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REVIEW

Heteromerization of TRP channel subunits: extending functional diversity

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ABSTRACT

Transient receptor potential (TRP) channels are widely found throughout the animal kingdom. By serving as cellular sensors for a wide spectrum of physical and chemical stimuli, they play crucial physiological roles ranging from sensory transduction to cell cycle modulation. TRP channels are tetrameric protein complexes. While most TRP subunits can form functional homomeric channels, heteromerization of TRP channel subunits of either the same subfamily or different subfamilies has been widely observed. Heteromeric TRP channels exhibit many novel properties compared to their homomeric counterparts, indicating that co-assembly of TRP channel subunits has an important contribution to the diversity of TRP channel functions.

KEYWORDS co-assembly, molecular mechanism, diversification, nonselective cation channel, polymodal receptor, multi-subunit protein complex

INTRODUCTION

The first member of transient receptor potential (TRP) channels was identified in 1969 in *Drosophila* mutants that displayed a transient response to light (Cosens and Manning, 1969). In the following 40 years, not only *Drosophila* TRP channels received intense attention but also their homologs in other species were eagerly identified in realization of their involvement of a wide spectrum of cellular sensing functions. To date, 28 mammalian TRP channel genes are known. Additional TRP channel genes are found in other species such as worm, fruit fly and zebra fish. Indeed, the TRP channel superfamily has bloomed to more than 50 members of mostly nonselective cation ion channels. According to similarity in their primary sequences, TRP channels are classified into seven subfamilies: TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPP (polycystin), TRPML (mucolipin), TRPA (ankyrin) and TRPN (NO mechanopotential) (Clapham, 2003; Nilius, 2007b). As expected for such a large family of cation channels, TRP channels are widely found throughout the biologic kingdom from yeast and fly to mouse and human. They are expressed in both sensory neurons and non-sensory cells (Stowers et al., 2002; Zhang et al., 2003; Zhou et al., 2003).

Structurally, TRPs are tetrameric cation channels (García-Sanz et al., 2004). Each channel subunit contains six transmembrane segments (S1–S6) and a pore-forming region formed by a loop between the S5–S6 segments. Both the amino (N) terminus and the carboxyl (C) terminus are intracellularly located. Most of the TRP channels have an N-terminal ankyrin repeat domain and a C-terminal TRP domain. The long N and C termini of TRPs also contain several additional regulatory domains which are relatively conserved in most of the TRPs (Fig. 1). For example, a coiled-coil domain present in TRPCs, TRPPs and TRPMs; TRPCs and TRPVs possess a PDZ-binding domain in their C-termini; TRPCs, TRPVs and TRPMs contain a calmodulin (CaM) binding domain; TRPMLs and TRPPs have a Ca^{2+}-binding EF-hand motif in their C-termini. Most of these domains mediate protein-protein interactions that allow intra- and inter-cellular associations.

Functionally, TRP channels are activated by diverse stimuli that are physical (e.g., temperature, voltage or mechanical stress) or chemical (e.g., pH, osmotic pressure, neurotransmitters, growth factors, environmental irritants) in nature. This enables TRP channels to act as multifunctional cellular sensors. TRP channels responding to combinations of stimuli serve as polymodal signal detectors for noxious stimuli from a cell's ambient environment (Tominaga et al., 1998; Bautista et

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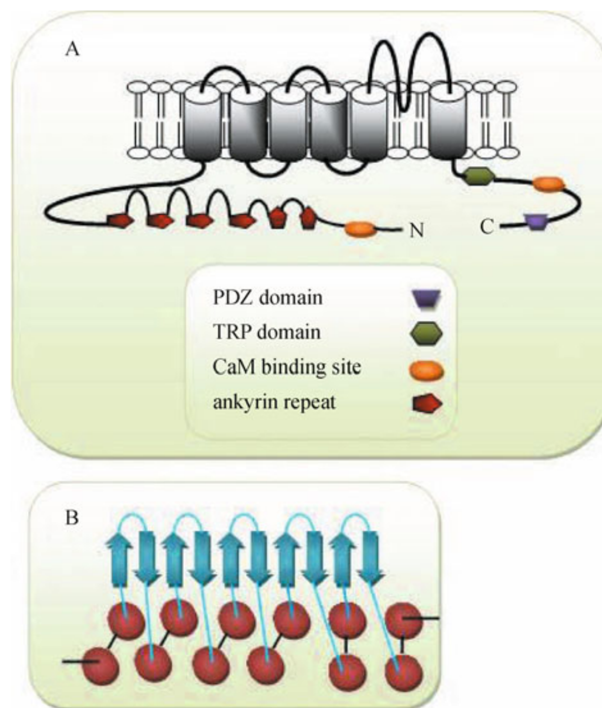

