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The TRP Channels, a Remarkably Functional Family

Minireview

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TRP cation channels display an extraordinary assortment of selectivities and activation mechanisms, some of which represent previously unrecognized modes for regulating ion channels. Moreover, the biological roles of TRP channels appear to be equally diverse and range from roles in pain perception to male aggression.

The TRP superfamily includes >20 related cation channels that play critical roles in processes ranging from sensory physiology to vasorelaxation and male fertility. Defects in TRP channels have been associated with changes in growth control and one TRP-related protein may be a tumor suppressor. Moreover, mutations in a member of the TRP superfamily are a common cause of polycystic kidney disease, while disruption of another is responsible for mucolipidosis, a neurodegenerative disease. TRP proteins are widely expressed in the nervous system, and, in non-excitable cells, TRP-related channels may be the primary mode of Ca^{2+} entry. TRP proteins are cation channels; however, they vary significantly in their selectivity and mode of activation. Nevertheless, members of the TRP superfamily share significant sequence homology and predicted structural similarities, such as six predicted transmembrane segments (reviewed in Montell, 2001).

In the Beginning

The founding member of the TRP superfamily was identified as a *Drosophila* gene required for visual transduction, which in the fly is a phospholipase C (PLC)-dependent process. The *transient receptor potential* (*trp*) locus is named based on the transient, rather than sustained, response to light in the mutant flies. Furthermore, *trp* mutants display a defect in light-induced Ca^{2+} influx. The predicted structure of TRP and the related protein, TRPL, raised the possibility that these proteins were Ca^{2+} influx channels (reviewed in Montell, 2001).

A variety of in vitro studies not only supported the proposal that TRP and TRPL were cation channels, but stimulated interest in these proteins among workers looking for proteins responsible for the many PLC-dependent Ca^{2+} influx pathways described in a wide range of mammalian cells.

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A Family with Many Cousins

As it turns out, there are not only mammalian homologs of TRP, but a panoply of TRP-related channels conserved in every metazoan organism that has been subjected to genome-wide sequencing efforts. These include proteins that fall into three subfamilies of channels that are the most related to TRP (TRPC, TRPV, and TRPM), and which have been the focus of a recent effort to unify the nomenclature (Montell et al., 2002). The mammalian proteins that display the greatest sequence similarity to *Drosophila* TRP belong to the TRPC subfamily. These proteins share 30%–47% amino acid homology over the N-terminal ~800 amino acids, which encompass 3–4 ankyrin repeats, the six transmembrane segments, and a highly conserved 25 amino acid segment referred to as the TRP domain (Figure 1). The TRPV proteins also include 3–4 ankyrin repeats but lack the TRP domain, while the TRPM proteins contain a TRP domain, but no ankyrin repeats (Figure 1).

Two other subfamilies (TRPP and TRPML), which include PKD2 and mucolipin respectively, are also conserved throughout animal phylogeny, but are more distantly related to TRP. A sixth subfamily, TRPN, is comprised of proteins in flies and worms, such as the mechanosensory channel NOMPC, which have large numbers of ankyrin repeats.

Remarkable Regulatory Mechanisms

A distinguishing and unanticipated feature of the TRP superfamily concerns the considerable diversity in selectivities and modes of activation of the channels. Given that *Drosophila* TRP requires PLC for activity in vivo, mammalian homologs of TRP channels were predicted to be PLC-dependent ion channels. While this is the case for some mammalian TRPs, there appears to be a greater variety of mechanisms linking PLC activation to cation influx than initially envisioned (Table 1). Most surprising of all is the medley of novel regulatory mechanisms that appear to be independent of PLC signaling pathways. The identification of some of the PLC-independent mechanisms provide insights into the molecular mechanisms underlying a variety of well-known but poorly understood physiological processes. These include processes as disparate as the sensation of pain and the control of necrotic and apoptotic cell death by the redox status of a cell.

Some Like It Hot and Some Like It Cold. While some mammalian TRP-related proteins are PLC-dependent channels, others are activated through a range of mechanisms, such as changes in cell volume (TRPV4; Table 1), which were not anticipated to be associated with TRP channels. Moreover, in many instances, a single TRP-related channel appears to be activated through a surprisingly broad range of stimuli. The breadth of regulatory mechanisms is particularly notable in the case of TRPV1, which is activated by ligands, including vanilloid compounds such as the active ingredient in hot chili peppers (capsaicin), and anandamide, the endogenous ligand for cannabinoid receptor 1, as well as by moderate temperatures exceeding 42°C.

