond (Schmiege et al. 2017). In the extracellular/luminal domains of TRPP, -C, -M and -ML channels, several intramolecular interactions have been characterized by functional and structural studies. More intramolecular interactions may potentially be identified in these channels and other TRPs as well.

2.2.2 Transmembrane Domains

Thermosensitive TRPVs (-V1 to -V4) possess hydrophobic residues located in the S1, S3, and S4 helices that are clustered, believed to stabilize the S1–S4 helices and are required for channel function (Boukalova et al. 2010; Liao et al. 2013); these channels also possess conserved hydrophilic aspartic acid in S5 and threonine in S6 that may be important specifically for channel opening (Shimada et al. 2020; Singh et al. 2018a). The S4 helix in TRPPs, as a voltage-sensing like domain (VSLD) reminiscent of voltage-gated channels, contains 2–3 cationic residues thought to interact with an aromatic or anionic residue located in S1, S2, or S3 (Shen et al. 2016; Su et al. 2018). Subsequent Rosetta structural modeling, a software for predictions of protein structure and function, together with the characterization of gating charge and channel function in HEK and SF9 insect cells showed that cationic residues in S4 mediate cation–π interaction with aromatic residues in S2 during polymodal activation of TRPP3 (Ng et al. 2019). Aromatic residues also form π–π interactions between S4 and S5 in TRPP2 and TRPP3 (Su et al. 2018). The thermosensitive TRPM8 S4 also acts as a VSLD and its cationic residues form salt bridges with anionic residues in S1, S2, or S3 (Yin et al. 2018). Also, a tryptophan in TRPM8 S3 interacts with aromatic residues in S4 and S5, presumably forming π–π bonds, which was thought to be important for cooling compound-induced channel gating (Yin et al. 2019). π–π interactions in S1/S4 and S5/S6 as well as salt bridges linking S4 to S2 and S3 in human TRPM4 were structurally revealed but with unclear functional implication (Autzen et al. 2018; Duan et al. 2018c). The van der Waals force formed by a valine:valine and valine:phenylalanine pair in S5/S6 of TRPML1 was thought to play an important role in stabilizing the channel in a closed state (Schmiege et al. 2017), and a V-to-P substitution in S5 resulted in gain of function of the channel, as shown by use of cell and animal models (Di Palma et al. 2002; Dong et al. 2009).

2.2.3 Intracellular Domains

The TRPA1 N-terminus contains as many as 18 ankyrin repeat domains (ARDs, a solenoid-like region mediating protein–protein interaction), which is longest in vertebrate TRP channels (Julius 2013), and extensive intramolecular interactions including TRP-like domain/helix-turn-helix (HTH) 2, HTH1/HTH2, HTH1/ARD16, and ARD15/ARD16 that are mediated by hydrophobic or polar residues and stabilize the stacking ARDs (Paulsen et al. 2015). Each of ARD16 and HTH1 is also close to, and presumably interacts with, the C-terminal coiled-coil (Paulsen et al. 2015). This interaction network possibly facilitates the channel assembly and transduces intracellular signals from ARDs and HTHs to the gating region (Paulsen et al. 2015). Similar structural arrangements among the stacking ARDs, N-linker domain (between ARD and pre-S1), and TRP helix are also present in TRPCs and TRPVs (Duan et al. 2018a, 2019; Zubcevic et al. 2016). In particular, TRPCs and TRPVs have 4 or 6 ARDs that are tightly stacked and presumably contain several intramolecular interactions (Bai et al. 2020; Cao et al. 2013; Duan et al. 2018a, 2019; Fan et al. 2018; Liao et al. 2013). In addition, the TRPC4 N-terminal region proximal to pre-S1 interacts with a distal C-terminal region, possibly via π–π interaction and hydrogen bonding (Duan et al. 2018a). In TRPV1, an aspartic acid and methionine located at the end of the S4–S5 linker and S6 helix, respectively, which are conserved in TRPVs, form a hydrogen bond mediating the binding between the two domains (Cao et al. 2013). In contrast, in $TRPV3_{apo}$, i.e. unbound state, the interaction between the S4–S5 linker and S6 helix is rather mediated by the phenylalanine:asparagine or phenylalanine:methionine pair (Zubcevic et al. 2018a).

