Perspectives on TRP Channel Structure and the TRPA1 Puzzle

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In this issue of the Journal, Rachelle Gaudet (Harvard University) and Moiseenkova-Bell and Theodore Wensel (Baylor College of Medicine) provide a critical overview on what is known and which are the new areas in need of further research regarding the three-dimensional structure of the superfamily of transient receptor potential (TRP) channels. Given the multifunctional and polymodal behavior of this class of ion channels, the elucidation of how these channels are assembled from a structural point of view is critical to understand the molecular workings of these proteins. It is also clear that a detailed crystal structure of the TRP channels will allow for the development of intelligent strategies for the design of drugs targeted to this class of ion channels. In addition, Ombretta Caspani and Paul A. Heppenstall (EMBL Monterotondo and Universitätsmedizin Berlin), Kelvin Y. Kwan and David P. Corey (Harvard University), and Sangsu Bang and Sun Wook Hwang (Korea University Graduate School of Medicine) share their views about the conundrum raised by the modulation of the functional properties of the TRPA1 channel and, in particular, that regarding its temperature sensitivity.

The TRP channel story began in the late 1960s with the report of Cosens and Manning (Cosens, D.J., and A. Manning. 1969. *Nature.* 224:285–287) of a spontaneous *Drosophila* mutant with an abnormal response to light. In the mutant fly, the photoreceptor potential showed a transient behavior during prolonged illumination. 20 years later this finding led to the discovery of the first TRP ion channel from *Drosophila* (Montell, C., and G.M. Rubin. 1989. *Neuron.* 2:1313–1323). Montell and Rubin reported that *trp* encodes a large membrane protein containing 1,275 amino acids. Its amino acid sequence did not present any clear homology to any known ion channel–forming protein. It was realized later that a new gene dubbed trp-like (*trpl*) showed structural homologies to *Drosophila trp* as well as with the superfamily of voltage-gated channel genes, raising the possibility that *trpl* encoded subunits of a calcium or a nonspecific cation channel (Phillips, A.M., A. Bull, and L.E. Kelly. 1992. *Neuron.* 8:631–642). 18 years since the first TRP channel was identified, we now know of more than 85 such channels (invertebrate and vertebrate combined; Montell, C. 2005. *Sci. STKE.* 2005:re3) organized by their sequence homology into seven subfamilies. The TRPC (classical) subfamily encompasses channels that present a great number of different activation modes. TRPC proteins also control growth cone guidance in both mammalian and amphibian model systems. Those from the TRPM (melastatin related) subfamily are designated as chanzymes because their C terminals contain catalytic domains with protein kinase activities (TRPM6 and TRPM7). The TRPV (vanilloid) subfamily has members activated by temperature that also sense osmolarity changes and mechanical stimuli. The TRPA (ankyrin) family contains only one member, TRPA1, which has been assigned many different functions. Because it is still unclear whether or not this channel is a noxious cold receptor, TRPA1 is one of the main actors in this Perspective. TRPN (NOMP-C homologues) has a single member, with homologues present in worms, flies, and zebrafish. The TRPP (polycystin) subfamily is formed by the polycystic kidney disease proteins or polycystins. The TRPML (mucolipin) subfamily consists of channels that appear to be only present in intracellular vesicles, and two of its members (TRPML1 and 2) have been implicated in hearing.

Transmembrane segment (TM) prediction algorithms indicate that TRP channels are related to the superfamily of voltage-gated cation channels. The putative

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organization of these channels consists of six TMs with the carboxy and amino terminals facing the intracellular side of the membrane (Harteneck, C., T.D. Plant, and G. Schultz. 2000. *Trends Neurosci.* 23:159–166; Ramsey, I.S., M. Delling, and D.E. Clapham. 2006. *Annu. Rev. Physiol.* 68:619–647). Family relatives are voltage-activated *shaker*-like channels, cyclic nucleotide–gated channels, and hyperpolarization-activated cyclic nucleotide–gated channels. According to the proposed six-TM architecture, the pore region would be a hydrophobic region somewhere between the fifth and sixth segments. Recent studies based on biochemical and optical methods strongly suggest that TRP channels are formed by four subunits (e.g., Amiri, H., G. Schulz, and M. Schaefer. 2003. *Cell Calcium.* 33: 463–470), and that active channels could assemble as homo- or heterotetramers (Hoenderop, J.G., T. Voets, S. Hoefs, F. Weidema, J. Prenen, B. Nilius, and R.J. Bindels. 2003. *EMBO J.* 22:776–785; Schaefer, M. 2005. *Pflüegers Arch.* 451:35–42).

As TRP channels are directly involved in sensory modalities such as vision, taste, olfaction, hearing, touch, thermal perception, and nociception, a recurrent idea is that multicellular organisms use TRP channel–dependent pathways to sense their surroundings, and at a more cellular level, that TRP channels allow cells to respond to changes in their the environment. But, despite the large body of literature on TRP channels, very little is known about the mechanisms that control their gating processes, and much work remains to be done in the fields of biophysics and protein structure.