The sensitivity of TRPV1 to capsaicin or heat is po-

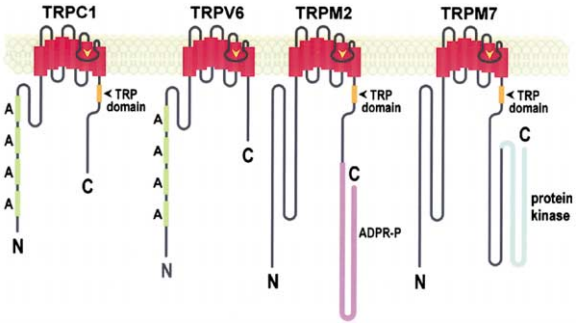

Figure 1. Domain Organization of Selected Members of the TRPC, TRPV and TRPM Subfamilies

The ankyrin repeats (A), TRP domain, protein kinase domains, and ADP-ribose pyrophosphatase (ADPR-P) domains are indicated.

tentiated by agents that change in concentration at sites of inflammation or ischemia. These include protons, bradykinin, and nerve growth factor (references can be found in Chuang et al., 2001). The observation that a single molecular entity, TRPV1, responds to each of these factors indicates that the integration of highly diverse chemical and physical stimuli that elicit pain occurs through a common pathway.

The effects of bradykinin and nerve growth factor on TRPV1 appear to occur through activation of PLCγ, which in turn relieves phosphatidylinositol-4,5-bisphosphate (PIP_2)-mediated inhibition of TRPV1 (Chuang et al., 2001). This is an intriguing observation, as it may explain the mechanism through which bradykinin and nerve growth factor promote sensitization to pain. Furthermore, these studies raise the possibility that similar mechanisms involving release of channel inhibition by PIP_2 may account for the activation of other members of the TRP superfamily that are gated in a PLC-dependent manner. Further, in vitro studies indicate that *Drosophila* TRPL is inhibited by PIP_2. Whether TRPL is inhibited by PIP_2 in vivo and whether PIP_2 binds directly to members of the TRP superfamily remains to be determined.

Another TRPV protein, TRPV2, is not activated by vanilloid compounds, but by noxious heat with a threshold of 52°C. Furthermore, TRPV2 can also be activated, at least in vitro, through regulated translocation of the protein from an intracellular compartment to the plasma membrane in response to either insulin-like growth factor-1, PDGF, or the neuropeptide head activator (references can be found in Boels et al., 2001). Regulated translocation of an ion channel in response to growth factors is unprecedented, though it is reminiscent of the insulin-induced translocation of the glucose transporter to the plasma membrane. The mechanisms underlying the regulated translocation of TRPV2 are unclear, but it appears to be dependent on the activity of phosphatidylinositol (PI) 3-kinase, as is the translocation of GLUT4.

The identification of TRPV1 and TRPV2 as heat receptors with moderate and high temperature thresholds raised the possibility that other TRP channels may serve as sensors for low temperatures. This prediction was recently borne out with the discovery that TRPM8 is activated by agents, such as menthol and icilin, that evoke a cool sensation as well as by temperatures below 26°C (McKemy et al., 2002; Peier et al., 2002 [this issue of *Cell*]). The findings that the same channel is activated by menthol and cold provides a plausible molecular explanation as to how both stimuli can evoke similar sensations. Furthermore, while the cold-activated currents are maximal at 8°C, in the presence of sub-activating concentrations of menthol, the threshold and saturation temperatures increase to 30°C and 15.5°C, respectively. Given that there are multiple conditions that potentiate the sensitivity of TRPV1 to heat, it will be of interest to determine whether there are additional stimuli that influence the response of TRPM8 to cold.

Chanzymes, a Marriage between Channels and Enzymes. Three TRPM proteins, TRPM2, TRPM6, and TRPM7, are distinguished from other known ion channels in that they consist of enzyme domains linked to the C termini of ion channel domains (Figure 1). The unusual architecture of TRPM7 was described contemporaneously by two groups, and in one case, by performing a yeast-two-hybrid screen for PLCβ-interacting proteins (Runnels et al., 2001). An atypical serine/threonine protein kinase domain was identified and shown to be joined to a domain similar to members of the TRPM subfamily. Interestingly, this atypical kinase, though lacking discernible primary amino acid sequence homology with classical protein kinases, displays a three-dimensional structure similar to typical protein kinases (Yamaguchi et al., 2001). TRPM7 is capable of autophosphorylation in vitro, and mutations that interfere with the protein kinase binding to ATP in TRPM7 greatly reduced channel activity (Runnels et al., 2001). These authors conclude that the kinase domain plays an important role in activation, although the detailed mechanism is not known.

Another study of TRPM7 did not invoke a requirement for the protein kinase in channel function (Nadler et al., 2001). Rather, it was concluded that TRPM7 is regulated by Mg^{2+}-ATP, which could provide a mechanism for coupling channel activity with the metabolic state of the cell. Furthermore, TRPM7 was reported to be permeant to Mg^{2+}, a highly unusual feature among ion channels. To account for the discrepancy between the two papers, Nadler et al. proposed that the addition of ATP by Runnels et al. contributed to channel activation by reducing the free Mg^{2+}, which inhibits TRPM7.

