TRPC Channel Structure and Properties

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Shengjie Feng

Abstract

TRPC channels are the first identified members in the TRP family. They function as either homo- or heterotetramers regulating intracellular Ca^{2+} concentration in response to numerous physiological or pathological stimuli. TRPC channels are nonselective cation channels permeable to Ca^{2+}. The properties and the functional domains of TRPC channels have been identified by electrophysiological and biochemical methods. However, due to the large size, instability, and flexibility of their complexes, the structures of the members in TRPC family remain unrevealed. More efforts should be made on structure analysis and generating good tools, including specific antibodies, agonist, and antagonist.

Keywords

TRPC • Structure • Property

TRP channel subunits are rather large, ranging from 70 kD to more than 200 kD [40]. Transmembrane (TM) segment prediction suggests TRP channels resemble voltage-gated K^+ or Ca^{2+} channels [74]. A consensus has been reached by researchers that putative organization of TRP channels consists of six transmembrane (TM) domains with the carboxyl (C-) and amino (N-) terminals facing the intracellular side of the membrane [20, 73, 74]. The features of TRP channels change from family to family. However, no matter how diverse these subunits are, they are conserved in their pore region, a hydrophobic region between fifth and sixth segment and their TRP domain on the proximal C-terminal region [40, 67]. The results obtained by biochemical and optical methods strongly suggest that TRP channels are formed by four subunits [2, 29, 35],

S. Feng (✉)
Department of Physiology, University of California,
San Francisco, CA, USA
e-mail: sjfeng100@gmail.com

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assembling as homo- or heterotetramers [22, 25, 40, 77].

2.1 TRPC Channel Structure

2.1.1 Structure of TRPC Channels

Three factors make structural studies of TRP channels a great challenge. First, structural biology techniques require an ample supply of highly pure and stable protein samples. TRP channels, however, are not endogenously expressed in bacteria. This is perhaps a major limiting factor for the lack of high-resolution structures of full-length TRP channel. Moreover, membrane proteins are extremely difficult to be produced in large quantities and be purified in a stable native state [18]. Second, TRP channels are very large tetramers with multiple domains in each subunit. Such large protein complexes cannot be accessible to high-resolution nuclear magnetic resonance (NMR) techniques of molecules in an identical conformation [16]. Third, flexibility also complicates single-particle electron microscopy (EM) studies. The flexibility afforded by multiple domains, which probably has functional significance, often hinders crystallization [16]. For TRP channels, especially, they respond to diverse chemical or physical stimuli and are therefore believed to be conformationally dynamic. An additional obstacle to coaxing these proteins is forming well-ordered crystal lattices required for X-ray and electron crystallographic analysis [46].

Three major techniques are commonly used to obtain structural information on macromolecules: X-ray crystallography, NMR spectroscopy, and electron cryomicroscopy (cryo-EM) – either single-particle EM or electron crystallography [45]. Currently, three approaches have yielded information on the structure of TRP channels: (a) X-ray crystallography and NMR spectroscopy have been employed effectively to obtain high-resolution structures of functionally important cytosolic domains of six TRP channels. Biophysical measurements of isolated cytosolic domains of several TRP channels also gain insights into their biological function [16]. (b) Single-particle EM studies provide six low-resolution structures (TRPA1, TRPC3, TRPV1, TRPV2, TRPV4, and TRPM2) (13.6–35 Å) for full-length TRP channels [16, 21]. (c) Recently, a breakthrough in electron cryomicroscopy makes it possible to obtain a high-resolution view (~3–4 Å) of an entire TRP channel. Till now, three high-resolution structures (TRPV1, TRPV2, and TRPA1) have been determined [46, 68, 106].

Similar to most TRP channels, TRPC channel subunits have six transmembrane domains and a putative pore region between fifth and sixth transmembrane domains and assemble into tetramers to form functional channels (Fig. 2.1a, b). Though no high-resolution structures of TRPC members have been obtained yet, in the six existing low-resolution structures, mouse TRPC3 has the second highest resolution (15 Å) and is the most unique [61]. TRPC3 has a bell-shaped structure containing ample water-filled spaces within the molecule (Fig. 2.1c). The structure can be further divided into two components, a dense globular inner core and a sparse outer shell with a mesh-like structure. Viewing from the top, the antenna-like outer columns radiate from the inner chamber like terminals in the airport. Four small extracellular segments are held away from the membrane surface by slender arms. These structures are assumed to function as signal sensing modules for agonists and regulators. The overall height of the TRPC3 molecule is 240 Å, the side length at the widest position is 200 Å, and a diagonal line yields 210 Å. Therefore, the reconstituted structure of TRPC3 is much larger than that of the other five TRP structures, even though the calculated mass of a TRPC3 tetramer is the smallest in the six molecules. One explanation for this disparity is the presence of many large water-filled cavities in the TRPC3 structure [45]. Another reason is its enormous volume mostly conformed by the cytoplasmic domain, which is made of a sparse external shell [40]. Though it is still not clear why the structures of TRPC3 and TRPM2 are so different from that of TRPV1/TRPV2/TRPV4 and TRPA1, it is hypothesized that the simultaneous association of TRPC3 with other protein complexes and its multimodal activation and modulation mechanisms may underlie its expanded structure. However, the use of an auto-