While TRPA1 and TRPVs possess ARDs TRPMs have their N-terminus that contain four featured TRPM homology regions (MHRs, a characteristic domain shared by TRPMs) that are important for intramolecular interactions (Huang et al. 2020). In fact, A432 in the TRPM4 MHR3 interacts with residues located at MHR1, -2, and -4 (Xian et al. 2018), which may be destabilized by substitution with a bulky residue at 432 resulting in gain of function (Xian et al. 2018). The same group subsequently reported that MHR4 interacts with the TRP helix in TRPM4 (Xian et al. 2020). Further, it was reported that MHRs in TRPMs also interact with the pre-S1, TRP, and distal C-terminal domains (Duan et al. 2018c; Yin et al. 2018). In TRPMLs, the cytosolic S2–S3 linker forms a unique HTH that interacts with a cationic residue-rich H1 helix right before the S1 helix (Chen et al. 2017; Zhou et al. 2017). Cryo-EM structures of TRPML1 reveal two stable closed conformations, assigned as closed state I and II, which are distinguished by the S4–S5 linker swinging away from the channel pore (Chen et al. 2017). In closed state II of TRPML1, interaction between the H4 helix of the S2–S3 linker and the S4–S5 linker was identified (Chen et al. 2017). In TRPML1 interaction between S3 and S4 through conserved π–cation bonding has been identified (Fine et al. 2018). For TRPPs, because all constructs used for structural determinations so far lack most of their cytosolic domains (Grieben et al. 2017; Shen et al. 2016; Su et al. 2018), the

intramolecular interactions among cytosolic domains identified by the functional study (Zheng et al. 2018) would have to be verified by future structural studies.

3 Regulation of TRP Intramolecular Interactions by Chemical Ligands

The TRP channels are polymodal sensors integrating numerous signals. Some chemical ligands such as endogenous PIP2, natural compound cannabinoid, and synthetic 2-APB are known to modulate a variety of TRP channels but the underlying mechanisms are not fully understood (Muller et al. 2018). There are other chemical ligands that specifically modulate a particular TRP channel or a subset of TRP channels, such as allyl isothiocyanate (TRPA1), resiniferatoxin (TRPV1), ZINC17988990 (TRPV5), BDTM/AM-1473/AM-0833 (TRPC6), menthol/icillin/WS-12 (modulating TRPM8), ADPR (TRPM2), and ML-SA1 (TRPMLs). Of note, the natural/endogenous compounds, such as PIP2, cannabinoid, menthol, ADPR, and resiniferatoxin are more relevant to physiological or pathological modulation of the corresponding TRP member(s), in contrast to artificial chemicals that are used as pharmacological tools. Some TRP structures are resolved in the presence of a ligand molecule, which when combined with functional studies provides unprecedented opportunities to determine whether and how intramolecular interactions are involved in ligand-induced channel gating (Table 1).

3.1 PIP2

PIP2 has three isoforms, namely PI(3,5)P2, PI(3,4)P2, and PI(4,5)P2, among which PI(4,5)P2 is the most abundant phosphoinositol lipid with two phosphate anchorings in the inner leaflet while PI(3,5)P2 is enriched in the endolysosome (Vanhaesebroeck et al. 2001). PIP2 is known to modulate almost all mammalian TRP channels but with distinct effects or underlying mechanisms (Cai et al. 2020; Nilius et al. 2008; Rohacs 2014; Zheng et al. 2018). Besides regulation of TRP channels, importance of PIP2 is involved in actin dynamics, focal adhesion assembly, membrane tubulation, intracellular trafficking as well as modulation of other ion channels (Mandal 2020; Suh and Hille 2008). This review will focus on direct effects of PIP2 on TRP channels.

The structure of three TRPs have been resolved in complex with PI(4,5)P2 including rTRPV5, hTRPM8, and hTRPML1 (Chen et al. 2017; Fine et al. 2018; Hughes et al. 2018; Yin et al. 2019). In particular, the TRPV5-PI(4,5)P2 complex was resolved with a high concentration (400 μM) of PI(4,5)P2 (Hughes et al. 2018). By binding to cationic residues in the N-linker, S4–S5 linker, and S6 helix, PI(4,5)P2 induces conformational changes in the pore region thereby enlarging the lower gate

Table 1 Ligands reported to bind specific domains in corresponding TRPs and the subsequent effects on intramolecular interactions or channel gating

Ligand	TRP in complex	Bound	Effects	Reference
PIP2	rTRPV5	N-linker, S4-S5 linker, S6 helix	Enlarge lower gate	Hughes et al. (2018)
	hTRPML1	Pre-S1, S1 helix, S2-S3 linker	S4-S5 linker moves away from central pore	Fine et al. (2018)
	hTRPM8	Pre-S1, S4-S5 linker, TRP helix	Pre-S1, S4-S5 linker move towards TRP helix	Yin et al. (2019)
Cannabinoids	rTRPV2	S5/S6 helix	S4-S5 linker moves to S5 helix & TRP helix rotates	Pumroy et al. (2019)
2-APB	rTRPV6	S4-S5 linker, TRP helix	S4-S5 linker moves closer to TRP helix	Singh et al. (2018b)
	hTRPV3	S1-S4 helices	S1-S2 loop moves away from membrane & shortened S6 helix	Singh et al. (2018a)
BDTM	hTRPC6	Pre-S1, S1/S4 helix, S4-S5 linker	Maintain the closed state	Tang et al. (2018)
AM-1473		S1-S4 helices, TRP helix		Bai et al. (2020)
AM-0833		PH, S6 helix	S4-S5 linker moves away from TRP helix	
ADPR	drTRPM2	MHR1/2	Release of S4-S5 linker/TRP helix interaction	Huang et al. (2018)
RTx	hTRPV1/2	S4 helix, S4-S5 linker	S4-S5 linker moves away from central pore	Gao et al. (2016)
ZINC17988990	rbTRPV5	S1-S4 helices	Tightened S1-S4 bundle	Hughes et al. (2019)
ML-SA1	hTRPML1	S5/S6 helix	Disrupted S5/S6 helix interaction	Schmiege et al. (2017)
JT-010/BITC	hTRPA1	CTD	Translocation of a loop, stabilized by loop/TRP helix interaction	Suo et al. (2020), Zhao et al. (2020)