The identification of TRPM7 was soon followed by the report of an equally intriguing chimeric protein, TRPM2, composed of a C-terminal ADP-ribose pyrophosphatase fused to a TRPM channel (Perraud et al., 2001). Expression of TRPM2 in HEK 293 cells leads to a Ca^{2+}-permeable cation conductance, which is induced in the presence of one of the products of NAD hydrolysis, ADP-ribose, but not by NAD or a variety of nucleotides, such as ATP (Perraud et al., 2001). However, in two other studies, TRPM2 was also stimulated by NAD; though, the basis of the discrepancy with NAD is unclear (Sano et al., 2001; Hara et al., 2002). Nevertheless, the activation by NAD is particularly intriguing in view of the findings that TRPM2 is also activated by H_2O_2 and other agents that produce reactive oxygen and nitrogen species (Hara et al., 2002). The activation by H_2O_2 might occur through an increase in production of NAD as a consequence of a shift of the redox state. Most interestingly, TRPM2-dependent Ca^{2+} influx may account for

Table 1. Features of TRP Proteins

(A) TRPC subfamily

Name	Selectivity P_{Ca}:P_{Na}	Mode(s) of Activation	Adult tissues with highest expression
TRPC1	non-selective cation	store-operated?	heart, brain, testis, ovaries
TRPC2	?	store-operated?	vomeronasal organ, testis
TRPC3	1.6	store-operated, DAG	brain
TRPC4	7 (>100 absent in KO)	store-operated?	brain, endothel, adrenal gland, retina, testis
TRPC5	9.5	store-operated?	brain
TRPC6	5	DAG	lung, brain
TRPC7	1.9 (spont.); 5 (ATP-enh.)	store-operated, DAG	eye, heart, lung

(B) TRPV subfamily

Name	Selectivity P_{Ca}:P_{Na}	Mode(s) of Activation	Adult tissues with highest expression
TRPV1	9.6 (vanilloids); 3.8 (heat)	heat (43°C), vanilloids, anadamide, PIP_2, H^+	trigeminal (TG) & dorsal root ganglia (DRG)
TRPV2	3	heat (52°C), translocation	DRG, spinal cord, brain, spleen, small & large intestine
TRPV4	6	osmolarity, cell volume, phorbol esters	kidney, lung, spleen, testis, endothelium, liver, heart
TRPV5	>100	low intracellular Ca^{2+}; hyperpolarization	kidney, duodenum, jejunum, placenta, pancreas
TRPV6	>100	similar to TRPV5, store-operated (CRAC?)	small intestine, pancreas, placenta, prostate cancer

(C) TRPM subfamily

Name	Selectivity P_{Ca}:P_{Na}	Mode(s) of Activation	Adult tissues with highest expression
TRPM1	non-selective cation	translocation?	eye, melanocytes
TRPM2	~0.3	ADP-ribose, NAD, redox	brain
TRPM3	?	?	?
TRPM4	non-selective cation	?	prostate, colon, heart, kidney,
TRPM5	?	?	small intestine, liver, lung
TRPM6	?	?	
TRPM7	divalent (Ca^{2+} and Mg^{2+})	phosphorylation, Mg^{2+}-ATP	kidney, heart
TRPM8	1–3.3	menthol, icilin, cold (26°C)	prostate, TG, DRG

necrotic and apoptotic death in many cell types in response to changes in the redox state (Hara et al., 2002).

A CRAC in a New Pair of SOCs. Activation of PLC could theoretically be coupled to TRP activation via production of inositol-1,4,5-trisphosphate (IP_3) and/or diacylglycerol (DAG). According to one mechanism, referred to as store-operated Ca^{2+} entry, transient release of Ca^{2+} from internal stores induces sustained Ca^{2+} influx. In fact, the desire to identify store-operated channels (SOCs) was the motivation for many workers to characterize mammalian TRP channels. Of particular interest was a highly Ca^{2+}-selective SOC, referred to as CRAC, as these latter channels have been implicated in a wide array of processes in non-excitable cells, ranging from T cell activation to salivary gland secretion and apoptosis.

While mammalian homologs of TRP were identified, in part, due to an interest in SOCs, current evidence indicates that *Drosophila* TRP is not a SOC after all. However, a surprising twist is that a significant subset of mammalian TRP channels appear to be SOCs. Moreover, the mechanisms through which the activity of TRP channels are coupled to Ca^{2+} release appear to be more diverse than originally anticipated. Some TRP channels seem to be coupled to Ca^{2+} stores via direct interactions with the IP_3 receptor (Boulay et al., 1999, and references therein), although, at least one, TRPC3, may also be activated through an interaction with the other known Ca^{2+} release channel, the ryanodine receptor. In addition, several TRPC channels may be regulated by diacylglycerol rather than a store-operated mechanism (reviewed in Montell, 2001).

