TRP Channels Entering the Structural Era

TRP channels entering the structural era

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Abstract

TRP channels are important in many neuronal and non-neuronal physiological processes. The past two years have seen much progress in the use of structural biology techniques to elucidate molecular mechanisms of TRP channel gating and regulation. Two approaches have proven fruitful: (i) a divide-and-conquer strategy has provided high-resolution structural details of TRP channel fragments although it fails to explain how these fragments are integrated in the full channel; and (ii) electron microscopy of entire TRP channels has yielded low-resolution images that provide a basis for testable models of TRP channel architecture. The results of each approach, summarized in this review, provide a preview of what the future holds in TRP channel structural biology.

Introduction

Transient receptor potential (TRP) channels form a diverse family of cation channels that respond to a variety of signals (Ramsey *et al.*, 2006; Venkatachalam & Montell, 2007). For example, some are involved in sensory perception and are directly activated by chemical ligands and/or physical sensory stimuli such as temperature, mechanical and osmotic stresses. Others are activated downstream of receptor stimulation through a phospholipase C (PLC)-dependent pathway. An intriguing feature shared by several TRP channels is that a given TRP channel gating response is the result of the integration of several signals of different nature (chemical or physical) and different sources (intra- or extracellular) (O'Neil & Brown, 2003; Soboloff *et al.*, 2007). TRP channels are particularly prominent in the genomes of the animal kingdom. In mammals, the TRP family contains nearly 30 members distributed into six subfamilies according to sequence and function (Montell, 2005): TRPA (ankyrin), TRPC (canonical), TRPM (melastatin), TRPML (mucolipin), TRPP (polycystin), TRPV (vanilloid). An additional subfamily, TRPN (NOMPC), is absent in mammals but found in many other organisms including worms and fish.

TRP proteins have six transmembrane segments homologous to the transmembrane domain of Shaker potassium channels (Long *et al.*, 2005; Long *et al.*, 2007). Just like Shaker channels, TRP proteins also assemble as tetrameric channels, as demonstrated by several biochemical studies (e.g. (Kedei *et al.*, 2001; Phelps & Gaudet,

2007)) and, very convincingly, by atomic force microscopy studies on TRPC1 (Barrera *et al.*, 2007). Major differences between TRP channel subfamilies lie in the large N- and C-terminal cytosolic domains which contain putative protein-interaction and regulatory motifs and have distinct features in different TRP subfamilies. Figure 1 illustrates the distinct sequence features of each TRP channel subfamily. Ankyrin repeats are present in the N-terminal cytosolic region of TRPC, TRPV, TRPA and TRPN channels. While the TRPC and TRPV channels have few repeats and irregular sequences (Phelps *et al.*, 2007; Phelps *et al.*, 2008), TRPA and TRPN have many regular repeats (see (Gaudet, 2008) for a recent review). TRPM channels also have a large, ~700-residue N-terminal intracellular region, which can be subdivided in four sub-domains labeled "TRPM homology regions" or MHRs, with similarity only to other TRPM channels (Clapham, 2003; Fleig & Penner, 2004). In their C-terminal intracellular region, TRPM channels have a coiled coil region (Jenke *et al.*, 2003; Montell, 2005). A few TRPM proteins also have a large extension of the C-terminal intracellular region beyond the coiled coil region, encoding an enzymatic domain (Cahalan, 2001): TRPM6 and TRPM7 have a C-terminal α-kinase domain (Nadler *et al.*, 2001; Riazanova *et al.*, 2001; Runnels *et al.*, 2001), and TRPM2 has a C-terminal NUDIX domain (Perraud *et al.*, 2001). Finally, both TRPP and TRPML channels have an extracellular domain inserted between transmembrane segments S1 and S2, although there is no significant sequence similarity between the extracellular domains of TRPP and TRPML proteins.

Until two years ago, three-dimensional structure information on TRP channels was largely limited to structures of homologous domains from other proteins (Gaudet, 2006), aside from the crystal structure of the TRPM7 α-kinase domain (Yamaguchi *et al.*, 2001), a domain unique to TRPM6 and TRPM7. However, TRP channels are now entering the structural era. Here I will introduce some of the methodologies available and approaches to TRP channel structural biology, review the recent literature on TRP channel structure, and discuss some of the challenges that lie ahead.

Structural biology of TRP channels

There are three major techniques to obtain structural information on macromolecules: x-ray crystallography, nuclear magnetic resonance (NMR) and electron

microscopy (EM) - either single particle EM or electron crystallography. A recent primer on structural biology for neuroscientists is an excellent source of information on these methods (Minor, 2007). Two factors make structural studies of TRP channels a particularly difficult challenge for structural biologists. First, structural biology techniques require an ample supply of highly pure and stable protein samples, and membrane proteins are notoriously difficult to produce in large quantities and purify in a stable native state (see (Grisshammer, 2006) for a recent review). Second, as introduced above, TRP channels are very large tetramers of 70-250 kDa subunits that are each formed of multiple domains (structural biologists usually define a protein "domain" as an independently folding unit, a protein segment that can take on its native conformation even when isolated from the rest of the protein). Such large proteins are currently inaccessible to high-resolution solution NMR techniques. In addition, the flexibility afforded by multiple domains, which likely has functional significance, often hinders crystallization - a necessary step in x-ray crystallographic analyses - because crystals are formed of an ordered array of molecules in an identical conformation. Flexibility also complicates single particle EM studies because the sample is then a heterogeneous mixture of distinct conformations, which have to be classified properly and averaged independently.