(Hughes et al. 2018). Residue K484 that mediates binding of the TRPV5 S4–S5 linker with PI(4,5)P2 is invariant in its closest homolog TRPV6 (as K484) and is also part of PI(4,5)P2 binding site in TRPV6 (Cai et al. 2020). Importantly, the autoinhibitory intramolecular S4–S5 linker/TRP helix interaction in hTRPV6 is mediated by the R470:W593 pair (Cai et al. 2020). Binding of PI(4,5)P2 to rTRPV5 increased the distance between R470 and W593 from 2.7 Å to 3.5 Å. Therefore, functional and structural data on TRPV5 and -V6 indicated that PIP2 attenuates the S4–S5 linker/TRP helix interaction through which it activates their channel function. TRPM8 structures in complex with icilin/PI(4,5)P2/Ca^{2+} or

WS-12/PI(4,5)P2 have been determined (Yin et al. 2019). In both complexes, a PI (4,5)P2 binding cassette is formed by residues from the pre-S1, S4–55 linker and TRP helix. Structural differences between $TRPM8_{apo}$ and $TRPM8_{icilin/PI(4,5)P2/Ca}$ indicate that the icilin/PI(4,5)P2 complex promotes the association of the pre-S1 and S4–S5 linker with the TRP helix (Yin et al. 2019), which is consistent with the functional data showing that PI(4,5)P2 strengthens the pre-S1/TRP helix interaction in TRPM8 (Zheng et al. 2018). TRPML is activated by PI(3,5)P2 in lysosome but is inhibited by PI(4,5)P2 on the cell surface (Zhang et al. 2012). PI(3,5)P2 interacts with the cytosol-facing cation-rich pre-S1 (also called H1 and H2), S1 helix and S2–S3 linker (also named H3-turn-H4). While no PIP2 density in the TRPML S4–S5 linker similar to TRPV6 was found, helix H4 can bind to the S4–S5 linker in the closed state II, which is a prerequisite for PI(3,5)P2-induced activation. Thus, the S4–S5 linker in PI(3,5)P2-activated TRPML1 moves away from the center pore and should be activated by PI(3,5)P2 indirectly (Fine et al. 2018). In summary, PI(4,5)P2 interacts with TRP channels through cytosolic domains or those proximal to the cytosol (Table 1) which further induces subsequent conformational changes. The TRP channels, underlying diverse physiological and pathological processes, with conformational changes caused by PIP2 would affect ion permeation resulting in alterations of the membrane potential and downstream signaling cascades, therefore regulating cellular functions.

3.2 Cannabinoids (CBD)

The cannabinoids are natural compounds derived from the plant *Cannabis sativa*, which have been widely used as medical interventions to alleviate diverse diseases via limiting the neurotransmitter release in presynaptic neurons (Whiting et al. 2015). In addition to the classical cannabinoid receptors (Mackie 2008), the thermosensitive TRP channels which include TRPA1, TRPV1-V4, and TRPM8 are either activated (A1 and V1-V4) or inhibited (M8) by cannabinoids and derivates and thus act as new cannabinoid-sensing integrators (Muller et al. 2018). TRPV2 has so far been the only TRP members with its structure resolved (at close-to-atomic resolution) in the presence of a cannabinoid ($TRPV2_{CBD}$) but was in a closed state (Pumroy et al. 2019). Specifically, based on the two rat TRPV2 structures resolved in the presence of 30 μM cannabidiol, the cannabidiol binding pocket was found to reside in a hydrophobic cavity formed by S5 and S6 helices from two neighboring monomers (Pumroy et al. 2019). Further, compared with $TRPV2_{apo}$, the first configuration with a bound cannabidiol ($TRPV2_{CBD1}$) only exhibits modest movements of the S4–S5 linker and TRP helix and the pore region remains unaffected. In the second structure ($TRPV2_{CBD2}$) the S4–S5 linker moves 2.6 Å toward S5 while the TRP helix rotates 8°.