Figure 1. Topological arrangement of a TRPV channel subunit. (A) Each channel subunit contains six transmembrane segments (S1–S6), and a pore region formed by a loop between the S5–S6 segments. Long intracellular N and C termini contain several relatively conserved domains such as ankyrin repeat domain (ARD), TRP domain, PDZ domain, CaM binding site. (B) Topological plot of the TRPV ankyrin repeat domain. Red circle represents α-helices, and blue arrows and lines represent β loop fingers.

al., 2006), or as coincidence detectors for simultaneous signal inputs that contribute to learning and memory (Chuang et al., 2004). As TRP channel subfamilies are defined by sequence homology, channels belonging to the same subfamily may exhibit distinct functional properties. For example, the TRPV subfamily members TRPV1–4 possess exquisite temperature sensitivity important for nociception and thermo-sensing, whereas TRPV5–6 are involved in epithelium Ca^{2+} entry with ordinary sensitivity to temperature. Similarly, the cold sensor TRPM8 belongs to the TRPM subfamily whose members do not all exhibit high temperature sensitivity.

The physiologic properties of most TRP channels are well studied. Readers are referred to excellent recent reviews on broad TRP channel subjects (Montell, 2005; Owsianik et al., 2006; Nilius, 2007a; Reaves and Wolstenholme, 2007; Gaudet, 2009; Moiseenkova-Bell and Wensel, 2009). In the present review, we focus on studies regarding heteromeric TRP channel formations, their functional diversity, as well as the molecular mechanism governing TRP channel subunits co-assembly.

TRP CHANNELS GENERATED BY SUBUNITS CO-ASSEMBLY

Subunits heteromerization in TRP channels has been widely reported. Heteromeric channels can form by subunits either within the same subfamily or between different subfamilies (Fig. 2). Compared with other channel families (e.g., the Kv voltage-gated potassium channel family and the ionotropic glutamate receptor family), co-assembly of TRP subunits exhibits substantial specificity.

TRPC

The TRPC channels are classical or canonical TRPs which are ubiquitously expressed. Based on sequence homology and functional similarities, TRPCs can be further divided into four groups: TRPC1, TRPC2, TRPC3/6/7 and TRPC4/5. Subunit heteromerization in the TRPC subfamily has been extensively explored (Goel et al., 2002; Riccio et al., 2002; Plant and Schaefer, 2003; Schaefer, 2005). A number of independent studies confirmed that co-assembly of TRPC subunits in both native cells and expression systems (Strübing et al., 2001; Goel et al., 2002; Hofmann et al., 2002; Strübing et al., 2003). Intensive interaction between TRPC subunits of three different subgroups (TRPC1, TRPC3/6/7 and TRPC4/5) were found (Lintschinger et al., 2000; Liu et al., 2005b; Plant and Schaefer, 2005; Poteser et al., 2006). However, TRPC2, which is thought to be a pseudogene in human, does not interact with any other known TRPC subunit. This is probably due to its distant relationship with other members of the canonical TRP family. Co-assembly of