Advances in our knowledge of the crystal structure of TRP channels have been hampered by the lack of adequate expression systems needed to obtain large amounts of protein, and attempts to overexpress these proteins in bacteria have so far been unsuccessful. Using mammalian cell (HEK293) transfection or yeast (*Saccharomyces cerevisiae*), however, sufficient amounts of protein have been collected to make it possible to determine by electron microscopy the structures of three TRP channels (albeit at low resolution): TRPC3 (Mio, K., T. Ogura, Y. Hara, Y. Mori, and C. Sato. 2005. *Biochem. Biophys. Res. Commun.* 333:768–777; Mio, K., T. Ogura, S. Kiyonaka, Y. Hiroaki, Y. Tanimura, Y. Fujiyoshi, Y. Mori, and C. Sato. 2007. *J. Mol. Biol.* 367:373–383), TRPM2 (Maruyama, Y., T. Ogura, K. Mio, S. Kiyonaka, K. Kato, Y. Mori, and C. Sato. 2007. *J. Biol. Chem.* 282:36961–36970), and TRPV1 (Moiseenkova, V.Y., and T.G. Wensel. 2008. *Proc. Nat. Acad. Sci. USA.* 105:7451–7455). In this series, Moiseenkova-Bell and Wensel give us a critical appraisal of the different techniques used to provide the first steps in the elucidation of TPR channel structure and speculate on the future of the field. Of particular interest is that the structures of TRPC3 and TRPM2 proved to be very different from that of TRPV1. The TRPC3 channel structure was determined using a single-particle reconstruction from images coming from cryoelectron microscopy. The bell-shape structure obtained in this way has an enormous volume mostly conformed by the cytoplasmic domain. This large cytoplasmic domain is made of a sparse external shell that appears as girdles connected through low density panels. This type of structure explains why large volume is at odds with the relatively low molecular weight of the channel-forming protein. Using electron microscopy in negative stain, a large volume bullet-shape structure was also found for TRPM2. The structure of TRPV1 is, in contrast, in many aspects reminiscent of that of the Kv1.2 (Long, S.B., E.B. Campbell, and R. MacKinnon. 2005. *Science.* 309:897–903), which includes a large "hanging gondola," most probably made up of the N and C terminus of the channel. The TRPV1 volume is several times smaller than that of TRPC3 or TRPM2. It has been argued that the large expansion of the TRP channel cytoplasmic surface allows the association with several macromolecules. Because TRPV1 has also evolved to be a specialized sensor of various signals, and is likely to be a macromolecular complex, one can only agree with Moiseenkova-Bell and Wensel that "it is not obvious why the TRPC3 and TRPV1 channel structures look so different."

When the first crystal structure of a voltage-dependent channel became available, its modular nature was instantly revealed (Long, S.B., E.B. Campbell, and R. MacKinnon. 2005. *Science.* 309:897–903). In the crystal of the Kv1.2 channel, two well-defined domains were apparent: the voltage sensor and a pore module. Because the transmembrane domain arrangement of TRP channels is homologous to that of the Kv ion channel family, similar modules have been proposed. Of particular importance, because they confer regulation by temperature and various ligands, are the N and C terminus (Brauchi, S., G. Orta, M. Salazar, E. Rosenmann, and R. Latorre. 2006. *J. Neurosci.* 26:4835–4840; Latorre, R., S. Brauchi, G. Orta, C. Zaelzer, and G. Vargas. 2007. *Cell Calcium.* 42:427–438). Here, Rachelle Gaudet describes how she and other groups have taken advantage of the modular nature of TRP channels to use a "divide and conquer" approach to determine the crystal structure of two TRP channel domains with high resolution: the ankyrin repeat domains (ARD) from TRPV channels and a coiled-coil from the TRPM7 channel. Importantly, a domain is defined as a structure that can fold in isolation and preserve the functional properties that it possessed while forming part of the whole protein. In this regard, the fact that the TRPV1-ARD crystal structure revealed a well-defined ATP binding site, and that the biochemical data indicate that the Ca–calmodulin complex binds to the same surface, led to an aesthetically pleasing explanation for the Ca^{2+}-dependent regulation of TRPV1. The model proposes that TRPV1-ARD is bound to ATP in the sensitized state, and that the Ca^{2+} influx through the open channel causes the release of ATP, followed by desensitization induced by binding of the Ca–calmodulin complex to the TRPV1-ARD.