3.3 2-ABP

The chemical 2-APB is synthesized from diphenylboronic acid generated from a reaction of methylborate with phenylmagnesium bromide (Maruyama et al. 1997). It is initially found as an antagonist of inositol trisphosphate receptor-induced calcium release (Maruyama et al. 1997). The IP3R is a ubiquitously expressed glycoprotein located on the endoplasmic reticulum (ER) membrane and its activation by IP3 releases Ca from ER and would affect intracellular Ca signaling pathways related to autophagy, cell death, or proliferation (Parys and Vervliet 2020). 2-APB is later found to exhibit functional effects on TRP channels, e.g., inhibiting TRPV6, TRPP2, TRPC1/3/5/6/7, and TRPM2/3/7/8, but activating TRPA1, TRPV1-V3, and TRPM6, and has limited or no effect on TRPV5 and TRPMLs (Clapham 2007; Colton and Zhu 2007). Several structures of the inhibited $TRPV6_{2\text{-}APB}$ and activated $TRPV3_{2\text{-}APB}$ were resolved (Deng et al. 2020; Singh et al. 2018a, b; Zubcevic et al. 2018a), which revealed that 2-APB binds to the S4–S5 linker and TRP helix of rat TRPV6 and brings them closer to each other (Singh et al. 2018b), presumably as part of channel pore closing process. This is consistent with an independent study which found that the disruption of the S4–S5 linker/TRP helix interaction is associated with TRPV6 channel opening (Cai et al. 2020). Compared with TRPV6, three putative 2-APB binding pockets were found to be distinctly located in an open TRPV3 $(Y564A)_{2\text{-}APB}$ structure (Singh et al. 2018a), among which the two pockets located within the S1–S4 helices are presumably involved in the pore gate opening. The $TRPV3(Y564A)_{2\text{-}APB}$ and $TRPV3_{apo}$ structures are basically identical, except that in $TRPV3(Y564A)_{2\text{-}APB}$ the S1–S2 loop is closer to the extracellular side and that the S6 helix is shorter and closer to S5 (Singh et al. 2018a). Based on currently available structures of 2-APB in complex with TRPV6 or TRPV3, different 2-APB binding cavities are revealed and distinct functional effects observed, presumably due to distinct conformational changes.

3.4 Specific Ligands

Apart from the promiscuous modulators discussed above, investigating regulation by member- or subfamily-specific ligands is crucial to understanding mechanisms underlying the function of TRP channels.

As the TRPV founding member, TRPV1 harbors a vanilloid-binding pocket that can interact with resiniferatoxin (RTx) and comprises residues in S4 and the S4–S5 linker (Gao et al. 2016). Compared with $TRPV1_{apo}$, the S4–S5 linker in $TRPV1_{RTx}$ moves away from the central pore which opens the lower pore gate (Gao et al. 2016) in a way that would involve in changes in the S4–S5 linker/TRP helix interaction based on related findings on TRPV6 (Cai et al. 2020). A similar vanilloid pocket is also identified in TRPV2 (Zubcevic et al. 2018b), a close homolog of TRPV1. Additionally, hydrogen bonds among S5, S6, and the pore helix in $TRPV2_{apo}$,

supposed to maintain channel symmetry, are disrupted in $TRPV1_{RTx}$ (Zubcevic et al. 2018b). The TRPV5-specific inhibitor ZINC17988990 binds with the S1–S4 helix bundle from the cytosol, which induces tighter interactions among the S1–S4 helices and thereby prevents channel activation (Hughes et al. 2019).

TRPC6 structures with a bound antagonist, $TRPC6_{BTDM}$ and $TRPC6_{AM\text{-}1473}$, where BTDM stands for (2-(benzo[d][1,3]dioxol-5-ylamino)thiazol-4-yl)((3 S,5 R)-3,5-dimethylpiperidin-1-yl)methanone a specific inhibitor, is almost identical to that of $TRPC3_{apo}$ (Bai et al. 2020; Tang et al. 2018), suggesting that these molecules may have maintained the channel in closed states. The BTDM binding pocket is composed of residues from the pre-S1, S1/S4 helices and S4–S5 linker (Tang et al. 2018), whereas AM-1473 interacts with a cavity formed by the S1–S4 and TRP helices (Bai et al. 2020). An analog of AM-1473 named SAR7334 inhibits TRPC6 based on a study using an acute hypoxic pulmonary vasoconstriction mouse model (Maier et al. 2015). In comparison with agonist-bound TRPC6 ($TRPC6_{AM\text{-}0833}$), in which AM-0833 is bound to the PH domain and S6, the major rearrangement is an upward movement of the S4–S5 linker away from the TRP helix (Bai et al. 2020). The nicotinamide adenine dinucleotide metabolite ADP-ribose (ADPR), which is a metabolite targeting ryanodine receptors in the ER and leads to Ca release underlying muscle contraction (Galione and Chuang 2020; Santulli and Marks 2015), activates TRPM2 (Huang et al. 2020). Structural comparison between EDTA-bound TRPM2 in the absence ($TRPM2_{EDTA}$) and presence of Ca^{2+} ($TRPM2_{ADPR/Ca}$) shows that upon cytosolic ADPR binding to the MHR1/2 domains, the S4–S5 linker/TRP helix association is released, which supposedly leads to upward movements of S5 and S6 and thereby opens the pore gate (Huang et al. 2018).

