

THE TRP ION CHANNEL FAMILY

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Mammalian homologues of the *Drosophila* transient receptor potential (TRP) channel gene encode a family of at least 20 ion channel proteins. They are widely distributed in mammalian tissues, but their specific physiological functions are largely unknown. A common theme that links the TRP channels is their activation or modulation by phosphatidylinositol signal transduction pathways. The channel subunits have six transmembrane domains that most probably assemble into tetramers to form non-selective cationic channels, which allow for the influx of calcium ions into cells. Three subgroups comprise the TRP channel family; the best understood of these mediates responses to painful stimuli. Other proposed functions include repletion of intracellular calcium stores, receptor-mediated excitation and modulation of the cell cycle.

POLYCYSTIC KIDNEY DISEASE PROTEINS

Also known as polycystins, these proteins form cation-selective channels that are permeable to calcium. Mutations in the polycystins lead to the formation of fluid-filled cysts in the kidneys.

The last bastion of ion channels is the transient receptor potential (TRP) channel family, and its walls are slowly crumbling. The TRP ion channels are a large class of channel subunits united by a common primary structure and permeability to monovalent cations and calcium ions, Ca^{2+} (REF. 1). Although at least 20 unique mammalian proteins have now been identified in this family, two central enigmas remain: what gates these channels and what do they do? The answers to these questions are still tentative. As we discuss here, TRP channels are linked to the phosphatidylinositol signal transduction pathway, and they bring Ca^{2+} into cells at hyperpolarized membrane potentials.

The TRP channels are made of subunits with six membrane-spanning domains, which are cast in the same evolutionary mold as voltage-gated K^+ channels (K_v), cyclic-nucleotide-gated channels (HCN and CNG), and the POLYCYSTIC KIDNEY DISEASE PROTEINS. By analogy to the relatively well-understood K_v class, TRP channels probably form tetramers in which the amino acids that link the fifth and sixth transmembrane domains line the pore. But these channels are less refined in their selectivity for ions, as most of them allow any cation, including Ca^{2+} , to move into the cell.

Ca^{2+} is the most common signal transduction element in cells ranging from bacteria to neurons; dozens of proteins are activated by its direct binding, and many more are activated by the binding of Ca^{2+} -adaptor pro-

teins such as **calmodulin**. Normal concentrations of free intracellular Ca^{2+} ($[\text{Ca}^{2+}]_i$), at around 100 nM, are 20,000-fold lower than extracellular concentrations. Cells tightly regulate intracellular levels through endogenous chelators and specialized extrusion proteins. Excitable cells have been equipped with specialized voltage-sensitive and highly selective Ca^{2+} channels. Opening of these channels provides the sharp, decisive rise in $[\text{Ca}^{2+}]_i$ required for rapid contraction in heart and skeletal muscle, as well as for exocytosis at nerve terminals. Non-excitable cells such as blood cells and fibroblasts use a distinct mechanism. Ligand binding to some membrane receptors initiates a sequence of events that lead to the activation of **phospholipase C** (PLC), generating inositol-1,4,5-trisphosphate ($\text{Ins}(1,4,5)\text{P}_3$), which opens an intracellular ion channel — the **$\text{Ins}(1,4,5)\text{P}_3$ receptor** (InsP_3R) — and liberates Ca^{2+} from the spidery distribution network of the endoplasmic reticulum² (FIG. 1). Over a few seconds, $[\text{Ca}^{2+}]_i$ rises to micromolar levels. Activation of the TRP channels accompanies this chain of events in a way that is not necessarily linked to Ca^{2+} depletion from intracellular stores. TRP channels do not have the sharp voltage sensitivity of the well-characterized voltage-gated Ca^{2+} channel family, and some of them pass more Ca^{2+} ions down the huge chemical gradient when the cell is more hyperpolarized. Most TRP channels are relatively non-selective to cations, and act to shift the membrane potential to around 0 mV, depolarizing cells from their