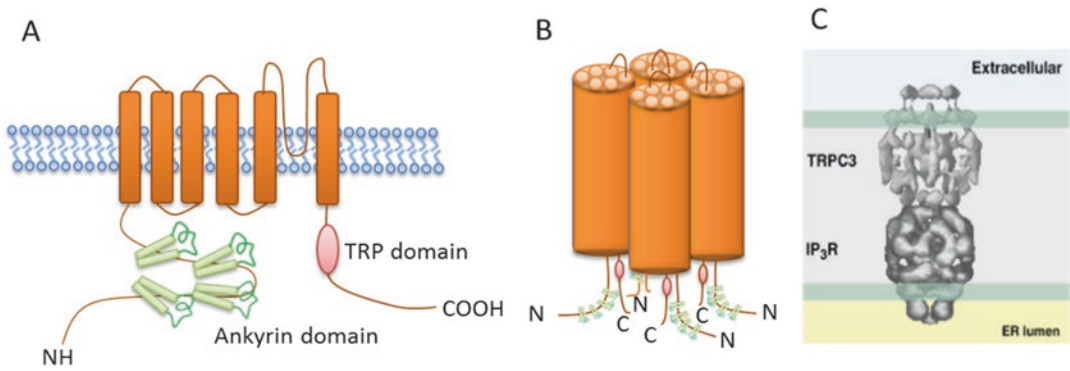

Fig. 2.1 Structures of TRPC channels. (**a**) Transmembrane topology of TRPC channels. The TRPC protein has six putative transmembrane domains, a pore region between the fifth and sixth transmembrane domains, four ankyrin domains (predicted), and a TRP domain in the proximal C-terminal region. (**b**) Structure of TRPC tetramer. The TRPC protein assembles into homotetramers or heterotetramers to form functional channels. (**c**) The large dimensions of TRPC3 [61]

mated particle-selection algorithm might have caused distortions in the data analysis and structural reconstruction of TRPC3 and TRPM2 [45].

2.1.2 Functional Domains of TRPC Channels

Structural biologists usually define a protein "domain" as an independently folding segment that can take on its native conformation even when isolated from the rest of the protein [16]. Though it has not been confirmed by experimental structure study, all members of TRPC channels were predicted to have 3–4 ankyrin repeat domains (ARD) on their N-terminal region and a conserved TRP domain at the beginning of the cytosolic C-terminal region [90] (Fig. 2.1a).

2.1.3 Ankyrin Repeat Domain

Ankyrin repeat (AR) sequences span ~33 residues and fold into a structural motif consisting of two α-helices folding back onto each other to form a helical hairpin, followed by a long hairpin loop that extends roughly perpendicular to the helical axes [63]. Several repeats of these structure motifs, ranging from 3 to over 30, are stacked side by side with their helices nearly parallel to each other, forming a modular, highly efficient, and specific protein-binding surface [15, 16]. The AR is one of the most common protein-protein interaction motifs [16, 40]. They function in various cellular processes, including regulation of transcription, cell cycle, development, cell-cell signaling, and transport.

Though little is known about TRPC ARs based on structure studies, the structure of several TRPV ARs has been published, which might give some hints to the understanding of TRPCs. When hundreds of chemicals were screened to optimize the TRPV1-ARD crystallization conditions, it was observed that the presence of ATP altered the crystal shape, likely by changing the packing interactions between protein molecules. The electron density map and biochemical assays further demonstrated that both ATP and calmodulin bind to the TRPV1-ARD [52] on the concave surface in a competitive manner. The TRPV2-ARD structure predicts that phosphorylation sites in its N-terminus (S116, Y200) should be on the surface of interaction of ARD with other proteins [31]. Furthermore, it is reported that ARDs play a role in promoting tetrameric assembly of TRPV5 and TRPV6 [6, 11]. Besides TRPVs, studies have shown that ARs of TRPA1 dictate sensitivity to thermal and chemical stimuli and ARs of TRPN (NompC) conveys force to gate the NOMPC mechanotransduction channel [8, 102].