The TRPML-specific agonist called mucolipin synthetic agonist 1 (ML-SA1) was found to partially bind to S5, S6, and the pore helix forming an intramolecular hydrophobic cavity (Schmiege et al. 2017). In the ML-SA1-bound state, the S5/S6 helix interaction presumably is mediated by a valine:phenylalanine bond present in the apo state, is disrupted during the ML-SA1-induced pore gate opening (Schmiege et al. 2017). As abnormalities of TRPML are related to lysosomal disorders, use of ML-SA1 might provide a promising intervention (Shen et al. 2012). TRPA1 is the only TRP member sensitive to electrophiles and contains cysteine residues in its C-terminus that can be covalently modified by 2-Chloro-N-[4-(4-methoxyphenyl)-2-thiazolyl]-N-(3-methoxypropyl)acetamide (JT-010) and benzyl isothiocyanate (BITC) (Suo et al. 2020; Zhao et al. 2020). Of note, JT-010 could induce TRPA1-dependent noxious pain (Heber et al. 2019), while BITC has been linked to alleviation of lipopolysaccharides or high-fat diet induced inflammasome formation independent of TRPA1 (Chen et al. 2020; Lee et al. 2016). The electrophiles of different sizes would interact with cysteine residue(s) and occupy the cavity, thereby inducing translocation of an activation-loop out of the pocket; this rearrangement can then be stabilized through the activation-loop interaction with the TRP helix (Zhao et al. 2020).

Importantly, it should be noted that there are ligands that modulate TRP channel function but do not apparently affect intramolecular interactions. For instance, the TRPA1 antagonist A-967079 that acts as a wedge to lock S5 and S6 in a

conformation that prevents the channel from activation (Zhao et al. 2020). Another example would be the TRPV6-specific inhibitors named (4-phenylcyclohexyl) piperazine derivatives (PCHPDs) exhibit similar functional effects as calmodulin but inhibit the channel function through directly blocking the ion permeation (Bhardwaj et al. 2020). The ATP-inhibitable TRPM4 channel function is proposed to undergo conformational changes in inter-subunit interfaces of the nucleotide-binding domain and ARDs (Guo et al. 2017). Therefore, through physical blockade of channel pore, inter-molecular interactions or locking of channel, these alternative mechanisms are also important for the regulation of specific TRPs and further underlie diverse biological or pathological processes in which TRPs play roles. In addition, there are intracellular proteins such as calmodulin interacting with multiple TRPs. But how these protein–TRP interactions relate to intramolecular interactions require further investigations.

4 Implications in TRP Causing Human Diseases

Given the crucial importance of TRP channels in integrating and transducing diverse cellular signaling events such as chemical, temperature, and mechano-stimuli, it is not surprising that altered function or expression of TRP channels is associated with a range of diseases including inflammation, noxious pain, and neuronal, metabolic, and cardiovascular disorders (Kaneko and Szallasi 2014). To date, 11 channelopathies are known to be genetically caused by pathogenic mutation (s) in 9 TRP channels (Fig. 6). TRPV4 mutations are associated with multiple diseases, namely spondylometaphyseal dysplasia (SMD, characterized by short-trunk short stature), scapuloperoneal spinal muscular atrophy limb (SPSMA, with progressive muscle atrophy and weakness), and Charcot–Marie–Tooth disease type 2C (CMT2C, with weakness in limb, diaphragm, and laryngeal muscle) (Deng et al. 2010; Krakow et al. 2009; Landoure et al. 2010). A progressive disease occurred in kidney, named autosomal dominant polycystic kidney disease (ADPKD), is featured with accumulating cysts and is associated with numerous gene mutations in TRPP2 or PKD1 (Mochizuki et al. 1996). The uncontrolled growth of cysts could result in high blood pressure and kidney failure. The focal segmental glomerulosclerosis (FSGS) is a renal syndrome linked to an inheritable TRPC6 gain-of-function mutation (Winn et al. 2005). Later, 24 more mutations in TRPC6 have been identified for this syndrome (Wang et al. 2020), which has nephrotic symptoms and can lead to kidney failure. Patients with the neurodegenerative diseases, known as Guamanian amyotrophic lateral sclerosis (ALS-G) and Guamanian parkinsonism dementia (PD-G), suffer from muscle weakness and loss of motor control, which are characterized as being due to interplays between environmental lack of Ca^{2+} and Mg^{2+} and missense mutations in TRPM2 and TRPM7 (Hermosura et al. 2005, 2008). A retina-specific autosomal recessive disease called congenital stationary night blindness (CSNB) has been revealed as genetic disorder leading to night blindness and is caused by TRPM1 mutations (van Genderen et al. 2009). A missense (E to K)