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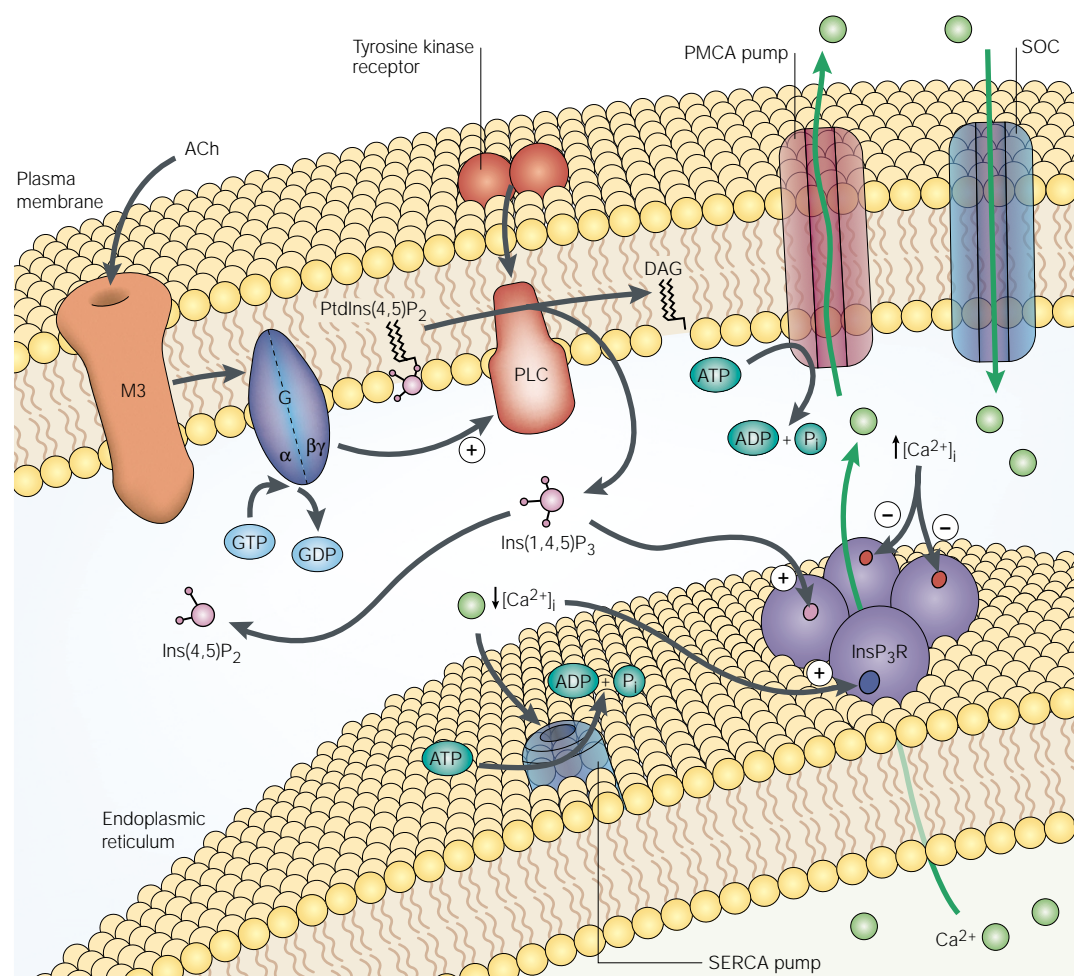


Figure 1 | **Calcium signal transduction.** Molecules in the cell membrane that relay hormonal and neurotransmitter signals bind to receptors on the cell surface (M3) that initiate the release of intracellular Ca^{2+} . ACh, acetylcholine; DAG, diacylglycerol; G, G protein; Ins(1,4,5) P_3 , inositol-1,4,5-trisphosphate; InsP₃R, Ins(1,4,5) P_3 receptor; M3, muscarinic type 3 ACh receptor; PLC, phospholipase C or PtdIns(4,5) P_2 phosphodiesterase; PMCA, plasma membrane Ca^{2+} -ATPase pump; PtdIns(4,5) P_2 , phosphatidylinositol-4,5-bisphosphate; SERCA, smooth endoplasmic reticulum Ca^{2+} -ATPase pump; SOC, store-operated Ca^{2+} channel; +, stimulatory regulation; –, inhibition.

resting membrane potentials. In other words, TRP channels short-circuit the cell battery and raise $[\text{Ca}^{2+}]_i$.

