Cell and Molecular Biology of TRP Channels


The TRP channel superfamily: insights into how structure, protein–lipid interactions and localization influence function

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Abstract
TRP (transient receptor potential) cationic channels are key molecules that are involved in a variety of diverse biological processes ranging from fertility to osmosensation and nociception. Increasing our knowledge of these channels will help us to understand a range of physiological and pathogenic processes, as well as highlighting potential therapeutic drug targets. The founding members of the TRP family, Drosophila TRP and TRPL (TRP-like) proteins, were identified within the last two decades and there has been a subsequent explosion in the number and type of TRP channel described. Although information is accumulating as to the function of some of the TRP channels, the activation and inactivation mechanisms, structure, and interacting proteins of many, if not most, are awaiting elucidation. The Cell and Molecular Biology of TRP Channels Meeting held at the University of Bath included speakers working on a number of the different subfamilies of TRP channels and provided a basis for highlighting both similarities and differences between these groups. As the TRP channels mediate diverse functions, this meeting also brought together an audience with wide-ranging research interests, including biochemistry, cell biology, physiology and neuroscience, and inspired lively discussion on the issues reviewed herein.

The TRP (transient receptor potential) superfamily
What has become known as the TRP superfamily started out with just one member, Drosophila TRP (dTRP), in 1989 [1]. Since then, the number of TRP channels has climbed to more than 30, with representative channels conserved across species from nematodes to humans [2]. On the basis of sequence homology, seven subfamilies have been proposed, and, initially, the search was on to identify the function of each of these groupings. Indeed, the TRP nomenclature is generally based on the founding member of the subfamily; for example, the TRPVs have high sequence similarity to the first member identified, the vanilloid receptor 1 [3]. It has become increasingly clear, however, that channel functions differ almost as widely within these subfamilies as they do between them. Although definitive functions have been assigned to some TRP channels, roles of others in a number of unexpected physiological and pathological processes have recently come to light; the following articles from the Cell and Molecular Biology of the TRP Channels Meeting held at the University of Bath highlight some of these in addition to exploring novel modes of channel activation, inactivation, structure and regulation.

TRP channel structure
It is generally accepted that TRP channels possess six transmembrane domains with N- and C-termini both located on the cytosolic/intracellular side of the membrane and...
a pore loop region between transmembrane segments five and six. By analogy with the Kv family of potassium channels, they probably form tetramers, although questions concerning subunit composition and channel assembly are still being addressed as described below. Although most of the TRP family members lack the full complement of charged amino acids in the S4 transmembrane domain that confers voltage-dependence on the Kv channels, a number of TRP channels do indeed exhibit voltage-dependent behaviour. Several conserved structural motifs are present in TRP family members such as coiled-coil domains, ankyrin repeats and the so-called ‘TRP domain’ [which seems to be missing in TRPP (TRP polycystin), TRPN (TRP NO-mechanopotential) and so-called ‘TRP domain’ [which seems to be missing in TRPP (TRP polycystin), TRPN (TRP NO-mechanopotential) and TRPML (TRP mucolipin) families!].

**TRP channel assembly**

Since they are on the same side of the membrane, the N- and C-termini of TRP channels are both available for subunit and regulatory/cytoskeletal interactions. In their reviews of the molecular determinants important for TRP channel assembly, Guylain Boulay ([4]; see pp. 81–83) and Rainer Schindl ([5]; see pp. 84–85) highlight the roles of the ankyrin repeats, coiled-coil and transmembrane domains in TRPV, dTRP, TRPM (TRP melastatin) and TRPC (TRP canonical) channels. Using a chimaeric approach, Lepage and Boulay [4] describe new evidence suggesting that there are two subunit-interaction sites which control the selectivity of TRPC subunit assembly, the first interactions between the ankyrin repeat region of the N-terminus and the second between adjacent N- and C-termini. Although the N-terminal interactions are crucial for channel function, interactions between the N-terminus and C-terminus appear to be independent of function. Characterization of the role of these latter associations remains to be determined, but it is suggested that they play a part in regulation rather than activity of the channel [6].