To compensate for the dearth of structural information on TRP channels, a number of studies have made use of various modeling approaches to provide a context for their mutagenesis data. These can be quite powerful especially when the structures of close structural homologs are available. In that respect, the structures of potassium channels like KcsA (Doyle *et al.*, 1998) - whose structure is expected to be similar to the pore domain of TRP channels, and the Shaker channels Kv1.2 and Kv2.1 (Long *et al.*, 2005; Long *et al.*, 2007) can be helpful. One example where a structural model was used to design and interpret experiments on a TRP channel is a mutagenesis study of the outer pore structure and ion selectivity of the calcium-dependent TRPV6 channel (Voets *et al.*, 2004). However, this review will focus on experimental structure determination results rather than modeling.

Two approaches have now yielded information on the structure of TRP channels. On one hand, high resolution structures and other biophysical measurements of isolated

cytosolic domains of several TRP channels have yielded insights into their biological function. On the other hand, several single-particle EM studies provide a low resolution view of entire TRP channels. The results available for each approach are summarized in the next two sections.

Divide and conquer: structures of TRP channel domains

Although the functional differences of various TRP channels are likely due in large part to their strikingly diverse cytosolic domains, their sequences reveal little about their structure and function. Determining the three-dimensional structure and functional properties of these domains is critical to deciphering their role in TRP channel function and regulation. This divide-and-conquer approach has yielded structural information on three types of TRP channel domains: (i) several structures of the ankyrin repeat domains of TRPV channels; (ii) structural information on the coiled coil domain of TRPM channels; and (iii) a structure of the α-kinase domain of TRPM7. These three cases are described below.

Ankyrin repeats, found in the N-terminal cytosolic segments of TRPA, TRPC, TRPN and TRPV channels, are identifiable in protein sequences as ~33-residue conserved sequence motifs found in tandem arrays ranging from three to more than thirty repeats (Mosavi *et al.*, 2004). Figure 2A shows the structure of a set of six ankyrin repeats as a ribbon diagram. The structure of each ankyrin repeat consists of a hairpin of two short α-helices, followed by a hairpin loop that projects out perpendicular to the helical axes. Tandem repeats then stack together side-by-side so that the first helices in each repeat - termed inner helices - form a concave surface, while the second, outer helices, form a convex surface. The concave surface formed by the inner helices is adjacent to the hairpin loops, and they are often referred to as the palm and fingers, respectively, of a hand-shaped domain. This analogy is particularly useful because most ligand interactions are observed on these surfaces, as if the hand grabs onto the ligand. The only known biochemical function of ankyrin repeat domains is to interact with ligands, although the nature of ligands is highly diverse, from small molecules to unusual oligonucleotides and large proteins (Myers & Julius, 2007; Gaudet, 2008).

To date, the structural data on TRP channel ankyrin repeats is restricted to the TRPV subfamily. TRPV channels are involved in pain, thermo- and mechanosensation, and calcium homeostasis (Niemeyer, 2005). In mammals there are six TRPV channels that partition into two groups: TRPV1-4, involved in sensory signaling; and the more distantly related TRPV5 and TRPV6, expressed in the intestinal tract and kidneys and important for calcium homeostasis. The structures of the N-terminal ankyrin repeat domain of three TRPV channels, TRPV1, TRPV2 and TRPV6, have been published (Jin *et al.*, 2006; McCleverty *et al.*, 2006; Lishko *et al.*, 2007; Phelps *et al.*, 2008). At a superficial level, the structures are very similar (Fig. 2*A*), as expected from their sequence homology. The TRPV ankyrin repeat domains have six ankyrin repeats, with unusually long finger loops (especially the first three loops). Amino acid residues that are conserved in TRPV proteins but diverge from the ankyrin repeat sequence consensus cause a pronounced twist between the fourth and fifth repeat (Phelps *et al.*, 2008). Since these residues are conserved in TRPV proteins, the overall shape of the TRPV ankyrin repeats is likely to be conserved in all TRPV family members. Of note, sequence analyses indicate that the ankyrin repeats from other TRP channel subfamilies are only distantly related to the TRPV ankyrin repeats, and therefore imply that their structures will also be quite different (Phelps *et al.*, 2007).