The identity of the CRAC channels has continued to be a focus in the field, and recently, a member of the TRPV family, TRPV5, was reported to display Ca^{2+}-selective permeation properties resembling those of CRAC channels (references can be found in Voets et al., 2001). In addition, another member of the TRPV subfamily, TRPV6 (Figure 1), exhibits several key features of CRAC channels when expressed in vitro (Yue et al., 2001). These include activation of TRPV6 through a store-operated mechanism, and a high degree of Ca^{2+} selectivity in the presence of divalent cations. Though some common features between TRPV6 and CRAC channels have been confirmed in an independent study (Voets et al., 2001), there are several differences between I_{CRAC} detected in RBL cells, and I_{TRPV6} (Voets et al., 2001).

It remains possible that TRPV6 is a subunit of a CRAC channel and the differences between I_{TRPV6} and I_{CRAC} are the consequence of heteromultimerization between TRPV6 and other channel subunits. There is precedent for interactions among members of the family. Heteromultimerization between the three *Drosophila* TRP channels occurs in vitro and in vivo and affects the biophysical properties of the channels (reviewed in Montell, 2001). Similar results are seen in mammalian systems.

In view of their significant structural similarities, it is remarkable that the mechanisms that regulate the various members of the TRPC, TRPV and TRPM subfamilies

are so diverse and, in many cases, represent heretofore unrecognized mechanisms. This diversity is likely to increase as additional members of the TRP superfamily are functionally characterized. An example is TRPM6, which is also predicted to encode a TRP channel linked to an atypical protein kinase.

An important limitation of most of the studies of mammalian TRP-related channels is that the analyses have focused on proteins overexpressed in heterologous systems. The initial exception is the identification of a TRPC3-dependent current in rat pontine neurons, which is activated in vivo through a pathway involving the TrkB neurotrophin receptor, PLCγ and the IP_3-receptor (Li et al., 1999). TRPC4-dependent store-operated currents have been identified in adrenal cells and macrovascular endothelial cells (references can be found in Freichel et al., 2001) and a TRPC2-dependent store-operated current has been described in sperm (Jungnickel et al., 2001). Similar analyses, using TRPV6 single- and TRPV5/TRPV6 double-knockout mice, will be required to provide definitive evidence that TRPV6 is a subunit of a CRAC channel. A challenge for the future involves the characterization of additional TRP-dependent cation influx pathways in native physiological contexts, as has been described for TRPC2, TRPC3, and TRPC4.

Emerging Roles for Mammalian TRP Channels

Of paramount importance is the identification of the biological functions of this heterogeneous family of proteins. Genetic approaches, in worms, flies, and mice demonstrate a role for many TRP proteins in an assortment of sensory processes ranging from vision to osmosensation, olfaction, mechanosensation, and pain reception. In addition, several recent studies have begun to unravel roles for TRP proteins in non-excitable cells, including a requirement in vasorelaxation, as indicated by analyses of TRPC4 knockout mice (Freichel et al., 2001).

Two recent studies have pointed to several intriguing functions of TRPC2 in the mouse. TRPC2 appears to be expressed in sperm, primarily in the anterior head, and application of an antibody that reacts to a presumed extracellular loop inhibits the sperm acrosomal reaction (Jungnickel et al., 2001). One isoform of TRPC2 is expressed exclusively in the mouse vomeronasal organ (VNO), which functions in the pheromone response. Moreover, in VNOs dissected from TRPC2 knockout mice, application of pheromone failed to evoke the usual excitatory response (Stowers et al., 2002). Most importantly, male TRPC2 knockout mice display several behavioral phenotypes. These include elimination of aggression among males, in response to pheromone, and an equal propensity to engage in sexual behavior with males and females (Stowers et al., 2002). Given that the VNO is a vestigal organ in humans, these studies would appear to provide an explanation for the observations that *TRPC2* encodes a functional protein in the mouse and is a pseudogene in humans.

There are also suggestions that a number of TRP-related proteins may have roles in growth control and changes in the expression of these channels may contribute to certain cancers. Studies in *C. elegans* indicate that at least one TRPM member may have a role in cell cycle control. Moreover, TRPM1 has been suggested to be a tumor suppressor and a decrease in expression of TRPM1 appears to be a prognostic marker for metastasis in patients with localized malignant melanoma. In addition, expression of TRPM8 and TRPV6, appears to be upregulated in prostate cancers and may represent new markers for prostate cancer (Wissenbach et al., 2001). Based on these early results, it seems likely that the biological functions of the TRP proteins are as diverse as their activation mechanisms.

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