In the human retina, photons strike the molecule of retinal that is nested in the opsin receptor, evoking a conformational change that leads to the exchange of GTP for GDP on the G protein α -subunit. GTP-bound $\text{G}\alpha$ activates cGMP phosphodiesterase which degrades cGMP, keeping CNG channels open in the dark. Vision in the *Drosophila* eye is processed in a related, but fundamentally different way. In invertebrates, the rhodopsin receptor is coupled to the same signal transduction system shown in FIG. 1, activating PLC- β and, in turn, TRP channels. TRP-deficient flies are blinded by intense light because sustained Ca^{2+} entry and subsequent Ca^{2+} -dependent adaptation are abrogated. The time course of the TRP/TRPL currents in *Drosophila* led investigators to propose that these channels were gated by the depletion of intracellular Ca^{2+} stores³. However, *Drosophila* mutants that lack InsP₃R do not provide support for this conclusion^{4,5}. Furthermore, the

immunohistochemical analysis of adult flies has shown that TRP and TRPL are distributed along the 2,000 nm-long microvilli, and are not localized only to their bases where the Ca^{2+} stores are present. Recent genetic analyses in flies have not resolved the mechanism of TRP activation, but have confirmed the importance of PLC- β and other components of the phosphatidylinositol pathway⁶.

Over the past few years, several TRP homologues have been cloned from *Caenorhabditis elegans* and from mammalian genomes. The primary structure shows the presence of seven hydrophobic segments, but, on the basis of analogy to other channels, the location of glycosylation sites and mutagenesis studies, it is generally assumed that TRPs have six transmembrane domains (S1-S6) with both carboxy and amino termini located intracellularly (FIG. 2). The TRP family can be divided by sequence homology into three subfamilies, designated by Harteneck¹ as short, long, and osm-9-like (a *C. elegans* TRP mutant). The most distinctive segments among the three subfamilies are located in the carboxyl

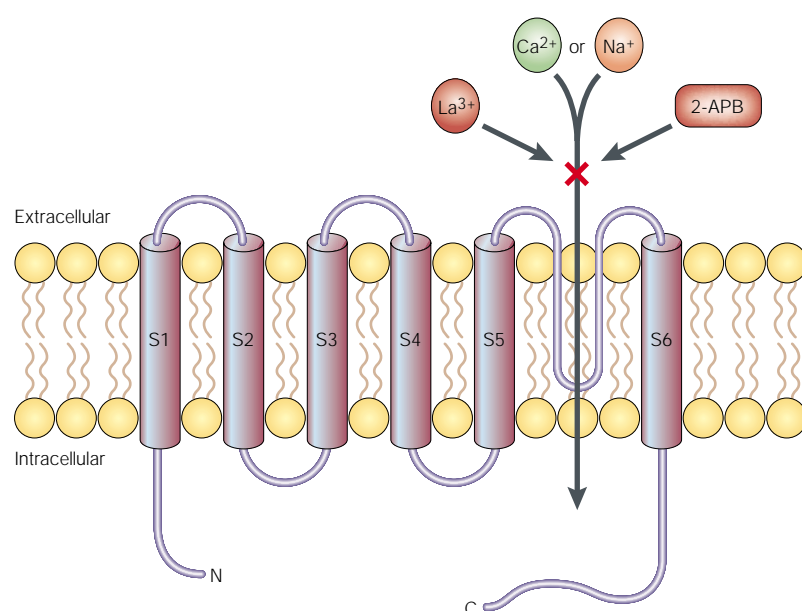


Figure 2 | Architecture of TRP channels. Like the broader class of ion channel subunits, TRP channels comprise six membrane-spanning domains. S1–S6 are transmembrane domains. Lanthanum ions (La^{3+}) and 2-aminodiethylidiphenyl borate (2-APB) often block these channels, although not specifically. Ankyrin repeats are found in the amino termini of TRPC and TRPV channels.

ANKYRINS

Adapter molecules that couple membrane proteins to the spectrin-based membrane cytoskeleton.

PLECKSTRIN-HOMOLOGY DOMAINS

Sequences of about 100 amino acids present in many signalling molecules. Pleckstrin is a protein of unknown function originally identified in platelets. It is a principal substrate of protein kinase C.

EF-HANDS

Ca^{2+} -binding domains originally identified in parvalbumin, which are also known as helix–turn–helix domains.

SH DOMAINS

Src-homology domains. They are involved in the interaction with phosphorylated tyrosine residues on other proteins (SH2 domains) or with proline-rich sections of other proteins (SH3 domains).

PERTUSSIS TOXIN

The causative agent of whooping cough, pertussis toxin causes the persistent activation of G_i proteins by catalysing the ADP-ribosylation of the α -subunit.

terminus, whereas the most conserved regions between these families are in the S6 domain, which is presumably the most important region for their gating. The short and osm-9-like families have two to four amino terminal ANKYRIN domains, and the short and long proteins contain a proline-rich cytosolic segment in the proximal carboxyl terminus. In this review, we will focus on the mammalian genes and use the nomenclature TRPCx, TRPVx, and TRPMx to denote the short (C), osm-9-like or vanilloid (V), and long or melastatin (M) families, respectively (FIG. 3). An alternative nomenclature (cation channel homologue or CCH) is still being debated at the time of this writing.