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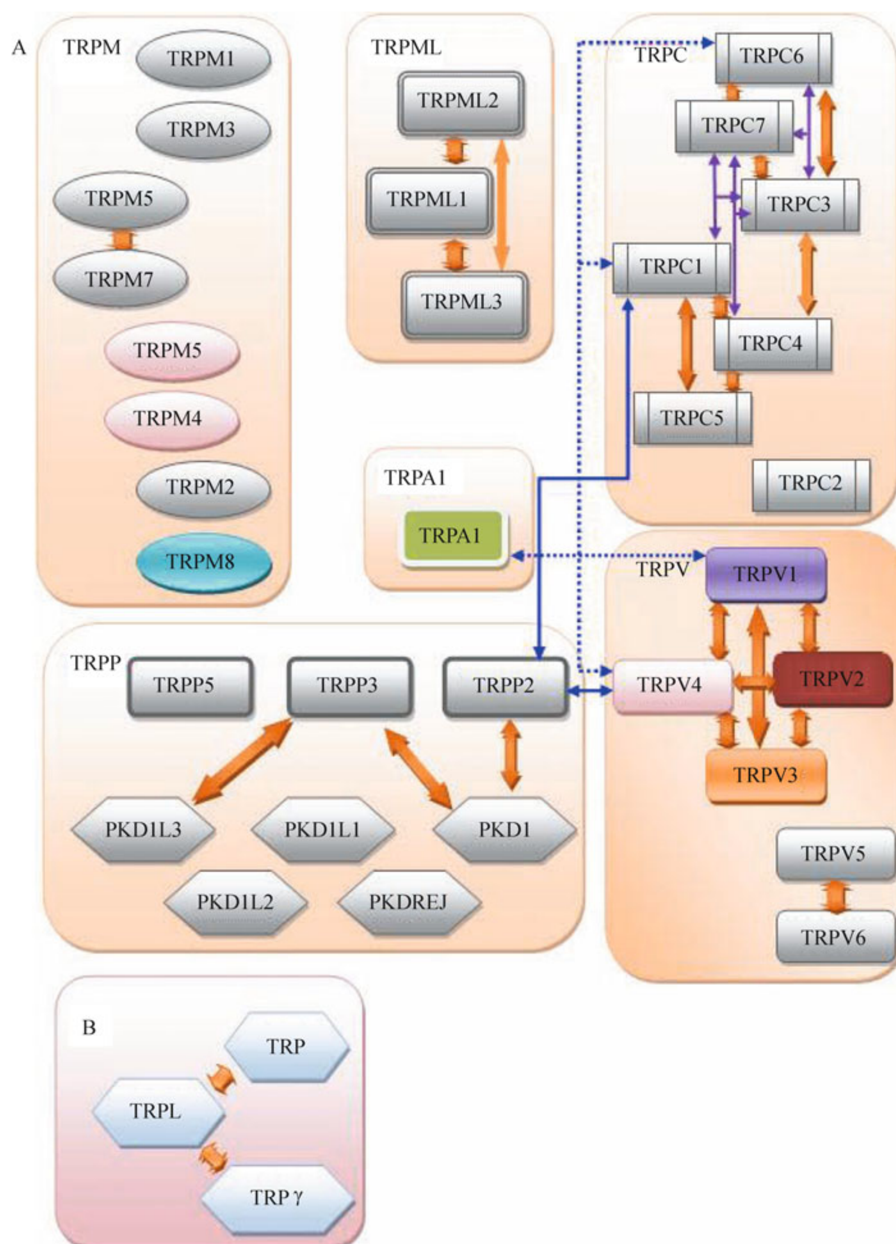

Figure 2. Subunit association between TRP channel subunits. (A) Heteromerization of mammalian TRPs. Orange arrows indicate co-assembly between two channel subunits. Purple arrows indicate interaction among three different subunits of the same subfamily. Blue solid arrows indicate interaction between subunits of different subfamilies. Blue dotted arrows indicate functional interaction for which heteromeric channel has not been identified. (B) Co-assembly of *Drosophila* TRPs.

TRPC1 and TRPC5 was shown in hippocampal neurons (Strübing et al., 2001; Goel et al., 2002). Similar associations were reported between TRPC1 and TRPC3 via an N-termini domain interaction in salivary gland cells lines (Liu et al., 2005b). In HEK293 cells co-transfection of TRPC1 and TRPC5 (Strübing et al., 2001) or TRPC1 and TRPC4 (Gudermann et al., 2004) yielded novel nonselective cation channels. Zagranichnaya et al. (2005) reported that TRPC1/3/7 can interact to form a store-operated channel complex (Zagranichnaya et al., 2005). Moreover, heteromerization of TRPC3 and TRPC4 seemed to produce channels with a distinct pore structure clearly different from those of the homomeric TRPC3 and TRPC4 channels (Poteser et al., 2006).