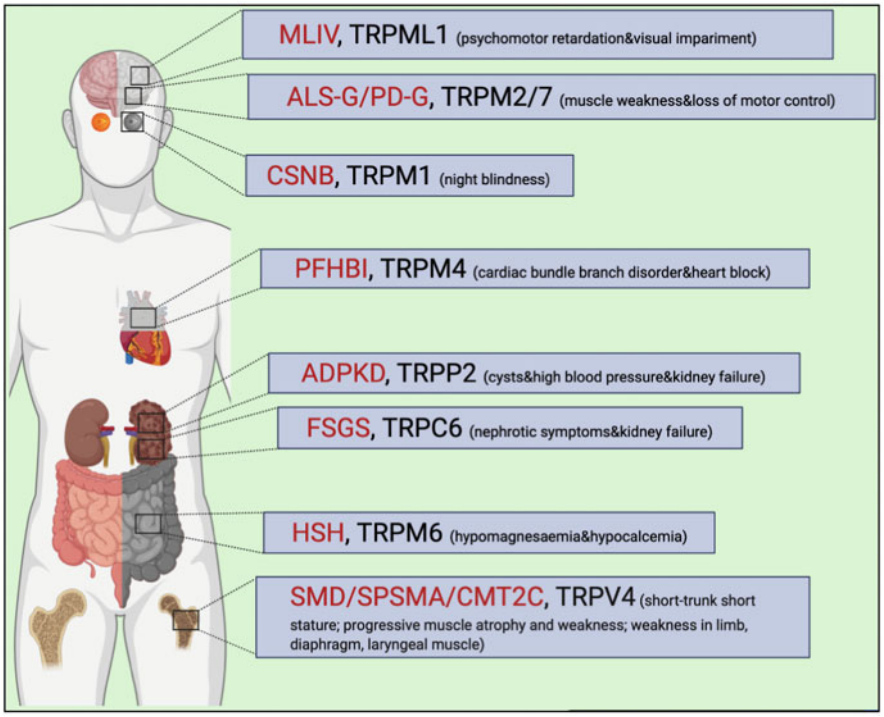

Fig. 6 TRP channelopathies in human. The diseases, namely mucolipidosis type IV (MLIV), Guamanian amyotrophic lateral sclerosis/Guamanian parkinsonism dementia (ALS-G/PD-G), congenital stationary night blindness (CSNB), progressive familial heart block type I (PFHBI), autosomal dominant polycystin kidney disease (ADPKD), focal segmental glomerulosclerosis (FSGS), hypomagnesemia with secondary hypocalcemia (HSH), spondylometaphyseal dysplasia/scapuloperoneal spinal muscular atrophy/Charcot–Marie–Tooth disease type 2C (SMD/SPSMA/CMT2C) are in red. The major organs affected by corresponding TRP(s) causing disorders are indicated as squares. The main clinical symptoms are described

mutation in TRPM4 is of gain-of-function and is observed in patients diagnosed with progressive familial heart block type I (PFHBI) (Daumy et al. 2016). Patients with PFHBI would develop into complete heart block from cardiac bundle branch disorder. The Mg^{2+} permeable TRPM6 is responsible for intestine absorption of dietary Mg^{2+}. The genetic autosomal recessive disorder called hypomagnesemia with secondary hypocalcemia (HSH) is due to mutations in TRPM6 (Schlingmann et al. 2002). Mutations in TRPML1 cause mucolipidosis type IV (MLIV), a neurodegenerative and autosomal recessive lysosomal storage disorder, and result in psychomotor retardation and visual impairment (Bargal et al. 2000). With the underlying mechanisms of pathogenesis remaining largely elusive, no effective treatment is currently available for these channelopathies.