Brief review of signal transduction

The three classes of phosphatidylinositol-specific PLC (PLC- β_{1-4} , PLC- $\gamma_{1,2}$ and PLC- δ_{1-4}) can be distinguished by their primary structure and modes of regulation⁷. The catalytic domain common to all mammalian PLCs contains two regions, X and Y, which show a conserved sequence identity of 40–60% among the different isoforms⁸. Flanking the X and Y regions, there are modular accessory domains (PLECKSTRIN HOMOLOGY DOMAIN (PH domain); EF-HANDS; C2 domain; and SH DOMAINS, SH2 and SH3) that control enzymatic activity and localization of PLC within cells. The mammalian PLCs show an absolute specificity for inositol lipids, hydrolysing phosphatidylinositol-4-phosphate (PtdIns4P) and phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5) P_2), and a dependence on Ca^{2+} within the physiological range (0.1–10 μM). PtdIns(4,5) P_2 is cleaved into two second messengers, the membrane-bound diacylglycerol (DAG) and soluble Ins(1,4,5) P_3 . DAG and intracellular Ca^{2+} liberated by Ins(1,4,5) P_3 activate **protein kinase C** (PKC) by

recruiting it to the membrane surface, bringing a wealth of PKC substrates into the Ca^{2+} signalling pathways. A complex set of enzymes mediates the generation of multiple inositol phosphates and lipid second messengers, but it is clear that Ins(1,4,5) P_3 is the dominant instigator of intracellular Ca^{2+} release⁹. At least 30 different metabotropic receptors initiate Ca^{2+} release by the stimulation of PLC- β through the PERTUSSIS-TOXIN-insensitive G_q family, but G protein $\beta\gamma$ -subunits can also activate PLC- β_2 or PLC- β_3 .

PLC- γ is not activated by G proteins⁷. Growth factors such as platelet-derived growth factor (PDGF) or epidermal growth factor (EGF) bring receptor tyrosine kinases together, allowing their kinase domains to phosphorylate each other. The phosphorylated tyrosines form docking sites for the SH2 binding domains of PLC- γ and usher its translocation to the membrane into proximity with PtdIns(4,5) P_2 . The tyrosine kinase receptor then phosphorylates PLC- γ to activate its enzymatic function. Again, Ins(1,4,5) P_3 and DAG are formed, but in general tyrosine kinases acting through PLC- γ increase Ca^{2+} more slowly and for longer periods than $G_{q\alpha}$ -mediated PLC- β stimulation.

Regulation of PLC- δ is poorly understood in comparison with PLC- β and PLC- γ , although PLC- δ is the archetypal enzyme from which PLC- β and PLC- γ evolved. PLC- δ is controlled by its own substrate, PtdIns(4,5) P_2 . Membrane targeting of PLC- δ is accomplished through its PH domain, which binds the polar head group of PtdIns(4,5) P_2 . Although Ca^{2+} activates all PLC isoforms *in vitro*, PLC- δ isoforms are more sensitive to Ca^{2+} than the other isoforms. This fact is highlighted by recent studies linking PLC- δ activation to the store-operated entry of extracellular Ca^{2+} (REF. 10).

Putney¹¹ proposed that empty Ca^{2+} stores gate the entry of external Ca^{2+} in order to replenish these reserves. The physiological hallmark of the store-operated Ca^{2+} entry process is a large receptor-mediated transient increase in $[\text{Ca}^{2+}]_i$, followed by a prolonged high $[\text{Ca}^{2+}]_i$ plateau phase that depends on the levels of extracellular Ca^{2+} . A very specific and highly Ca^{2+} -selective current (Ca^{2+} -release-activated current or I_{CRAC}) is activated by a variety of store-depletion protocols in whole-cell recordings from single blood cells^{12,13}, but store-operated entry might not occur solely through I_{CRAC} channels. From the start, TRPs have been the major candidates for store-operated channels, including I_{CRAC} . At odds with this supposition is the high Ca^{2+} -selectivity of I_{CRAC} compared with the cationic non-selectivity of most members of the TRP family. There are three dominant hypotheses for the store-operated activation of channels: first, direct gating by the Ins P_3 R; second, exocytotic fusion of vesicles containing store-operated channels; and third, generation of a second messenger that diffuses from the endoplasmic reticulum to the channel. None of these hypotheses has yet been proven.