The TRPV ankyrin repeat structures do have notable differences in their details. More importantly, the structural differences result in drastically different biochemical properties. For instance, the structure of the TRPV1 ankyrin repeats shows a bound ATP molecule (Fig. 2*B*), and accompanying data support a role for this ATP-binding site in regulating TRPV1 sensitivity (Lishko *et al.*, 2007). TRPV1 is expressed in nociceptor neurons and is activated by heat and capsaicin, the pungent compound causing the "hot" taste of chili peppers (Caterina *et al.*, 1997). Biochemical and electrophysiology experiments showed that intracellular ATP can sensitize the TRPV1 response to capsaicin, whereas calcium-bound calmodulin, acting through the same binding region on the TRPV1 ankyrin repeats, causes desensitization (Lishko *et al.*, 2007). This ATP-binding site is conserved in TRPV1 from other species (Phelps *et al.*, 2007), more so than the capsaicin-binding site, which is not conserved in chicken TRPV1 for instance (Jordt & Julius, 2002). However, this ATP-binding site is not conserved in several other TRPV

channels, including TRPV2, a close homolog of TRPV1 also involved in sensing hot temperatures, and TRPV5 or TRPV6, involved in calcium homeostasis (Lishko *et al.*, 2007; Phelps *et al.*, 2008). Similarly, the TRPV1 ankyrin repeats bind to calmodulin, as do - although weakly - the TRPV5 ankyrin repeats, whereas the TRPV2 and TRPV6 ankyrin repeats do not (Lishko *et al.*, 2007; Phelps *et al.*, 2008). These structural and biochemical studies demonstrate that studying isolated domains can provide useful information about a domain's function in the context of the whole ion channel, and that high resolution information provides the level of detail required to grasp some of the unique features of each channel.

Most TRPV channels have 50-150 amino acid residues of poorly conserved sequence N-terminal to the six ankyrin repeats. An N-terminal deletion analysis on TRPV1 indicated that removing the ~100 residues N-terminal to the ankyrin repeats had little effect on its electrophysiological properties (Jung *et al.*, 2002). Furthermore, although the protein crystals used to determine the structure of human TRPV2 ankyrin repeats also included the 68 residues N-terminal to the ankyrin repeats, the resulting x-ray diffraction data provided no interpretable electron density corresponding to these 68 residues, implying that they are flexible and disordered (McCleverty *et al.*, 2006). In this case the combination of functional and structural data suggests a flexible N-terminal appendage that may not be important in channel function. In TRPV4, the junction between the N-terminal region and the ankyrin repeats is proline-rich and interacts with the Src Homology 3 (SH3) domain of PACSIN3 (Cuajungco *et al.*, 2006). The TRPV4-PACSIN3 interaction selectively inhibits TRPV4 basal activity and activation by cell swelling and heat, but not its activation by phorbol ligands (D'Hoedt *et al.*, 2008). SH3 domains typically interact with a proline-rich peptide segment in an extended polyproline type II helix (Musacchio, 2002), which fits with at least the linker between the N-terminal region and ankyrin repeats of TRPV4 being flexible.

The TRPV ankyrin repeat structures can also provide a useful perspective on available functional data. For instance, TRPV1 activation by allicin, the pungent compound in garlic, has been attributed to the covalent modification of cysteine 157 in the N-terminal ankyrin repeat domain (Salazar *et al.*, 2008). The sulfhydryl group of C157 is located in close proximity to the regulatory ATP- and calmodulin-binding region,

although it is completely buried between repeats 1 and 2 of the structure (Fig. 2*C*). Salazar and colleagues hypothesize that the ankyrin repeats can undergo a large conformational change (Salazar *et al.*, 2008). Although there is little precedent for such large conformational changes in ankyrin repeats (Gaudet, 2008), there is evidence that some ankyrin repeats are unstable and only fold stably in the presence of ligands (Croy *et al.*, 2004). The first ankyrin repeat of the TRPV1 has high B-factors - a crystallographic parameter that indicates the degree of disorder of a protein region, and by inference, can hint at its stability. This suggests that the first ankyrin repeat may be unstable under some circumstances, which could reconcile the apparent discrepancies between the structural information on TRPV1 and the function assigned to C157. This hypothesis is readily testable, providing a basis for further experiments on the molecular mechanism of TRPV1 activation by allicin.

TRPM proteins are implicated in a range of physiological processes, including response to oxidative stress (TRPM2), T-cell activation (TRPM4), taste (TRPM5), magnesium homeostasis (TRPM6 and TRPM7) and temperature sensation (TRPM8) (see (Harteneck, 2005) for a review). TRPM channels share a coiled coil domain located C-terminal of the transmembrane domain (Jenke *et al.*, 2003; Montell, 2005). When expressed by themselves, these coiled coil domains formed tetramers, as observed using a range of biophysical techniques (Tsuruda *et al.*, 2006). While these domains have so far resisted high-resolution structure determination, the biophysical observations can be used to generate molecular models of these homotetramers to inform functional studies on TRPM channels. For example, engineered or natural mutations in the coiled-coil region can be assigned as either exposed to solvent (and potential ligands) or buried and participating in tetramerization. This assignment could then be directly tested by studying the mutant versions of the coiled-coil domain using the same biophysical techniques. Recent reports on the coiled-coil domain of TRPM8, TRPM2 and TRPM4 have shown that the coiled coil is important for TRPM protein integrity and function (Launay *et al.*, 2004; Erler *et al.*, 2006; Mei *et al.*, 2006). A TRPM8 deletion construct missing the C-terminal coiled coil still tetramerizes and traffics to the plasma membrane when expressed heterologously in insect cells, but the coiled coil is necessary for channel activation (Phelps & Gaudet, 2007). In aggregate, studies of the TRPM channel coiled

coils indicate that they have two important roles: (i) the coiled coil helps direct subunit assembly into functional channels; and (ii) it is also necessary for channel activation, at least in TRPM8's response to cool-tasting ligands or cool temperatures.