Recent advances in cryo-EM shed light on detailed architectures at near-atomic scales of 7 out of the 9 TRPs (except TRPM1 and TRPM6) (Cao 2020). Structures of

pathogenic mutants of TRPV4, TRPP2, TRPC6, TRPM4, and TRPML1 are also available (Bai et al. 2020; Deng et al. 2018; Duan et al. 2018c; Schmiege et al. 2017; Shen et al. 2016; Zhang et al. 2017). The locations of known pathogenic mutations are clustered in interfaces between the S4–S5 linker and TRP helix, between S5 and S6, and in ankyrin repeats of TRPV4 (Deng et al. 2018); in the polycystin domain, pore helix/S6 interface and S4 of TRPP2 (Shen et al. 2016); in interfaces between ankyrin repeats and the C-terminal coiled-coil of TRPC6 (Bai et al. 2020); in the S4–S5 linker and N-terminus of TRPM4 (Duan et al. 2018c); in luminal domains, pore helix, S5 and S1–S4 helices in TRPML1 (Schmiege et al. 2017; Zhang et al. 2017). Fewer disease-causing mutations are found in TRPM2 and TRPM7, compared with other TRP channels reported with unclear mechanism. The P1018L mutation in TRPM2 (Hermosura et al. 2008) is located in the pore loop near S6 helix and may affect the stability of the S5/S6 helix interaction, given the close proximity (Huang et al. 2018). Notably, S6 helices of tetrameric TRPM2 form the channel pore and the stability of S5/S6 helix interaction potentially affects its channel function, which has been reported in TRPV6 and proposed to be conserved across TRPs (Cai et al. 2021). The TRPM2 P1080L mutant has been shown as of loss-of-function and is believed to interfere TRPM2 ion influx, causing ALS-G and PD-G diseases (Hermosura et al. 2008). The T1482I mutation in TRPM7 (Hermosura et al. 2005) is located in the C-terminus and has not been revealed in the reported structure which lacks the C-terminus (Duan et al. 2018b). These pathogenic mutations should have affected intra- and/or inter-molecular interactions in close proximity to their locations. Because these interactions maintain the physiological function of TRPs, the induced conformational changes would modulate the channel function and potentially disturb the physiological homeostasis.

In addition to the diseases caused by genetic mutations in TRP channels, there are multiple pathophysiological implications associated with certain TRP members. For instance, psoralen and ultraviolet A therapy induce pigmentation disorders through the intervention of TRPA1 and TRPV1 involved melanogenesis (Jia et al. 2021). The chondrogenesis or chondrification, which stands for mesenchymal stem cells undergoing differentiation to form cartilage, is recently proposed to rely on the presence of TRPV4 protein and its Ca permeability (Willard et al. 2021). Among all TRP channels, knockout of TRPA1, TRPV1, or TRPM3 in mice abolishes acute noxious heat sensing while retaining the sensation of cold-induced or mechanical pain (Vandewauw et al. 2018), which provides a way to alleviate chronic pain (Bamps et al. 2021). Additionally, TRPA1 and TRPV1 have been suggested to mediate nociception in the pain syndromes associated with cancer cell inoculation (de Almeida et al. 2021). Due to the involvement of multiple TRP channels in adaptive and innate immune systems, TRPA, -V, -C and -M are known to play notable roles in atherosclerosis, atopy, chronic fatigue syndrome, hypertension, inflammatory bowel disease, and myalgic encephalomyelitis, which have been reviewed in-depth (Froghi et al. 2021).

5 Discussions and Perspectives

Thanks to advances in structural determination techniques notably cryo-EM and the associated reconstruction algorithms, the majority of the TRP channels have now been uncovered at near-atomic resolutions. This review has focused on TRP intramolecular interactions and their functional importance. These intramolecular interactions are either shared by members across subfamilies or within a subfamily or unique to individual members. We have covered relationships between ligand binding and changes in intramolecular interactions as well as those between pathogenic mutations and changes in structures or intramolecular interactions. Thus, the presence of a ligand (or mutation)-intramolecular interaction relay should be a mechanism shared among all TRPs and be very important in transducing upstream signals or genetic mutations into conformational and thereby functional changes in TRPs. In fact, functional importance of intramolecular interactions has also been recognized in other ion channels including mechanosensitive Piezo1 (Lewis and Grandl 2020), calcium (Kim et al. 2018; Singh et al. 2006), sodium (Kass 2006), potassium (Sharmin and Gallin 2017), and chloride channels (Dhani and Bear 2006). Therefore, intramolecular interactions present in various types of ion channels should be important for protein stability, function, and regulation.

PIP2 is an important ligand that regulates most, if not all, TRP channels. Our recent studies allowed proposing a PIP2-intramolecular interaction relay that converts extracellular stimuli into electrical signals, which is shared by TRP members from different subfamilies including TRPV1, TRPV6, TRPP3, TRPP2, TRPC4, and TRPM8. Specifically, PIP2 affects the strength of intramolecular interactions in a TRP protein thereby regulating its channel function. It can thus be reasonably postulated that the PIP2-intramolecular interaction relay exists in most or all TRP members as a shared mechanism that mediates the functional regulation by extracellular stimuli or upstream factors. Thus, any upstream cascade, e.g. GPCR-phospholipase C (PLC) (Kadamur and Ross 2013), that affects the PIP2 level directly or indirectly, would regulate the channel function through the relay. On the other hand, hydrolysis of PIP2 by PLC produces diacylglycerol (DAG) and IP3 and would potentially affect the function of some TRP channels through PKC-dependent phosphorylation (Mandadi et al. 2011; Venkatachalam et al. 2003; Zhang and Saffen 2001).