TRPC channels likely have four ARs, which have weak similarity to the AR consensus [40]. The structure of AR in TRPC channel is therefore likely to have some unusual kinks and loops, as is observed in TRPV channels. Even there is still lack of structural evidence, biochemical assays uncover the function of TRPC ARD. TRPC1 negatively regulates TRPV6 by interaction with TRPV6 on its N-terminal ankyrin-like repeat domain [80]. The heteromerization of TRPC3 with TRPC1 was shown by GST pull-down assay of TRPC3 portions with TRPC1. The portion containing the AR region of TRPC3 was bound to TRPC1. The heteromeric TRPC3/TRPC1 is shown to participate in regulating the resting cytosolic Ca^{2+} levels in skeletal muscle [96].

The first ankyrin-like repeat is the minimum indispensable key structure for functional assembly of homo- and heteromeric TRPC4/TRPC5 channels assayed by confocal Förster resonance energy transfer (FRET) and total internal reflection fluorescence (TIRF) microscopy [79]. Consistently, by using GST pull-down, yeast two-hybrid, circular dichroism approaches and chimeras, studies show that the N-terminus of TRPC4 self-associates via the AR domain and the coiled-coil domain (CCD) to assemble the tetrameric channel of TRPC4 [43, 44]. These two domains are responsible for the association between TRPC4 and TRPC6 (members of distinct subgroups of TRPCs) [43]. TRPC4 can form complexes with TRPC6 subunits containing the N-terminal ankyrin and coil-coiled domain (residues 1-304) of TRPC4 [43]. Using both GST pull-down assay and immunoprecipitation, researchers show that MxA and RNF24 interacted with the ankyrin-like repeat domain of all TRPCs and regulated their activity or trafficking [55, 56]. The cGK-Iα interacts with the AR domain in the N-terminus of TRPC7 and phosphorylated TRPC7 at threonine 15, which contributes to the quick and accurate regulation of calcium influx and CREB phosphorylation [99]. In conclusion, ARs of TRPCs may play roles in the regulation of channel assembling, activity, and trafficking.

2.1.4 TRP Domain

Most TRP families display a conserved sequence on their proximal C-terminal region which has been regarded as a signature sequence for TRP channels. This ~25-amino acid intracellular region, just after the sixth transmembrane domain, is called the TRP domain [62]. This domain contains the TRP box, a conserved motif defined by the consensus sequence "WKFQR." All members or relatives of the mammalian TRPC family contain this highly conserved TRP box. It is possible to identify a second conserved region on the carboxy-terminal end of the TRP domain with proline-rich sequence of LPPPF (leucine on the first position is highly conserveative) [40]. However, the second TRP box is only conserved in TRPC and TRPM subfamilies. Although the main function of the TRP domain remains elusive, it may be required for PIP2 binding and regulation of channel gating [75].

TRPC3 and TRPC6 TRP domains differ in seven amino acids. Assayed by chimera and mutation experiments, the TRP domain of TRPC3, but not that of TRPC6, is found essential for association with cytoskeleton and the increased channel translocation to cell surface in response to Epo stimulation [24].

2.1.5 Pore Region

Few studies have been aimed at the identification of the pore region and the description of the pore properties of TRPC channels [67]. A theoretical prediction of pore elements seems to be ineffective to TRPC channels. Unlike TRPV channels, the segment between TM5 and TM6 of TRPC members does not share significant homology to the sequence of the pore region of bacterial K^+ channels [67]. This loss of homology to the bacterial archetype pore may signify that TRPCs are phylogenetically younger than TRPV channels [67].

The ultimate proof that a region contributes to the pore of an ion channel is demonstrating that pore properties, e.g., ion selectivity, can be altered by mutations to the putative pore-forming

region [94]. To this extent, the location and structure of the pore region and selectivity filter of most TRP proteins, including all members of the TRPC subfamilies, are currently unknown. Till now, three studies have addressed the role of several amino acids in the TM5-TM6 linker of three TRPC members, which gives some hints about the pore region.

To determine the degree of cooperativity within a TRPC channel pore complex, researchers generated a dominant-negative construct of TRPC6 (TRPC6DN) by exchanging three highly conserved residues, L678, F679, and W680, in the putative pore region for alanine residues. The TRPC6DN protein is correctly inserted into the plasma membrane and was functionally silent. Transient expression of TRPC6DN nearly abrogates TRPC3- and TRPC6-dependent currents, but does not compromise TRPC4 or TRPC5 activity [27]. It is shown that substitution by positively charged lysines at Glu576 and Asp581 in TRPC1, both located just outside the putative pore region, reduced the Ca^{2+} permeability [53]. For TRPC5, mutating glutamate residues (Glu543, Glu595, and Glu598), which are located close to either TM5 or TM6, affect the potentiation and inhibitory effects of extracellular La^{3+} on channel activity [32].