TRPV

The vanilloid TRPV channels include six members that can be divided into two groups: TRPV1/2/3/4 and TRPV5/6. The

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overall sequence similarity of this subfamily is more than 40%. TRPV1–4 subunits contain 3–6 ankyrin repeats in the intracellular N-terminal region. Activation of TRPV1–4 channels is driven by diverse stimuli including heat, voltage, pH, as well as natural endogenous/extrogenous ligands and synthetic compounds. Indeed, the very noticeable polymodal feature of TRPV1–4 channels indicates fascinating functional implications. As major cellular sensors for thermosensation of dorsal root ganglion (DRG) neurons and epithelial cells, TRPV1–4 channels formed by homomeric subunits exhibit disparate activation temperature thresholds and kinetic properties. TRPV1–4 channels are nonselective cation channels with a permeability ratio for P_{Ca}/P_{Na} between one and ten, whereas TRPV5/6 channels are Ca^{2+}-selective cation channels with the P_{Ca}/P_{Na} ratio of 100 or more. Several studies revealed that TRPV5/6 subunits not only are co-localized in the same cells but also can form heteromeric channel complexes (Hoenderop et al., 2003; Hellwig et al., 2005; Schaefer, 2005). Co-localization and association between TRPV1 and TRPV2 as well as TRPV1 and TRPV3 were also reported (Smith et al., 2002; Liapi and Wood, 2005; Rutter et al., 2005). Hellwig et al. (2005) reported no association between TRPV1–4 subunits expressed in HEK293 cells except between TRPV1 and TRPV2 subunits (Hellwig et al., 2005). A later study using fluorescence resonance energy transfer (FRET) as well as single-channel recording demonstrated wide-spread interaction between any two members of TRPV1–4 (Cheng et al., 2007). This study provided evidence that thermosensitive TRPV channel subunits can form heteromeric channels with intermediate conductance levels and gating kinetic properties compared to homomeric channels. The disparity between these two studies is attributed to how fluorescence signals were analyzed (Cheng et al., 2007).

TRPP

Mutations in the polycystic TRPP channels can cause polycystic kidney diseases. The TRPP subfamily contains eight members that can be further divided into two groups by sequence similarities: the PKD1-like group and the PKD2-like group. The PKD1-like group contains five members, PKD1, PKDREJ, PKD1L1, PKD1L2 and PKD1L3; the PKD2-like group contains TRPP2 (previously known as PKD2), TRPP3 (previously known as PKD2L1) and TRPP5 (previously known as PKD2L2). Subunits of the two groups are structurally quite different. PKD1-like group members have 11 transmembrane segments, a very long (up to ~3000 amino acids) extracellular N-terminal segment, and an intracellular C-terminal segment which can interact with that of TRPP2. PKD2-like group members resemble other TRP channels, with intracellular N and C termini, six transmembrane segments and a central channel pore region. Members in this group share a coiled-coil domain in their C-termini. Heteromeric interaction between PKD1 and TRPP2 has been identified by several groups throughout the past decade (Qian et al., 1997; Tsiokas et al., 1997; Hanaoka et al., 2000; Grimm et al., 2003; Delmas et al., 2004; Sharif-Naeini et al., 2009). PKD1 can also interact with PKD2L1 (Murakami et al., 2005); the interaction is essential for PKD2L1 trafficking and channel formation. Along these lines, studies also showed that PKD1L3 and PKD2L1 co-localized in taste receptor cells (Ishimaru et al., 2006; LopezJimenez et al., 2006).

TRPM

The long or melastatin TRPM subfamily composes of eight members that can be divided into four groups: TRPM1/3, TRPM2/8, TRPM4/5 and TRPM6/7. They are structurally very similar to the well-studied voltage-gated potassium channels, and contain six transmembrane segments, intracellular N and C termini, and a channel pore domain. Unlike those of TRPV and TRPC subfamilies, TRPM subunits contain a TRP motif with very low sequence conservation but a highly conserved coiled-coil domain within their C termini. In addition, TRPM channels lack an ankyrin repeat domain seen in the N-terminal of many TRP channels. Furthermore, members of the TRPM3/6/7 group have a functional enzymatic domain in their C termini. Most TRPMs except TRPM4/5 (TRPM4 and TRPM5 are the only monovalent-selective ion channel of the TRP family) are cation channels permeable to Ca^{2+} and Mg^{2+}. All TRPM subunits can form functional homotetrameric channels. Whether TRPM subunits can heteromerize remains far less clear. The only known combination is between TRPM6 and TRPM7 (Chubanov et al., 2004; Chubanov et al., 2005; Li et al., 2006; Jiang, 2007), in which case heteromeric channels with intermediate conductance and gating properties exhibit distinct properties compared to the parental homomers, such as different sensitivity to low PH, different permeability to divalent cation and different regulation by 2-APB (Li et al., 2006).