Two TRPM channels important in magnesium homeostasis, TRPM6 and TRPM7, have an unusual kinase domain - termed α-kinase - appended to their C-terminus (Nadler *et al.*, 2001; Riazanova *et al.*, 2001; Runnels *et al.*, 2001). The structure of the isolated TRPM7 α-kinase domain, determined by x-ray crystallography, revealed a domain-swapped dimer: the N-terminal α-helix of one subunit interacts with the second subunit and vice-versa (Fig. 2*D*; (Yamaguchi *et al.*, 2001)). In a tetrameric TRPM7 channel, there would therefore be two such dimers. The structure represents the first example of an α-kinase, and revealed significant structural homology to other kinases, with N- and C-lobes that sandwich the nucleotide substrate (Fig. 2*D*). A conserved glycine-rich loop, which had been hypothesized to interact with the triphosphate moiety of ATP, is instead poised to interact with the peptide substrate (Yamaguchi *et al.*, 2001). Furthermore, a zinc-binding site appears to play a structural role, stabilizing the C-lobe. Follow-up studies used the structure to generate mutant channels with impaired kinase activity and examine the role of the kinase domain in TRPM7 function. On one hand, kinase activity was not required for modulation of TRPM7 activity through internal magnesium concentrations (Matsushita *et al.*, 2005). On the other hand, the kinase activity is necessary for modulation through G-protein- and protein kinase A-dependent cyclic AMP levels (Takezawa *et al.*, 2004).

The examples above highlight how structures of isolated protein domains can provide information on TRP channel function. There are still many TRP channel domains to which this divide-and-conquer strategy could be applied productively. For example, TRPM2 is regulated by ADP-ribose and related compounds through its NUDIX domain (Eisfeld & Lückhoff, 2007). Structural and biochemical studies of the isolated NUDIX domain could help resolve molecular mechanisms of these regulatory processes. TRPA1, a pain receptor and sensor of pungent compounds, is activated through the modification of cysteines in the ankyrin repeat region (Hinman *et al.*, 2006; Macpherson *et al.*, 2007), and structures of the modified and unmodified ankyrin repeats could shed light on the activation mechanism. Moreover, both the structure and function of the

MHRs of TRPM channels remain rather enigmatic, although mutations in the MHRs of TRPM6 channel are associated with hypomagnesemia (Schlingmann *et al.*, 2007) and deletions impair trafficking of TRPM2, TRPM4 and TRPM8 channels (Perraud *et al.*, 2003; Launay *et al.*, 2004; Phelps & Gaudet, 2007). Structural information could also help elucidate the role of the extracellular loops of TRPML and TRPP channels. Finally, many protein partners have been identified that interact with and regulate TRP channels. Structures of isolated TRP channel domains in complex with these binding partners will be important in mapping out the structural basis of TRP channel regulation. In summary, although the divide-and-conquer approach of seeking structural information on isolated domains of TRP channels has already reaped some results, it is likely that we have only seen the tip of the iceberg.

Blurry images? Single-particle electron microscopy of TRP channels

Electron microscopy (EM) can be a powerful technique to study large macromolecules and complexes (see (Henderson, 2004; Chiu *et al.*, 2005; Jiang & Ludtke, 2005) for recent reviews of the technique), and has proven quite useful in the study of membrane protein structure. In single particle EM, images of thousands of single macromolecules are classified according to the orientation of the macromolecules, and then used to reconstruct the three-dimensional structure of the macromolecule. Single particle EM reconstructions typically range in resolution from 20-30 Å for negative stain samples - where the sample is embedded in a thin layer of a heavy metal salt like uranyl acetate to enhance constrast, after adhering to a carbon-coated EM grid - to near-atomic resolution for some cryo-EM studies. Thus far only cases with a high degree of symmetry have yielded the near-atomic resolution necessary to trace the path of the protein chain with certainty, because the symmetry averaging enhances the signal to noise of the images. Examples of cryo-EM structures with near-atomic resolution include the 7-fold symmetric chaperone GroEL (Ludtke *et al.*, 2008), the rotavirus inner capsid particle using 13-fold averaging (Zhang *et al.*, 2008), and the infectious epsilon15 particle with icosahedral symmetry (Jiang *et al.*, 2008). With current methodologies, resolution values often range from 10-20 Å for cryo-EM structures, where the samples are imaged after freezing in a thin layer of vitrified ice, although cryo-EM structures now

regularly provide reconstructions at better than 10 Å resolution. Of note, although they share the same units, these resolution values do not correspond to the numbers quoted for x-ray crystal structures since the two techniques use different data and statistics, and therefore should not be directly compared. For reference, in cryo-EM, resolution in the range of 6-9 Å is required to visualize secondary structure elements like rods corresponding to α-helices, ~4 Å to resolve individual strands of a β-sheet, or ~8 Å to see the grooves in RNA.