A conserved glycine residue within the cytosolic S4–S5 linker of both TRPC4 and TRPC5 proteins is important for their mysterious pore function. Mutating the glycine residue by a serine forces the channels into an open conformation. Expression of the TRPC4G503S and TRPC5G504S mutants causes cell death, which could be prevented by decreasing extracellular Ca^{2+} concentration in the culture medium. Current-voltage relationships of the TRPC4G503S and TRPC5G504S mutant channels resemble that of wild-type TRPC4 and TRPC5 channels [4]. Introduction of a second mutation (S623A) into TRPC4G503S suppressed the constitutive activation. Therefore, it is likely that the S4-S5 linker is a critical constituent of TRPC4/TRPC5 channel gating and that disturbance of its sequence allows channel opening independent of stimulation [4].

Given that these different residues are not conserved within the TRPC subfamily, it is unlikely that they form part of the actual selectivity filter, but rather contribute to the extracellular mouth of the pore [94].

2.1.6 Coiled-Coil Domain

Coiled coil is a protein structure in which α-helices wrap around each other in a helical coil conformation [45]. Sequences with a propensity to assume coiled-coil structures are characterized by recurring pattern of aliphatic residues alternating every third and then fourth residue to form seven residue repeats [45]. Coiled-coil structures, functioning as oligomerization domains, are found in a variety of proteins including transcription factors, motor proteins, structural proteins, cellular and viral membrane fusion proteins, and ion channels [45, 64]. Coiled-coil domains have been implicated in subunit interaction and assembly of ion channels, including TRPV1 [14], TRPM2 [58], TRPM7 [13], and TRPM8 [10, 87].

Coiled-coils are predicted in TRPC channels at either or both the N-terminal intracellular linker between the AR and the transmembrane domain and the C-terminal domain [42, 81]. Though these TRPC coiled-coil regions still need to be confirmed through biochemical and/or structural experiments, the Orai1-activating region of STIM1 interacts with the TRPC channel coiled-coil domains (CCDs). This interaction is essential for opening the channels by STIM1 [41]. Disruption of the N-terminal CCDs by mutations eliminated TRPC surface localization and reduced binding of STIM1 to TRPC1 and TRPC5 while increasing binding to TRPC3 and TRPC6 [41]. Using a yeast two-hybrid assay, the coiled-coil domain is found to facilitate homodimerization of the N-terminus of mTRPC1 and is required for structural organization, thus forming functional channels [9]. The CCD is one of the two domains responsible for the association between TRPC4 and TRPC6 [43, 44]. Thus, TRPC channel CCDs can participate in channel gating and assembling.

2.1.7 Other Functional Domains

Binding domains for various signaling molecules exist in the N-termini and C-termini of certain TRPC proteins. In addition to these structurally defined domains mentioned above, functional domains, including PIP2- and calmodulin (CaM)-binding domains, CRIB domain, and PDZ domain, also yield insights into the biological function of TRPC channels [73].

Because of their common interaction with ion channels, PIP2 and calmodulin may be considered as channel components [23, 76]. TRPC proteins contain multiple putative CaM-binding sites in their N- and C-termini, some of which overlap with an IP3 receptor (IP3R)-binding site (CRIB domain) [85]. The GST fusion proteins of all TRPC channels interact with CaM in vitro. By using CaM and IP3R peptides, in vitro Ca^{2+}-dependent competition experiments demonstrated that Ca^{2+}/CaM and IP3Rs may dynamically regulate TRPC through competitive interactions [85]. However, the function of TRPC and IP3R interactions has been challenged when receptor-activated TRPC3 functions have been found totally normal in avian DT40 cells lacking all three IP3R isoforms [89].

In *Drosophila* photoreceptors, TRP channels are tethered into signaling complexes via PDZ interactions with the scaffolding protein INAD [19]. Both TRPC4 and TRPC5 contain a carboxyl terminal PDZ-binding motif (VTTRL) which is absent in other TRPCs. This motif in both channels mediates interactions with NHERF/EBP-50 and PLCβ1 [86]. The PDZ domain of TRPC4 controls its localization and surface expression in HEK293 cells [59]. Although PDZ-binding motifs of TRPC channels have been demonstrated to participate in plasma membrane localization [59, 82], few data so far directly implicate PDZ proteins in the control of TRPC channel activity.