Focusing specifically on the TRPM family, Lin Hua-Jiang ([7]; see pp. 86–88) explores a potentially powerful regulatory system affecting channel assembly: expression of short non-functional subunits which are capable of inhibiting the assembly of their full-length counterparts. In the case of TRPM1, association of a short subunit occurs directly with the full-length subunit and inhibits the formation of functional channels by preventing translocation to the plasma membrane. Indeed, a common, but often poorly characterized, aspect of many of the TRP channel members is the existence of multiple splice variants and their roles in regulating both channel assembly and activity. In his article focusing on TRPM3, Johannes Oberwinkler ([8]; see pp. 89–90) brings together data from a number of different laboratories suggesting that this is one family member with numerous and somewhat unique splice variants (see also the review by Christian Harteneck [9] on pp. 91–95). In this case, alternative exon usage results in changes within the pore region creating channels with very different permeability characteristics and therefore potentially very different functions.

**Novel (and not so novel) activation/regulation mechanisms**

Since the theory of SOCE (store-operated calcium entry) was formalized by Jim Putney in 1986 [10], the search for the molecule responsible has been intense. Based on their calcium permeability and role in insect photoreceptor activation, TRP channels became very attractive candidates for channels mediating calcium entry in response to store depletion. In spite of tremendous efforts to pin down the role of these channels in this process, the contribution of TRP channels to SOCE remains unclear. Indu Ambudkar ([11]; see pp. 96–100) reviews the wealth of experimental evidence implicating TRPC1 in SOCE and relates this to the current work on STIM and Orai, two proteins which have emerged in the last few years with proposed roles as a calcium sensor and the SOCE channel respectively [12].

As the number of TRP family members has increased, so has the number of proposed activators. In his review focusing on TRPC5, David Beech ([13]; see pp. 101–104) describes a novel agent causing activation, LPC (lysophosphatidylcholine), identified in a screen using inducible TRPC5 expression. Evidence suggests that TRPC5 is actually capable of sensing LPC directly, and therefore the possibility that it acts as a lipid ionotropic receptor for both extracellular and intracellular LPC is put forward. Another lipid detected in the same screen, S1P (sphingosine 1-phosphate) is also capable of activating the channel, but lacks the direct extracellular effect. In contrast with TRPC5, Harteneck and Reiter [9] report that TRPM3 is activated specifically by sphingosine, but not by S1P. These findings provoke the intriguing speculation that differences in activation/function in different cell types may be due to the lipid environment in which the TRP channels find themselves.

In the light of the central role of phosphoinositides in a myriad of cellular processes, including classical signal transduction pathways, receptor desensitization and internalization [14], it is no surprise that TRP channels are also regulated by phosphoinositides [15]. Bernd Nilius ([16]; see pp. 105–108) expands and extends previous data linking PIP2 (phosphatidylinositol bisphosphate) with TRP channels. He describes elegant experiments exploring the effects of increasing PIP2 levels on TRPM4 activity. PIP2 not only increases the calcium-sensitivity of TRPM4, but also shifts the voltage-dependence towards more negative, and hence more physiological, potentials. The region responsible for PIP2 binding is also addressed; it consists partially of the TRP domain, but also key is the plekstrin homology domain located in the C-terminus.

Under the category of ‘novel’ activators of TRP channels is included the adenosine-based second messenger, ADPR (adenosine diphosphoribose). Andreas Guse ([17]; see pp. 109–114) highlights the evidence for the activation of TRPM2 by ADPR. The observation that a domain in the C-terminus of TRPM2 is homologous with the mitochondrial ADPR-metabolizing enzyme, NUDT9, prompted the initial studies into this link. It was shown subsequently that ADPR

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binds directly into a cleft in this region of TRPM2 and induces channel opening [18]. Activation of TRPM2 by ADPR was also shown to be dependent on the concentration of calcium, binding of calcium/calmodulin and varying levels of cADPR, thus demonstrating fine-tuning of the gating of this channel by cross-talk between various adenine-based second messengers. An important physiological function mediated by TRPM2 which has recently been identified is a response to oxidative stress, resulting in apoptosis.

Switching from the TRPM family to the TRPV family, René Bindels ([19]; see pp. 115–119) describes several mechanisms for regulation of TRPV5 and TRPV6 which act as ‘gatekeepers of calcium entry across epithelia’. An extremely novel mechanism is based on the regulation of cell-surface protein by the anti-aging hormone, klotho. Klotho resembles β-glucuronidase in function, i.e. it acts in carbohydrate processing. Exactly how clipping of carbohydrate moieties attached to TRPV5 results in a decrease in internalization and essentially trapping of the channel at the cell surface is still a mystery, but a very intriguing one indeed. Equally intriguing is the regulation of channel activity by calbindin-D28k which translocates to the plasma membrane and interacts directly with TRPV5 after a decrease in the intracellular calcium concentration.