TRPML

The mucolipin TRPML family exhibited high homology with TRPPs but low similarity to other TRPs. This family of nonselective cation channels consists of three members, referred to as TRPML1, TRPML2, and TRPML3. Mutation of human TRPML1 can cause mucolipidosis type IV, a neurodegenerative lysosomal storage disorder disease (Bargal et al., 2000). Mutation of TRPML3 in varitint-waddler mouse resulted in deafness and pigmentation defects (Di Palma et al., 2002). Using a FRET-based approach Montell and coworkers demonstrated that TRPMLs can interact to form heteromultimers (Venkatachalam et al., 2006). Moreover, the presence of either TRPML1 or TRPML2 specifically make TRPML3 trafficking to lysosomes from endoplasmic reticulum (Venkatachalam et al., 2006). Recent study which

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using immunocytochemical analysis as well as single-channel recording confirmed that heteromultimeric TRPMLs exhibit intermediate conductance and kinetic properties (Curcio-Morelli et al., 2010).

Heteromerization between TRP subunits of different subfamilies

Since the early reports of TRP subunits heteromerization in *Drosophila* (Gillo et al., 1996; Xu et al., 1997), there are extensive documentations appeared in the past decade on TRP channel subunits co-assembly into heteromeric complex. Studies continuous to reveal wide-spread heteromerization within the TRP channel superfamily. TRPγ (a *Drosophila* TRP-related subunit) can co-assemble with TRPL to form a channel stimulated by phospholipase C- (PLC) (Xu et al., 2000). Independent studies from several groups focusing on TRPC1 and TRPP2 have demonstrated that heteromeric channels formed by these two subunit types at a 2:2 stoichiometry exhibited a new receptor-operated channel property (Tsiokas et al., 1999; Bai et al., 2008; Kobori et al., 2009; Zhang et al., 2009). Köttgen et al. (2008) reported that TRPV4 and TRPP2 formed heteromeric channel complex both *in vivo* and *in vitro* (Köttgen et al., 2009). Then using atomic force microscopy, it was found that TRPP2 and TRPV4 form heteromeric complexes with a 2:2 stoichiometry and alternating subunit arrangement (Stewart et al., 2010). Moreover, novel combinations including TRPC1/TRPC6/TRPV4 and TRPA1/TRPV1 have been reported by recent studies (Alessandri-Haber et al., 2009; Salas et al., 2009).

MOLECULAR MECHANISMS GOVERNING TRP CHANNEL SUBUNITS ASSEMBLY

What are the molecular mechanisms governing TRP channel assembly? Recent studies have applied a combination of biochemistry, electrophysiology, X-ray crystallography as well as single-molecule optical imaging to elucidate the answer. These studies have revealed a number of molecular determinants (Lepage and Boulay, 2007; Schindl and Romanin, 2007) that could directly or indirectly contribute to TRP channel subunits assembly.