A finding shows that 437–508 aa of TRPC6 is important for its inhibition of Aβ production [95]. This domain contains the first and second transmembrane regions and the first extracellular loop. When the second transmembrane (TM2) region is mutated, by point mutation, replacement, or reversal, TRPC6 is not able to reduce Aβ levels, indicating that the TM2 domain is essential for TRPC6 to regulate Aβ production.

2.2 TRPC Channel Properties

2.2.1 Expression Pattern of TRPC Channels

2.2.1.1 Tissue Distribution

TRPC channel proteins are expressed in both excitable and non-excitable cells. While mRNA and protein of TRPC1 are widely expressed in mammalian tissues, those of TRPC3 and TRPC5 are predominantly detected in the brain. A relatively weak signal of TRPC3 mRNA is present in the ovary, colon, small intestine, lung, prostate, placenta, and testis [103]. TRPC5 can also be detected in the liver, kidney, testis, and uterus in much lower levels [66]. TRPC6 mRNA is detected in the lung and at a lower level in the brain, muscle, placenta, and ovary [5, 90]. TRPC7 mRNA expression is in the heart, lung, and eye and moderate expression in the brain, spleen, and testis [65]. TRPC2 is a pseudogene in human, and its protein localizes to neuronal microvilli in rat vomeronasal organ and in the head of mouse sperm [33, 50, 54] (Table 2.1).

2.2.1.2 Expression Pattern During Development

During fetal development, TRPC1 is expressed at the highest level in the brain and at lower levels in the liver and kidneys. In the adult, TRPC1 is expressed at the highest levels in the heart, testes, ovary, and many regions of the brain [105]. In rat hippocampus, TRPC1 and TRPC3 proteins are detectable at postnatal day 14 and 7 [84], and their expression levels remain high into adulthood. By contrast, the peak expression of TRPC4, TRPC5, and TRPC6 is between postnatal days 7 and 14 [84].

Table 2.1 Properties of TRPC Channels Homotetramer

Name	Selectivity	Conductance (pS)	Effects of trivalent cations	Activation mechanism	Expression
TRPC1	Non	16	La^{3+}, Gd^{3+}	Store operated/STIM1 Mechanical stimuli	Widely expressed
TRPC2	2.7	42	La^{3+}, Gd^{3+}	Store operated /STIM1, receptor operated	VNO, sperm, testis, heart, brain
TRPC3	1.6	23/66	La^{3+}, Gd^{3+}	Store operated/IP3, Receptor operated, Mechanical stimuli	Brain (mainly), heart, placenta, muscle, lung, ovary, colon, prostate, small intestine, testis
TRPC4	1.05/7	41	La^{3+}	Store operated/STIM1, receptor operated	Brain, testis, placenta, adrenal gland, retina endothelia, testis
TRPC5	9.5/1.79	63	La^{3+}, Gd^{3+} (GTPγS) La^{3+}, Gd^{3+s} (ATP)	Store operated/STIM1, receptor operated	Brain (mainly), liver, kidney, testis, uterus [66]
TRPC6	5	35	La^{3+}, Gd^{3+}	Store operated, Receptor operated Mechanical stimuli	Lung, brain, muscle, placenta, ovary
TRPC7	1.9/5	25-50	La^{3+}, Gd^{3+s}	Store operated, Receptor operated	Heart, lung, eye, brain, spleen, testis

Non nonselective, *s* slightly, *red* inhibition, *green* activation

2.2.2 Electrophysiological Properties of TRPC Channels

Interpretation of the functional data of TRPC channels is often difficult and complex due to a noisy background caused by endogenous cation-selective, Ca^{2+}-permeable channels regulated by store depletion and/or products of PLC-dependent pathways [67]. This has led to conflicting descriptions of the pore properties of TRPC channels. For example, TRPC4 and TRPC5 have been described as either Ca^{2+} selective or nonselective between mono- and divalent cations.

2.2.2.1 Activation Properties of TRPC Channels

There are two major mechanisms proposed for the activation of TRPC channels, either through receptor-operated or store-operated Ca^{2+} channel [7]. However, the activation mechanism of mammalian TRPC channels remains controversial. The results conflict with each other when the systems are varied. TRPC homologs have been found to be gated by a direct interaction with inositol 1,4,5-trisphosphate (IP3) receptors, ryanodine receptors, or diacylglycerol [26, 38, 39, 65, 66, 78]. But studies of *Drosophila* mutants lacking its only IP3 receptor indicate that TRP activation is independent of Ca^{2+} store depletion in its native environment in photoreceptor cells [1, 60, 72]. TRPC2 can function as a store-operated channel in transfected cells and is likely involved in sperm function [33, 88]. However, within its native environment in vomeronasal neuron, TRPC2 acts as a diacylglycerol-gated cation channel participating in the chemoelectrical transduction [54]. TRPC3 was used as a representative of a store-operated Ca^{2+} channel (SOC) to study the property and machineries of SOC [70]. But abundant evidence has been shown that

TRPC3 can be activated in a receptor-operated way [7, 70].