The function of TRPV5 is regulated by trafficking; however, so is that of another family member, TRPV4. This process is outlined by Harteneck and Reiter [9]. This channel is also expressed predominantly in epithelial cells and elegant experiments described in this review suggest that it functions in regulating hypotonic stress. The initial activation of TRPV4 specifically on the basolateral surface results in a transcellular permeability by triggering calcium-activated potassium channels in the apical membrane. As was discussed for TRPV4 above, the presence of a CK2 phosphorylation site allows for binding of two proteins, PACS-1 and -2, which are also involved in TRPP2 localization and trafficking.

Mutations in TRPP channels have been associated with pathological conditions not only in humans, but also in nematode worms. Mutations in TRPP2/PKD2 were first associated with defects in male mating behaviour in Caenorhabditis elegans [23]. In their review on TRPM channels in C. elegans, Howard Baylis and Kshamata Goyal ([24]; see pp. 129–132) report on the affects of mutations in three members of this family, gon-2, gtl-1 and gtl-2. In addition to a role for gon-2 in gonad formation, an additional and novel function for TRPM channels emerges from studies on the gon-2 and gtl-1 mutants: regulation of calcium-dependent rhythmic processes, specifically ultradian defecation rhythm in the worm. It will be interesting to see whether this can be correlated with similar functions in mammalian systems, perhaps in the intestine where TRPM6 functions. Not only are C. elegans’ TRPM channels associated with problems of the reproductive tract, but also TRPM8 in humans is highly expressed in prostate and this expression increases dramatically in prostate cancer. As described by Natalia Prevarskaya and colleagues ([25]; see pp. 133–135), silencing experiments using siRNA (small interfering RNA) to TRPM8 suggest a role in calcium homeostasis in prostate epithelial cells and link this to cell survival. Interestingly, TRPM8 provides another example of localization to both the ER and plasma membranes, although the significance of this has yet to be elucidated; a potentially important observation, however, is that TRPM8 localization is almost exclusively to the ER in dedifferentiated metastatic LNCaP prostate cells.

When TRP channels cease to function

As TRP channels are ubiquitous and involved in essential functions involving calcium, it comes as no surprise that they are also implicated in a number of pathophysiological conditions. This has been highlighted by the creation of transgenic animals with mutations in TRP genes. The information gained on the normal and pathophysiological functions of various TRP family members has been gathered from innumerable studies and compiled into an extremely accessible and useful format by Marc Freichel and Veit Flockerzi ([21]; see pp. 120–123). Examples include links to human disease, cell proliferation and tumour progression, as well as unravelling roles for TRP channels in processes ranging from thermosensation to smooth-muscle-dependent contractility.

An in-depth focus on mutations in TRPP2 that are linked with autosomal dominant polycystic kidney disease is given in the review by Richard Sandford and colleagues ([22]; see pp. 124–128). In association with polycystin-1, TRPP2 localizes to a unique structure, the renal primary cilium. This is a prime location for responding to mechanical stimuli which is then translated into calcium influx and release from intracellular stores. In addition, TRPP2 is also localized to the endoplasmic/sarcoplasmic reticulum where it can also regulate intracellular calcium signalling. The localization patterns are governed by specific signals, e.g. a C-terminal ER (endoplasmic reticulum) retention motif confers ER localization which can be masked, resulting in transport to the plasma membrane. As was discussed for TRPV4 above, the presence of a CK2 phosphorylation site allows for binding of two proteins, PACS-1 and -2, which are also involved in TRPP2 localization and trafficking.

The end of the TRP

In one of the very early reviews on TRP channels, Roger Hardie and Baruch Minke [26] speculated on the potential of TRP channels to be responsible for the general phenomenon of phosphoinositide-regulated calcium influx. This was at a time when the existence of mammalian homologues was just starting to be discovered [27]. Had they been trapped in a time-warp for the last decade and emerged to join in the proceedings of this Focused Meeting, the breadth and depth of topics associated with activation, regulation, trafficking and function of the now superfamily of TRP channels would surely have come as a bit of a pleasant
surprise. The molecular identity of TRP channels was first elucidated in *Drosophila* and this organism continues to give insight into TRP function; however, other model organisms, including nematode worms and zebrafish, are also contributing to an exponentially expanding database of TRP activities and mechanisms of activation. The identification of interacting proteins and lipids, as well as information on their subcellular distribution and trafficking, continues to shed light on how TRP channels are regulated. On the basis of the previous 10 years, research into TRP channel activity over the next decade will surely uncover some unforeseen roles for this versatile and unpredictable superfamily.

References


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