Thus far, one representative for each of the three central families of TRP channels has been imaged by single particle EM (Fig. 3*A*-*C* and *3G*): TRPC3 (Mio *et al.*, 2007), TRPM2 (Maruyama *et al.*, 2007) and TRPV1 (Moiseenkova-Bell *et al.*, 2008). The TRPC3 structure was determined by the Sato group using cryo-EM at a stated resolution of 15 Å using protein purified from transiently-transfected HEK293 cells (Mio *et al.*, 2007). The resulting structure, measuring 240 x 200 x 200 $Å^3$, is surprisingly large for a 388 kDa tetramer. For comparison, the crystal structure of the 228 kDa Shaker channel is approximately 135 x 95 x 95 $Å^3$ (Long *et al.*, 2005) and a compact globular 30 kDa protein domain would fit in a ~ 33 x 33 x 33 $Å^3$ cube or a 40-Å diameter sphere. The TRPC3 EM reconstruction is also mesh-like, and the density in the region assigned as transmembrane is rather different than the structure of the Shaker channel with which it shares sequence homology (compare Fig. 3*A* and 3*D*). The TRPM2 structure was determined by the same group to a stated resolution of 28 Å using 3-D reconstruction from negative-stained samples also isolated from transfected HEK293 cells (Maruyama *et al.*, 2007). Of note, different resolution criteria were used for TRPC3 and TRPM2; if the more stringent criterion applied to the TRPC3 structure (and the TRPV1 structure described below) is also applied to TRPM2, the corresponding resolution is 37 Å. The bell-shaped TRPM2 structure is also very large at 250 x 170 x 170 $Å^3$, although its 689-kDa tetrameric molecular mass is significantly larger than TRPC3. The TRPM2 structure is surprisingly featureless for a protein complex of that size (Fig. 3*B*), suggesting that some of the features may have been averaged out during the particle alignment and/or symmetry averaging of the particles, which could happen with a sample that has multiple heterogeneous conformations. There is also no obvious structural feature resembling the NUDIX domain, for which a homologous crystal structure is available (compare Fig. 3*B*

and 3*F*). The most recent addition to the collection of TRP channel EM reconstructions is a cryo-EM structure of the ~400 kDa TRPV1 tetramer purified from a heterologous *S. cerevisiae* expression system (Moiseenkova-Bell *et al.*, 2008). The native state of the purified TRPV1 protein was validated by measuring calcium flow in response to resiniferatoxin after reconstitution into proteoliposomes. The resulting 19 Å structure is ~150 x 100 x 100 $Å^3$ and the particles had clear signs of the expected four-fold symmetry before averaging was applied. The TRPV1 shape is likened to a "hanging gondola" (Fig. 3*C*), a term that was coined for the similarly-shaped EM reconstructions of voltage-gated channels (Kobertz *et al.*, 2000; Kim *et al.*, 2004).

What can be learned from these low-resolution structures of complete TRP channels? In the case of the TRPV1 structure, the crystal structures of the transmembrane domain of the Shaker channel (Fig. 3*D*; (Long *et al.*, 2005; Long *et al.*, 2007)) and the TRPV1 ankyrin repeat domain (Fig. 3*E*; (Lishko *et al.*, 2007)) could be placed within the model with reasonable confidence, providing a hypothesis of their relative position within the intact protein (Moiseenkova-Bell *et al.*, 2008). The TRPV1 density assigned to the N-terminal ankyrin repeats is part of a continuous and hollow bowl-shaped density that forms the cabin of the "gondola" (Fig. 3*C* and 3*E*). Together with the fact that the isolated TRPV1 ankyrin repeats do not oligomerize (Lishko *et al.*, 2007), a testable hypothesis emerges that the N- and C-terminal cytosolic segments of TRPV1 are in contact with each other, forming an intricate quaternary structure. In this context, it is interesting to consider a study showing that the swapping of the C-terminal domains of TRPM8 (containing the coiled coil discussed above) and TRPV1 (which as no predicted coiled coil) results in the swapping of their thermosensing properties (Brauchi *et al.*, 2006). A chimera containing the TRPV1 N-terminal and transmembrane domains with the C-terminal domain of TRPM8 was sensitive to capsaicin and cold, whereas a chimera of the TRPM8 N-terminal and transmembrane domains with the C-terminal domain of TRPV1 was sensitive to menthol and heat (Brauchi *et al.*, 2006). If the N- and C-terminal domains of TRPV1 interact in the native TRPV1 channel as hypothesized above, it is surprising that the TRPM8 C-terminal domain can effectively replace that of TRPV1. Further studies are required to determine the impact of the chimeras on channel structure.

Unless structures of homologous proteins or fragments are available, it is difficult to assign particular protein domains to distinct densities of low-resolution EM reconstructions. One approach is to combine the reconstruction with electron microscopy imaging of complexes of the macromolecule under study with antibodies. By locating the antibody-binding site on the reconstructed model, the relevant density can then be assigned to a particular protein fragment. This approach was used in the 3D reconstructions of TRPC3 and TRPM2 (Maruyama *et al.*, 2007; Mio *et al.*, 2007), although the information obtained thus far is somewhat limited because only antibodies to C-terminal tags were used.