Yet, there are similarities among TRPC channels, as all members are activated through pathways coupled to stimulation of phospholipase C (PLC) [90]. Consistently, PLC-deficient mutants also show that *Drosophila* TRP is gated in some manner downstream of PLC. Notable findings are the identification of STIM1, a Ca^{2+} sensor of endoplasmic reticulum essential for SOC and Icrac, and Orai1 or CRACM1, functioning as Icrac channels or as an Icrac subunit [12, 92, 101]. STIM1 selectively binds to TRPC1, TRPC2, and TRPC4, but not to TRPC3, TRPC6, or TRPC7, providing evidence for TRPC1, TRPC2, and TRPC4 being SOC [28]. It is worth mentioning that although the Orai and TRPC channels can function independently of each other to mediate the CRAC current [100] and nonselective Ca^{2+}-permeable current, it was shown that all Orai channels interact with TRPC channels to complex with STIM1 and enhance TRPC channels' store dependence [47–49]. In most cells, both Orai and TRPC channels appear to be required for SOC by affecting the activity of each other. Deletion of Orai1 inhibits all forms of Ca^{2+} influx in these cells. For the TRPC3/TRPC6/TRPC7 channels, the majority of published results suggest that endogenous DAG, produced upon PLC activation, or exogenous DAG (usually oleyl acetyl glycerol, or OAG) can be the signal activating these channels [7, 70, 90]. It turned out that TRPC3 can function as STIM1-dependent SOC channel only by assembling with TRPC1. TRPC6 functions as STIM1-dependent channel only in the presence of TRPC4 [98]. Notably, in the absence of TRPC1 and TRPC4, activation of TRPC3 and TRPC6 is STIM1 independent. Since TRPC4 and TRPC5 channels are highly sensitive to protein kinase C (PKC), it is difficult to obtain evidence that DAG can activate TRPC. Indeed, OAG inhibits the activation of TRPC4 or TRPC5, and this inhibition is blocked by inhibitors of PKC [70, 91]. The Gαi/o proteins, including Gαi2 and Gαi3, are proposed to be important for the activation of TRPC4 and TRPC5 [30, 37].

It is reported that TRPC1 can be activated with a latency of a few milliseconds by stretch in patches from *Xenopus* oocytes [57]. In another study, TRPC1 is found forming mechanosensitive channels in growth cones of *Xenopus* spinal neurons [36]. Recently, it has been shown that the sensitivity of podocyte cells to stretch is reduced by TRPC6 RNAi or by the expression of podocin, a protein that interacts with TRPC6 [3]. In a study, TRPC3 and TRPC6 are proposed to be essential for normal mechanotransduction in subsets of sensory neurons and cochlear hair cells [71]. These findings suggest that some members of TRPC channels can be activated in response to mechanical stimuli.

The activation mechanism of TRPC channels is summarized in Fig. 2.2 and Table 2.1.

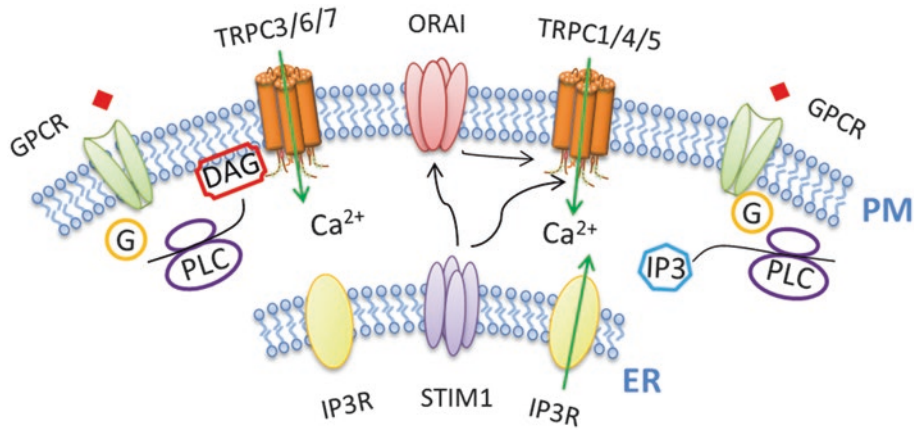

Fig. 2.2 Activation mechanisms for TRPC channels

2.2.2.2 Properties of TRPC Channel Homotetramer

TRPC1

TRPC1 has no bias of Ca^{2+} and Na^{+} selectivity. By performing noise analysis of the currents, the relation between the currents and their variances is roughly linear, and the slope of this relation indicates a single-channel amplitude of 1.1 pA at −70 mV. Assuming a linear amplitude-voltage relation, the predicted single-channel conductance is 16 pS [105].