The TRPC family

The **TRPC** group can be divided into four subfamilies (TRPC1, TRPC4,5, TRPC3,6,7 and TRPC2) on the basis of sequence homology and functional similarities.

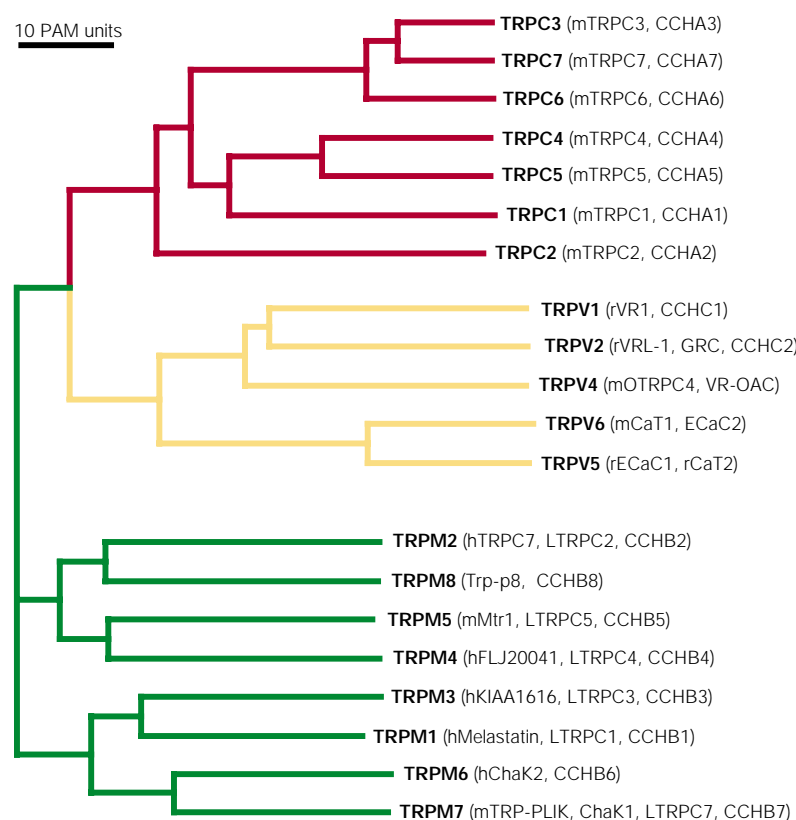


Figure 3 | Phylogenetic relationship in the TRP protein family. The evolutionary tree is calculated by the neighbour-joining method⁷⁰. The evolutionary distance is shown by the total branch lengths in point accepted mutations (PAM) units, which is the mean number of substitutions per 100 residues. Other more distantly related members of the mammalian superfamily are PKD (TRPP) and mucolipidin (TRPML). The corresponding GenBank accession numbers for the proteins used are (in parentheses): mTRPC3 (NP_062383), mTRPC7 (AAD42069), mTRPC6 (AAC06146), mTRPC4 (AAC05179), mTRPC5 (AAC13550), mTRPC1 (AAB50622), mTRPC2 (AAG29950), rVR1 (T09054), rVRL-1 (NP_035836), mOTRPC4 (AAG17543), mCaT1 (BAA99538), rECaC1 (BAA99541), LTRPC2 (NP_003298), Trp-p8 (see REF. 51), mMtr1 (AAF98120), hFLJ20041 (NP_060106), hKIAA1616 (BAB13442), hMelastatin (NM_002420), hChaK2 (AAK31202), mTRP-PLIK (F376052).

PDZ DOMAIN

A peptide-binding domain that is important for the organization of membrane proteins, particularly at cell–cell junctions, including synapses. They can bind to the carboxy termini of proteins, or can form dimers with other PDZ domains. PDZ domains are named after the proteins in which these sequence motifs were originally identified (PSD95, Discs-large, zona occludens-1).

TRANSDUCISOME

Signalling complex in the *Drosophila* eye that is formed by the components of the phototransduction cascade, including the ion channels, the phospholipase C β and protein kinase C.