Clearly, we are still in the early days of electron microscopy of TRP channels. There are several previous examples of membrane protein families where the early reconstructions from different laboratories were difficult to reconcile with each other, including ABC transporters (some of the early work includes (Rosenberg *et al.*, 1997; Rosenberg *et al.*, 2001; Velarde *et al.*, 2001; Chami *et al.*, 2002)), inositol 1,4,5-trisphosphate (IP3) receptors (much of the EM work is reviewed in (Bosanac *et al.*, 2004)), and AMPA ionotropic glutamate receptors (Tichelaar *et al.*, 2004; Nakagawa *et al.*, 2005). The bottom line is that these "blurry images", or low-resolution views generated by single-particle EM reconstructions, should be considered working models that can and should be tested by further experiments - using functional, biochemical, biophysical and structural approaches. And just like any model, they should not be used at the exclusion of others, until they are supported by coherent datasets from multiple approaches and/or reach a near-atomic resolution that leaves little doubt about the details of the protein's fold, intra-molecular packing and domain organization.

Outlook

Determining the three-dimensional structures of TRP channel cytosolic domains alone and in complex with small molecule or protein ligands is an important step in understanding the molecular basis of channel gating and regulatory mechanisms. The cytosolic domains serve as important regulatory modules sensing specific aspects of the cell state, such as the intracellular concentration of signaling molecules or metabolites. Structural information obtained on the cytosolic domains of TRP channels can be

combined with data from biochemical and electrophysiology assays to define the molecular mechanisms of channel modulation by intracellular signals. An inherent problem with this divide-and-conquer approach is that it provides few insights into how the domains communicate within the context of the whole channel. Electron microscopy studies of TRP channels, although still in their early days, are providing some views of entire TRP channels that can provide a context for much of the accumulated structural and functional data. Ultimately, the Holy Grail of TRP channel structural biology is high-resolution structures of entire TRP channels. Although membrane protein structure determination remains a challenge, the relevant methods are improving (see (Lacapere *et al.*, 2007) for a recent review). Advances in cryo-EM and membrane protein production methodologies could lead to significant improvements in the resolution of three-dimensional reconstructions of TRP channels. X-ray crystallography is another obvious approach that is actively being explored by many labs interested in TRP channels. The progress in the field, illustrated by recent structures of eukaryotic potassium channels (Long *et al.*, 2005; Long *et al.*, 2007) and acid-sensing ion channel (ASIC; (Jasti *et al.*, 2007)), provides encouraging signs that such endeavors are feasible. Furthermore, membrane proteins can readily be restricted to a two-dimensional space when embedded in a lipid bilayer, making them excellent candidates for electron crystallography of two-dimensional crystals, a technique that is seeing a revival (Hite *et al.*, 2007; Ford & Holzenburg, 2008).

The interest in structural biology of TRP channels also prompts the question: What are some of the important physiological functions of TRP channels that can be better understood through structural approaches? First, some TRP channels are gated by temperature (Tominaga & Caterina, 2004; Latorre *et al.*, 2007) and others may be gated by mechanical stimuli (Christensen & Corey, 2007). Understanding the molecular basis of sensing and responding to these physical stimuli by a conformational change will require both detailed structural models of the relevant TRP channels as well as biophysical experiments interpreted in light of the structural information. Second, TRP channel structures, perhaps in the presence of phosphoinositides, will help us understand whether there is a common mechanism for the regulation of many TRP channels by phosphoinositides. Similarly, many TRP channels are regulated by calcium and

calmodulin (Zhu, 2005). Is the structural basis of this regulation the same? Functional data suggest that it differs, and structural analyses of complexes of TRP channels and/or isolated domains with calmodulin will provide additional insights. Third, high resolution structures of TRP channel pores will help elucidate the structural basis for their selectivity, which varies from TRP channel to TRP channel, with some like TRPV5 and TRPV6 being highly calcium-selective, others like TRPM4 and TRPM5, highly monovalent-selective, and yet others like TRPV1-4 and many TRPCs, non-selective cation channels (see (Venkatachalam & Montell, 2007) for a recent review). Finally, many TRP channels have been implicated in pain, inflammation and disease, either directly through TRP channel mutations or indirectly by contributing to the symptoms and progression of disease (Nilius *et al.*, 2007). This makes TRP channels important targets in the development of new drugs and therapeutic approaches. All high-resolution TRP channel structures can contribute to the development of new pharmaceutics through structure-based drug design. In summary, TRP channels have definitely entered the structural era and we can anticipate many more mechanistic insights from TRP channel structural biology.