Despite the tremendous details revealed by all the TRP structures by means of cryo-EM or crystallography, these techniques have limitations in reflecting the natural configurations of these channels. For instance, while ligand 2-APB functionally activates TRPV3 the structure of TRPV3 bound with 2-APB ($TRPV3_{2\text{-}APB}$) exhibits a closed conformation (Singh et al. 2018a). Interestingly, the $TRPV3_{2\text{-}APB}$ complex becomes an open channel in the presence of the K169A mutation to stabilize the open state or the Y564A mutation to increase the 2-APB binding affinity (Singh et al. 2018a; Zubcevic et al. 2018a). Another example is TRPV5 of which a cryo-EM structure did not reveal a PIP2 binding site until a high concentration of 200 μM diC8-PIP2 was used, which is around three folds of the PIP2 EC_{50} value for

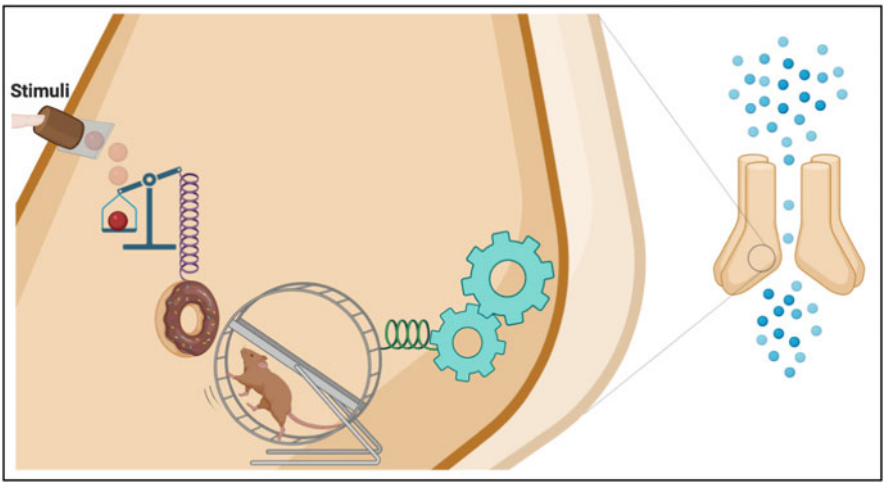

Fig. 7 Cartoon illustrates the proposed intramolecular interactions within one subunit modulating the TRP channel gating processes. The environmental stimuli induce series of subsequent changes in intramolecular interactions and protein conformation, represented by scale, donut, mice, and gear, which mediate the channel gating regulation

TRPV5 (Hughes et al. 2018). Further increase in the diC8-PIP2 dose to 400 μM enabled resolution of a $TRPV5_{diC8}$ structure (Hughes et al. 2018). In addition, although PIP2 can activate purified TRPM8 channels as shown by planar lipid bilayer electrophysiology (Zakharian et al. 2010) the $TRPM8_{PIP2}$ complex structure cannot be obtained until ligand icillin or WS-12 was added (Yin et al. 2019). Therefore, structures would have to be determined under more physiological conditions to better reconcile with functional determinations in living cells.

Based on the currently available structural and functional data, we propose that TRP channels sense extra- or intracellular stimuli, which induces conformational changes including critical intramolecular interactions and thereby results in changes in channel gating (Fig. 7). Genetic mutations associated with human channelopathies may have also altered intramolecular interactions. Notwithstanding, this hypothesized ligand-intramolecular interaction-gating scheme warrants further verifications. TRP structures resolved by currently available techniques are only a few snapshots of highly dynamic configurations during the unknown and sophisticated channel gating cycles, yet under very different experimental conditions. Structural data should be interpreted with caution when comparing or combining with functional data obtained using living cells or with data from numerical simulations. It should also be noted that although the regulation mediated through intramolecular interactions is important, other mediating routes are important parts of functional regulation. For example, a chemical antagonist can directly block the TRPV6 pore and some hydrophilic mutations can directly alter a TRP hydrophobic pore gate (Bhardwaj et al. 2020).

In summary, while the TRP superfamily has shared intramolecular interactions that act as a crucial molecular switch responding to common environmental and physiological stimuli, some TRP subfamilies or individual members have distinct intramolecular interactions or stimuli (Fig. 5). Understanding the underlying roles of these intramolecular interactions would not only help elucidating the TRP gating mechanisms but would also provide novel therapeutic targets in clinical interventions.

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Conflicts of Interest The authors declare no conflict of interest.

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