Ankyrin repeat domain

Ankyrin repeat domain (ARD) is a repetition of 33-residue motif. Each ankyrin repeat is characterized by a pair of antiparallel α-helices followed by a loop linking to a β-hairpin (Sedgwick and Smerdon, 1999). Generally, most proteins contain tandem arrays of two-to-seven repeats (Groves and Barford, 1999). ARD is one of the most common domains mediating protein-protein interaction. For the majority of cases, ARD interacts with a short peptide segment; ARD-ARD interactions are rarely observed. In TRP channels, the ARD resides in the intracellular N terminus. The number of repeats varies from 3–6 for TRPC/TRPV, 14–15 for TRPA and ~29 for TRPN. TRPM, TRPP, and TRPML channels lack the ARD. It has been reported that ARD plays a key role in heteromerization of TRPC1/3, and the first ankyrin repeat of TRPC1 is found to interact with the N terminus of TRPC3 (Liu et al., 2005b). Other studies demonstrated that not only N-terminal ankyrin repeat domain (Hellwig et al., 2005; Arniges et al., 2006) but also the C-terminal domain from position 716 to position 828 participates in oligomerization and trafficking of TRPV4 (Hellwig et al., 2005; Arniges et al., 2006; Becker et al., 2008). Meanwhile, Lepage et al. (2006) found that both N-terminal ARD and C terminus are responsible for TRPC oligomerization (Lepage et al., 2006). Two studies reported that ARD is critical for channel assembly of TRPV5 and TRPV6 (Chang et al., 2004; Erler et al., 2004). Specifically, the first ankyrin repeat (residues 64–77) of TRPV5 and the third ankyrin repeat (residues 116–140) of TRPV6 are important for subunit assembly. However, these studies did not exclude other factors which may regulate channel assembly. A recent study using analytical size exclusion chromatography as well as crystallization provided evidence that TRPV6-ARD is monomeric in solution, does not form tetrameric complexes, and may regulate channel assembly partly (Phelps et al., 2008). Furthermore, for TRPV2, both solution studies and crystal packing interactions revealed that the ankyrin repeat domain is a regulatory domain but not a determinant of subunit assembly (Jin et al., 2006; McCleverty et al., 2006).

Coiled-coil domain

Coiled-coil domain is a structural motif that consists of two or more α-helices that wrap together like the strands of a rope. They are one of the most common and best understood protein-protein interaction domains. Usually it contains a heptad repeat pattern of $(abcdefg)_n$ in its amino acid sequence. The first and fourth positions of the heptad repeat are occupied by hydrophobic amino acids while the fifth and seventh position residues are charged or polar. Coiled-coil domain has been found in members of TRPC, TRPM and TRPP subfamilies. It has been reported that the N-terminal coiled-coil structure domain of TRPC1 facilitated the homo-dimerization process (Engelke et al., 2002). Interesting studies with TRPM2 and TRPM8 demonstrated that the coiled-coil domain in the intracellular C terminus instead of the N terminus is essential for channel tetramerization (Mei et al., 2006; Tsuruda et al., 2006; Mei and Jiang, 2009). The C-terminal coiled-coil domain of TRPP2 has been demonstrated to be essential for interaction with the C-terminal part of PKD1 (Hanaoka et al., 2000; Delmas, 2005). A recent study provides evidence that the C-terminal coiled-coil domain directs assembly of homotrimer of TRPP2, which in turn interacts with C-termini of PKD1 (Yu et al., 2009). The case is

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very similar to the rod cyclic nucleotide-gated channels in which the C-terminal coiled-coil domain dedicates the assembly of three CNGA1 subunits and one CNGB1 subunit (Zhong et al., 2002).

TRP domain

The TRP domain is a 25-amino acid motif rich in charged residues. It contains the TRP box (characterized by the specific amino acid sequence EWKFAR), which is fully conserved in TRPC channels, but is less conserved among members of the TRPV and TRPM subfamilies. The TRP domain is localized in the C terminus close to the sixth transmembrane segment (Clapham et al., 2001). Studies indicated that the TRP domain may be essential for TRP channel assembly. It has been recently shown that the TRP domain of TRPV1, in particular the segment from D684 to R721, is involved in assembly of the channel subunits into functional channels (GarcíaSanz et al., 2004). The TRP domain may also serve as a regulatory domain targeted by PIP2 and other channel regulators (Liu and Liman, 2003; Liu et al., 2005a; Rohács et al., 2005).