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Figure and legends

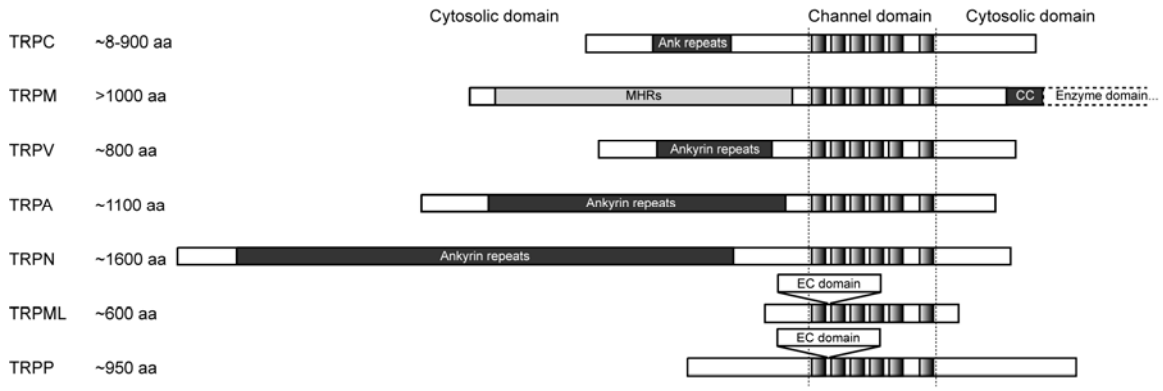

Figure 1. Primary structures of the seven TRP channel subfamilies.

Lengths are approximately to scale. CC is coiled coil region, EC domain is an extracellular domain, and the dotted lines indicate C-terminal extensions containing enzymatic domains in some TRPM channels.

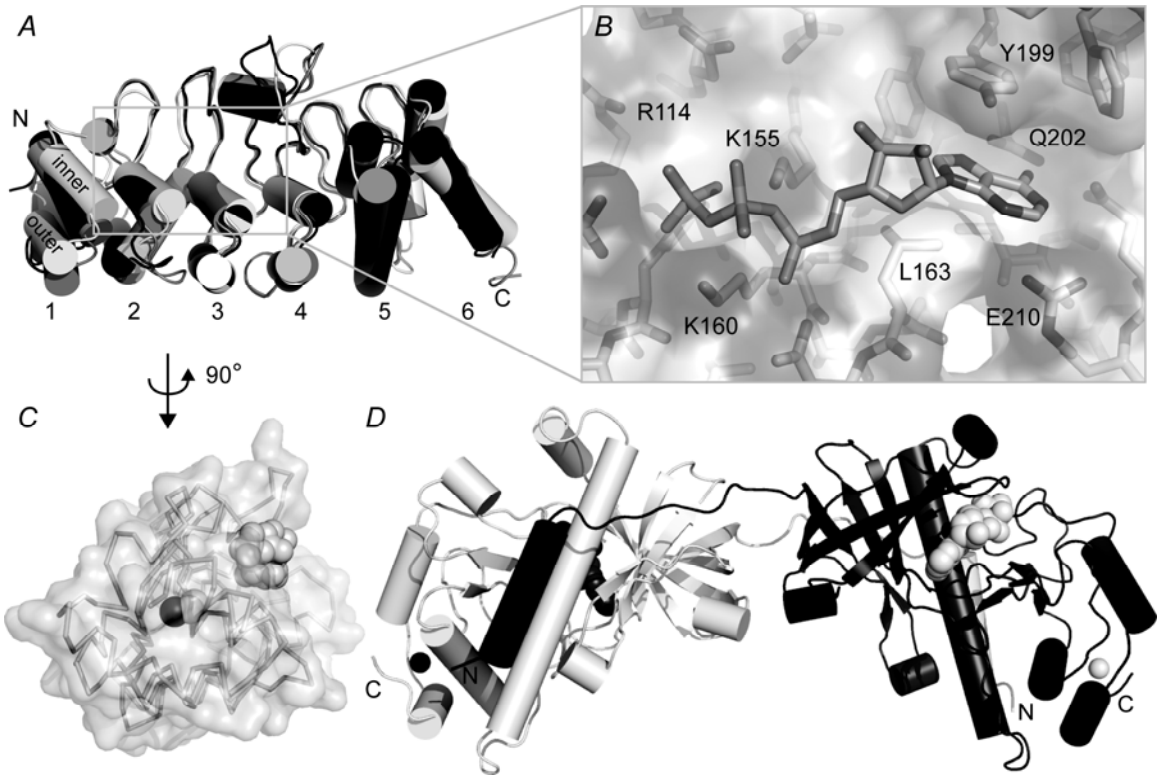

Figure 2. High resolution structures of TRP channel fragments.

A, Superposition of three TRPV ankyrin repeat domains: TRPV1 (black; (Lishko *et al.*, 2007)), TRPV2 (dark grey; (Jin *et al.*, 2006)) and TRPV6 (light grey; (Phelps *et al.*, 2008)). The cylinders represent α-helices. Each repeat consists of an inner helix lying above an outer helix, followed by a finger loop - finger loops are located in the back of the molecule in this illustration. The six repeats, numbered below the structures, are stacked side-by-side from left to right, with the palm surface facing the reader and formed by the fingers and the top of the inner helices. The box indicates the approximate position of the ATP-binding site on the TRPV1 palm surface. *B*, A surface representation of the ATP-binding site on the TRPV1 ankyrin repeats, with the ATP shown in dark grey sticks. *C*, A surface representation of the TRPV1 ankyrin repeats viewed end-on from the N-terminal side (corresponding to a 90° rotation along the y-axis of the view shown in *A*). Cysteine 157, in spheres with its sulfur atom in black, is buried in the middle of the structure. The ATP is also shown in grey spheres, for reference. *D*, The TRPM7 α-kinase dimer (Yamaguchi *et al.*, 2001), with the two subunits in light grey and black. The N-terminal helices are swapped between the two subunits. The ATP nucleotide

substrate (AMPPNP in the structure; spheres) is sandwiched between the N-lobe (proximal to the dimerization axis) and the C-lobe, which contains a zinc ion (isolated sphere).