In the TRPM subfamily, the C terminus coiled-coil domains self-assemble in tetramers and modulate channel trafficking and assembly. In discussing the work of Fujiwara and Minor (Fujiwara, Y., and D.L. Minor. 2008. *J. Mol. Biol.* 383:854–870), Gaudet emphasizes the point that despite the fact that TRP channels appear to have a fourfold rotational symmetry of the pore, the homotetrameric crystal structure of TRPM7 coiled-coil shows a twofold symmetry. The coiled-coil is anti-parallel with two α-helical strands going in one direction and two strands going in the opposite direction. However, this twofold symmetry matches the structural symmetry of the kinase domain that follows the C terminus of the TRPM7 coiled-coil, a dimer held together by an exchange of the N terminus α-helix of each monomer. Notably, an analysis of the primary structure of the other members of this subfamily suggests that they can be divided in two groups: TRPM1,3,6,7 and TRPM2,4,5,8. This observation suggests that the quaternary structure of the coiled-coil domain of the TRPM8 group will be different from that of TRPM7 and, therefore, it is unlikely that heterotetramers will form between groups. In other words, coiled-coil domains bear channel assembly information specificity.

TRPA1 is expressed in DRG neurons that behave as polymodal nociceptors because they also express TRPV1. TRPA1 channels are also expressed in the sensory epithelium of the utricle, a membranous structure located in the vestibule of the inner ear. Conflictive results on the exact role of this channel started when this channel was identified in sensory neurons that were also activated by capsaicin. In contrast to the findings of Story et al. (Story, G.M., A.M. Peier, A.J. Reeve, S.R. Eid, J. Mosbacher, T.R. Hricik, T.J. Earley, A.C. Hergarden, D.A. Andersson, S.W. Hwang, et al. 2003. *Cell.* 112:819–829), Jordt et al. (Jordt, S.E., D.M. Bautista, H.H. Chuang, D.D. McKemy, P.M. Zygmunt, E.D. Högestätt, I.D. Meng, and D. Julius. 2004. *Nature.* 427:260–265) were unable to obtain reliable responses of TRPA1-expressing neurons to noxious cold. The Julius and Patapoutian groups (and everybody else) agreed, however, that TRPA1 is the molecular target of pungent-natural compounds, such as the isothiocyanates contained in wasabi (see below). Here, Caspani and Heppenstal argue at length in favor of the view that TRPA1 is activated directly by internal Ca^{2+}, and that the apparent cold sensitivity occurs indirectly through the increase in cytosolic Ca^{2+} that occurs during cooling in heterologous systems. But, whether or not TRPA1 is actually gated by noxious cold is still a big unknown because TRPA1 can be activated by deep cooling in inside-out patches of both dorsal root ganglion neuron and HEK 293 cells in Ca^{2+}-free solutions. The puzzle of the cold sensitivity of TRPA1 is also discussed in detail by Kwan and Corey, paying special attention to the results obtained in TRPA1 knockout mice. Unfortunately, the answer to this problem did not come from *trpa1* knockout mice because the behavioral responses of *trpa1*$^{-/-}$ knockout mice to noxious cold temperatures also turned out to be inconclusive. Kwan and Corey, however, underscore the fact that two different groups have found TRPA1 cold activation in excised patches in the absence of Ca^{2+}, implying that if TRPA1 lacks an intrinsic temperature sensor, its cold sensitivity must be due to some membrane-delimited component.

TRPA1 is activated by several pungent-natural compounds that are used in cooking, including wasabi and mustard, owing their pungency to isothyocyanate compounds. This list of compounds has recently been extended to include a series of environmental irritants, such as air pollutants and chemotherapeutic agents (for review see Bandell, M., L.J. MacPherson, and A. Patapoutian. 2007. *Curr. Opin. Neurobiol.* 17:490–407). A fascinating new discovery is that cysteine residues in TRP channels can be covalently modified by a great variety of chemically unrelated agonists, having in common only that they are electrophyles (they love electrons of other molecules) as part of their mechanism of activation. Thus, unlike other natural agonists such as menthol or capsaicin, which appear to act in a lock and key fashion, TRPA1 agonists act by covalently modifying their target cysteines by binding "irreversibly" to the channel. Mutagenesis experiments showed that modification of only three cysteines of the 31 present in the cytoplasmic aspect of mouse TRPA1 led to agonist activation. All three cysteines are located in the N-terminal region of the channels. This subject is discussed in detail in Bang and Hwang's Perspective, noting that the data strongly suggest that TRPA1 works as a chemical nociceptor in the nervous system, and is able to detect chemicals that mediate tissue damage and raise the voice of alarm to the whole organism by allowing the propagation of the pain response.

Letters to the editor related to these Perspectives will be published in the June 2009 issue of the *Journal of General Physiology*. Letters to the editor should be received no later than Friday, April 17, 2009, to allow for editorial review. The letters may be no longer than two printed pages (approximately six double-spaced pages) and will be subject to editorial review. They may contain no more than one figure, no more than 15 references, and no significant references to unpublished work. Letters should be prepared according to the Journal's instructions and can be submitted electronically at www.jgp.org, or as an e-mail attachment to jgp@rockefeller.edu.