TRPC2

The current-voltage (I-V) relationship of the SAG-activated TRPC2 conductance was nearly linear, with a reversal potential of 1.72 ± 2.4 mV. SAG activates a nonselective cation conductance that is permeable for Na^{+}, Cs^{+}, and Ca^{2+} but not for $NMDG^{+}$. Under bi-ionic conditions, the relative permeabilities PCa/PNa and PCs/PNa were 2.7 ± 0.7 and 1.5 ± 0.3, respectively. SAG-induced single-channel currents with a mean unitary current amplitude of −3.3 ± 0.5 pA at a holding potential of −80 mV, exhibiting a nearly linear I-V relationship with a slope conductance of 42 pS in symmetrical 150 mM Na^{+} solution [54].

TRPC3

The permeability for monovalent cations is PNa > PCs ≈ PK >> PNMDG, and the relative permeability PCa/PNa is 1.62 ± 0.27. The trivalent cations La^{3+} and $Gd^{3+}$are potent blockers of TRPC3 current (the IC50 for La^{3+} was 24.4 ± 0.7 μM). The single-channel conductance of bTRPC3 (cloned from bovine retina) activated by ATP, assessed by noise analysis, is 23 pS [34]. Stimulated by intracellular Ca^{2+}, hTRPC3 in inside-out patches shows cation-selective channels with 66-pS conductance and short (<2 ms) mean open times [104].

TRPC4

Following GTPγS stimulation, the PCa/PNa value calculated using the Goldman equation for divalent and monovalent cations is 1.05 for mTRPC4 [78]. According to the constant-field theory, the permeability ratio PCs/PNa of bTRPC4 is about 0.9 at nominal extracellular free Ca^{2+}. Extrapolating the ratio to higher divalent ion concentrations, the lower limits for the relative permeabilities PCs/PNa/PCa/PBa of bTRPC4 is 1:1.1:7.7:12.3 [69]. In contrast to TRPC3, La^{3+} enhances mTRPC4 current [78]. Under symmetrical buffer conditions, the single-channel I-V relation for mTRPC4 closely resembled those for whole-cell currents, showing a doubly rectifying shape and a reversal potential close to 0 mV. The single-channel chord conductances at 260 mV are 41 ± 1 picosiemens for mTRPC4 [78].

TRPC5

The PCa/PNa value calculated using the Goldman equation for divalent and monovalent cations is 1.79 for mTRPC5 following GTPγS stimulation. However, in response to ATP, on the assumption that activity coefficients are 0.3 for Ca^{2+} and 0.75 for both Na^{+} and Cs^{+}, the reversal potentials of 8 mV in the $0Ca^{2+}$ external solution and 17 mV in the 10 mM Ca^{2+} solution lead to permeability ratios PCa/PNa/PCs = 14.3:1.5:1 [66]. Similar to TRPC4, the GTPγS-induced TRPC5 current at both positive and negative membrane potentials is potentiated by La^{3+} without changing the reversal potential. Carbachol-induced Mn^{2+} entry through mTRPC5 in fura-2-loaded HEK cells was not impaired by La^{3+} at concentrations up to 300 mM [78]. However, La^{3+}, but not Gd^{3+}, significantly suppresses Ca^{2+} influx induced by ATP in TRPC5-transfected cells [66]. Like mTRPC4, the single-channel I-V relation for mTRPC5 shows a doubly rectifying shape and a reversal potential close to 0 mV. The single-channel chord conductance at 260 mV was 63 ± 1 pS for mTRPC5 [78].

TRPC6

The hTRPC6 is a nonselective cation channel that is permeable for Ca^{2+}, Cs^{+}, Na^{+}, and K^{+}, but not NMDG. The relative permeabilities PCa/PNa and PNa/PCs are 5 and 0.7, respectively, under bi-ionic conditions with Cs^{+} as the main cation in the pipette. The current-voltage (I-V) relation reveals dual inward and outward rectification.

The reversal potential of the hTRPC6 current is −3.6 ± 0.8 mV. In inside-out patches, single-channel currents with a mean unitary current amplitude of −1.7 ± 0.1 pA are detected at a holding potential of −60 mV. The hTRPC6 exhibited a linear I-V relationship with a calculated slope conductance of 35 pS and 37.5 pS in symmetrical 120 mM Cs^+ and in the bath solution with 120 mM Na^+. The open probability for hTRPC6 is higher at positive (+60 mV) than at negative (−60 mV) holding potentials, and the mean open time is within 1 ms [26].