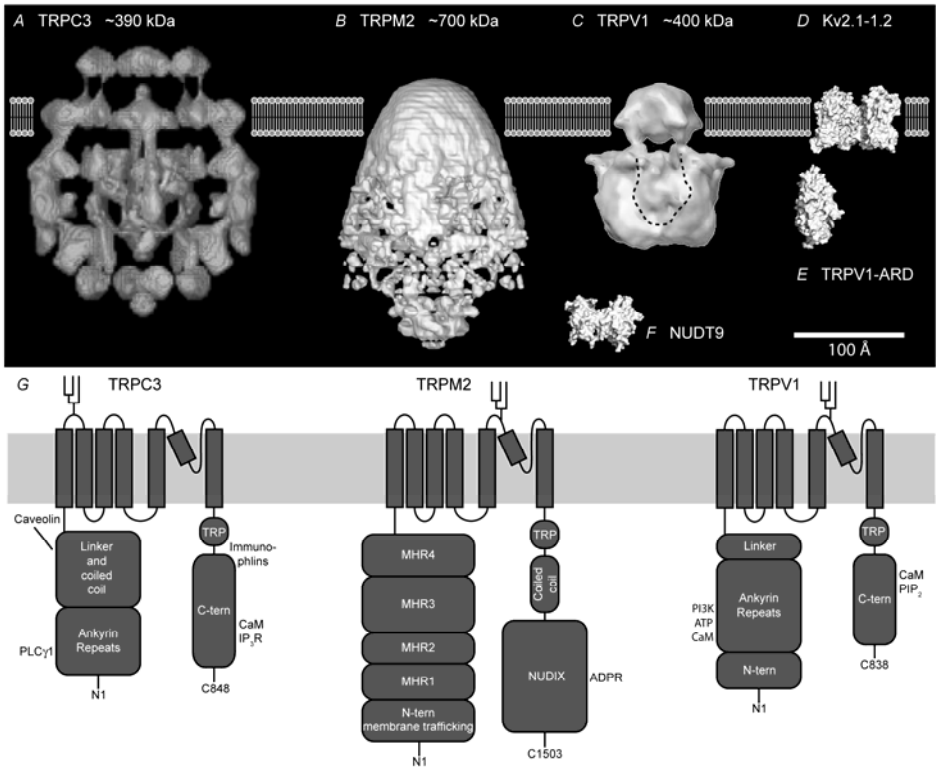

Figure 3. Electron microscopy of TRP channels.

A, Cryo-EM reconstruction of TRPC3 (Figure adapted from (Mio *et al.*, 2007) with permission). *B*, Reconstruction of TRPM2 by negative-stain EM (Figure adapted from (Maruyama *et al.*, 2007) with permission). *C*, Cryo-EM reconstruction of TRPV1 (Figure adapted from (Moiseenkova-Bell *et al.*, 2008) with permission, copyright (2008) National Academy of Sciences, U.S.A.). The dotted line indicates the approximate position of an internal cavity creating the bowl shape within the "hanging-gondola" structure. *D*, Molecular surface representation of the tetrameric channel domain of the Kv2.1-1.2 chimera structure (Long *et al.*, 2007). The approximate position of the lipid bilayer, as assigned in the original work, is indicated in *A* through *D*. Note that the bottom of the TRPM2 structure in *B* is tantalizingly similar to the top of the TRPV1 structure in *C*. *E*, Molecular surface representation of the TRPV1 ankyrin repeats (Lishko *et al.*, 2007), in an orientation compatible with the TRPV1 structure in *C*, with the N-terminus towards the bottom and the C-terminus at the top. *F*, Molecular surface representation of NUDT9, a homolog of the TRPM2 NUDIX domain. No density in the

TRPM2 structure in *B* can readily be assigned to the four copies of the NUDIX domain expected in the tetrameric channel. All structures are represented to scale, with the scale bar indicated at the bottom right. *G*, Topology diagrams single subunits of TRPC3, TRPM2 and TRPV1, with the boxes drawn approximately to scale to illustrate the expected relative size of the proteins. Identified regulatory ligands are indicated next to their interacting region. Ligands for TRPC3 and TRPM2 are described in (Eder *et al.*, 2007) and (Nazıroğlu, 2007) and references therein, respectively. For TRPV1, see (Rosenbaum *et al.*, 2004) and (Lishko *et al.*, 2007) for the interaction of the ankyrin repeats with ATP and TRPV1, (Stein *et al.*, 2006) for PI3K, and (Numazaki *et al.*, 2003) and (Kwon *et al.*, 2007) for PIP_2 and calmodulin and the C-terminus.