Other factors

Effects to identify the determinants of TRP subunits assembly also led to other regions. Boulay and coworkers identified two regions in TRPC that are involved in subunits assembly (Lepage et al., 2006). One region is in the N-terminal region that includes the ankyrin repeat domain and the coiled-coil domain; the other region is made of the pore region and the C-terminal tail (Lepage et al., 2006). Studies suggested that both the C-terminal and the N-terminal may be necessary for tetrameric assembly of TRPP2,TRPV4, and TRPV5 (Chang et al., 2004; Becker et al., 2008; Feng et al., 2008; Yu et al., 2009). For TRPV5, Chang et al. (2004) narrowed down the essential sequence to parts of the N terminus (residues 64–77) and the C terminus (residues 596–601) (Chang et al., 2004). Previous studies also identified the transmembrane domain as a candidate for the molecular determinants (Xu et al., 1997, 2000; Hellwig et al., 2005). The PDZ domain of TRPC4, which controls the channel's cellular localization and surface expression in HEK293 cells, was suggested to be a potential determinant of channel assembly as well (Mery et al., 2002). Additional efforts are needed to carefully examine the identified candidate structures and identify potential new structures that may contribute to TRP channel assembly.

HETEROMERIZATION EXTENDS THE FUNCTIONAL DIVERSITY OF TRP CHANNELS

While much remains to be learned about the physiologic consequences of heteromultimerization among TRP subunits, it is clear that subunits co-assembly yields a variety of channel types with functional properties distinct from their homomeric counterparts. In the early study of *Drosophila* heteromeric TRP channels, it was shown that the combination of TRP/TRPL subunits can produce a distinct store-operated conductance (Xu et al., 1997). In addition, co-assembly of TRPL and TRPγ yielded a channel that was activated by PLC stimulation (Xu et al., 2000). Meanwhile, heteromerization of mammalian TRPV1/3, TRPV5/6, TRPML1/2, TRPML1/3, TRPML2/3,TRPC1/4, TRPC1/5, TRPC3/4, TRPC3/6, TRPC3/7 or TRPC4/5 has been identified to produce channels with novel properties (Lintschinger et al., 2000; Strübing et al., 2001; Hoenderop et al., 2003; Strübing et al., 2003; Plant and Schaefer, 2005; Poteser et al., 2006; Cheng et al., 2007; Curcio-Morelli et al., 2010). TRPV3 was found to co-assemble with TRPV1 to form heteromeric channels with altered pharmacological properties (Smith et al., 2002). Heteromultimeric TRPP2/TRPC1 channels have been identified to be new receptor-operated channels implicated in mechanosensation (Tsiokas et al., 1999; Bai et al., 2008; Kobori et al., 2009). Furthermore, TRPP2 associates with TRPV4 to produce a channel that functions as a mechanosensitive and thermosensitive molecular sensor in the primary cilium of renal epithelium cells (Köttgen et al., 2008). TRPC1/3/7 can interact to produce a channel complex with store-operated channel properties (Zagranichnaya et al., 2005). Alessandri-Haber et al. (2009) found that TRPC1 and TRPC6 with TRPV4 are frequently co-expressed in DRG neurons; TRPC1 and TRPC6 subunits incorporate with TRPV4 to mediate mechanical hyperalgesia and primary afferent nociceptor sensitization (Alessandri-Haber et al., 2009). Surprisingly, the noxious cold-sensitive TRPA1 channel subunit may functionally interact with the noxious heat-sensitive TRPV1 channel subunit, despite their substantial sequence dissimilarity. It is found that co-expression of TRPA1 and TRPV1 contributes to TRPA1-mediated responses in trigeminal sensory neurons (Salas et al., 2009). Moreover, PKD1L3 was found co-localized with PKD2L1 in taste receptor cells (Ishimaru et al., 2006; LopezJimenez et al., 2006) and may play a potential role in taste sensory transduction. Taken together, heteromerization of TRPs serves as an important mechanism for the regulation of TRP channel function.

While most studies so far focus on the static TRP channels subunit composition, what is perhaps more interesting is the possibility that the subunit composition of TRP channels in living cells may be dynamically regulated. Indeed, upon stimulation the cell surface TRP channel density increases dramatically and rapidly (Vetter et al., 2008; Schmidt et al., 2009). Little is known about whether and how TRP channels change subunit composition in response to environmental stimuli.

CONCLUSION

TRP channels are widely expressed in virtually all cell types

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and carry out diverse fundamental functions in the human body. Heteromerization of TRP subunits emerges as one way to produce diverse functions of this fascinating family. This is the area that still needs much work. This review provides an overview of the current understanding of TRP channel assembly and function. Further studies will fully reveal the scope of subunits co-assembly and the molecular mechanism governing specific subunits interactions.

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