TRPC7

The current-voltage relationship of TRPC7 current induced by ATP was almost linear, showing a slight flattening approximately between 0 and 20 mV and outward rectification at more positive potentials. Divalent cations, Ca^{2+}, Ba^{2+}, and Mn^{2+}, permeate the TRPC7 channels responsible for the spontaneous and ATP-enhanced inward currents. The calculated relative permeabilities (PCs/PNa/PCa/PBa) are 1:1.0:1.9:3.5 for the spontaneous current and 1:1.1:5.9:5.0 for the ATP-enhanced current. In the presence of 2 mM Ca^{2+}, further addition of 100 μM Gd^{3+} only slightly inhibits the currents induced by ATP [65].

2.2.2.3 Properties of TRPC Channel Heterotetramer

The combinatorial rules within the TRPC subfamily put forward by Hofmann et al. appear to apply in vivo, since the complex formed by TRPC1/TRPC4/TRPC5 is found in the embryonic brain [27, 83]. Although some ruled out heteromerization between distant relatives in the TRPC family, the nature of the heterotretramer is not clear so far. The evidence provided by several groups indicates that distant TRPC members can form heteromeric channels. For example, TRPC3 can assemble with TRPC1 [51], and a TRPC3/TRPC4 complex is able to form redox-sensitive channels in endothelial cells.

Moreover, TRPC heteromultimers with functional properties are distinct from homodmeric channels. Co-expression of TRPC1 and TRPL in *Xenopus* oocytes produced a thapsigargin-stimulated current that was not observed in oocytes expressing either TRPC1 or TRPL alone [17]. The co-expression of TRPC1 and TRPL in 293T cells produces a novel current not present in either TRPC1-expressing or TRPL-expressing cells [97, 93]). Co-expression of TRPC1 and TRPC3 results in a constitutively active cation conductance higher than that in TRPC1 or TRPC3 expression [51]. Coincidently, co-expression of TRPC1 and TRPC4 or TRPC5 results in outwardly rectifying nonselective cation conductances [83]. Homomeric TRPC5 is inwardly rectifying and has a conductance of 38 pS; TRPC1/TRPC5 is outwardly rectifying and displays an eightfold smaller conductance. The whole-cell I-V curve of the TRPC1/TRPC5 current is not changed upon removal of intra- and extracellular Mg^{2+}, indicating that the rectification mechanisms in TRPC1/TRPC5 may be different from TRPC5 homomers. The recombinant TRPC1/TRPC5 channel is activated by Gq-coupled receptors, and its activity is independent of calcium store depletion, which supports the hypothesis that TRPC heteromers form receptor-modulated currents in the mammalian brain. Moreover, biochemical analyses have identified TRPC1, TRPC4, and TRPC5 heteromultimers in rat embryonic brains, which form channels with novel conductances when expressed in vitro [83].

Together, heteromerization of TRPCs can form channels that have unique properties not exhibited by homomeric TRPC channels. Such a mechanism of homo- and heteromultimeric channel formation should create an incredible diversity of channels with an array of distinct biophysical properties and biological functions.

2.3 Prospectives

More efforts are required on structure analysis and in vivo study to have a better understanding of the TRPC channels. The good news is that there is great progress made by cryo-EM. Therefore, determining the structures of TRPCs is more than promising.

Though lots of efforts have been made to figure out the properties of TRPC channels, conflict

results obtained from varied systems make the whole field confusing. Many reasons can be listed to explain the contradiction. Firstly, due to various splice variants of TRPC homologs, it is possible that the expressed TRPC homolog may differ from that utilized to make the native channel. Each cell line has its own preference of protein expression, and even the same cell line cultured in various labs or by different people may have its unique expression profile. Secondly, it is not the exogenously expressed TRPC channels but the endogenous regulatory proteins that cause the difference. Thirdly, even when channel activities detected by electrophysiology are not present in the control cells, it does not necessarily imply that the activity belongs to the exogenously expressed protein. Fourthly, characterization of TRPC channels relies on "transient" overexpression or downregulation TRPC proteins. However, the "transient" time is enough to change the expression of numerous endogenous proteins or the activation of endogenous channels in response to the overexpression protein. In addition, for TRPCs, the universal expressed channels which have relatively small current amplitudes, it is hard to tell how much contribution belongs to these endogenous channels. Generating good antibody, especially functional antibody, and screening specific agonists and inhibitors, along with determining the structures, may be helpful for resolving the differences.

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