TRPC1 was the first member of the mammalian TRP family purported to form an ion channel¹⁴. In the initial study, expression of TRPC1 gave rise to a linear non-selective cationic current activated by the depletion of Ca²⁺ stores. The TRPC1 single-channel conductance was estimated to be 16 pS by noise analysis, but has not been measured directly so far. In a more recent study, Lintschinger *et al.*¹⁵ found no evidence of store-dependent regulation of TRPC1, but reported activation of the channel by DAG in Ca²⁺-free solution. When cells were stimulated with agonists that activate PLC- β under physiological ionic conditions, we found that expression of TRPC1 alone did not result in measurable ion currents¹⁶. Given its widespread expression (TABLE 1), and its ability to co-assemble with other TRPC subunits *in vitro*¹⁵ and *in vivo*¹⁶, TRPC1 might be a component of different heteromeric TRP complexes. Whether TRPC1 can form functional channels in the absence of other TRP subunits is therefore not established.

The second TRPC subfamily most closely related to TRPC1 comprises TRPC4 and TRPC5. Murine TRPC4

and TRPC5 can form homomeric cation channels that are activated following stimulation of G_q-coupled receptors^{17,18} (FIG. 4a) and by receptor tyrosine kinases¹⁸. But despite intensive study, the details of their activation mechanism remain elusive. As G_q and receptor tyrosine kinase signalling pathways converge at the level of PLC activation, TRPC4 and TRPC5 are likely to be activated by a common messenger generated by different PLC isoforms. However, the two primary products of PLC enzyme activity — Ins(1,4,5)P₃ and DAG — were not found to activate TRPC4 and TRPC5 (REFS 18,19). This intriguing result suggests that an as yet unknown PLC-dependent mechanism or a combination of messengers link membrane receptors to TRPC4 and TRPC5 activation. Both TRPC4 and TRPC5 contain a carboxy terminal PDZ-binding motif (VTTRL) not present in other TRPs. PDZ DOMAIN scaffolding proteins such as the Na⁺/H⁺ exchanger regulatory factor, and signalling molecules like PLC- β 1, co-immunoprecipitate with TRPC4 and TRPC5 (REF. 20), indicating that the channels might be part of multimolecular signalling complexes similar to the TRANSDUCISOME of *Drosophila* photoreceptors. Deciphering the functional role of the protein–protein interactions within these signalling complexes will be crucial to understanding TRPC4 and TRPC5 regulation.

The biophysical properties of heterologously expressed murine TRPC4 and TRPC5 have been confirmed in independent studies, but it is not yet certain that these are the channels formed by TRPC4 and TRPC5 *in vivo*. Strübing *et al.*¹⁶ showed that TRPC5 and TRPC1 are subunits of a receptor-operated heteromeric channel *in vivo* that was not activated by store depletion. Both TRP proteins have overlapping distributions in the hippocampus, and their co-expression gave a novel non-selective cationic channel with a voltage dependence similar to that of NMDA (*N*-methyl-D-aspartate) receptor channels, but unlike that of any reported TRP channel (FIG. 4b). TRPC1/TRPC4 heteromers might play a similar role in the cerebral cortex. Accordingly, we proposed that many TRPC heteromers form diverse receptor-regulated non-selective cationic channels in the mammalian brain. The first knockout of a TRP gene in mice showed that elimination of TRPC4 reduced (but did not abolish) an I_{CRAC}-like store-operated current in endothelial cells²¹, consistent with the previous claim of activation of bovine TRPC4 by store depletion²². Other ion currents in the knockout mouse should be examined to settle the question of whether TRPC4 forms store- or receptor-operated channels, or both.

TRPC3, TRPC6 and TRPC7 are ~75% identical and form a cationic non-selective channel that shows RECTIFICATION in both the inward (negative voltages) and outward (positive voltages) directions. TRPC3 channels have short mean open times ($\tau \approx 0.1$ ms) and a single-channel conductance of 66 pS (REF. 23). The channel is predominantly expressed in rat brain during a narrow developmental period around birth²⁴. Although exogenously applied DAG enhanced channel activity of expressed TRPC3¹⁹, it is not established that DAG is the *bona fide* physiological activator of native

RECTIFICATION

The property whereby current through a channel does not flow with the same ease from the inside as from the outside. In inward rectification, for instance, current into the cell flows more easily than out of the cell through the same population of channels.

TRPC3-containing channels. For example, a recent study of *Drosophila* TRPL showed that high concentrations of DAG might further stimulate PLC, even in excised membrane patches²⁵. Moreover, depending on expression levels and co-assembly with other subunits, TRPC3 might be activated by an alternative InsP_3 -dependent route²⁶.

TRPC6 and TRPC7, like TRPC3, are inwardly and outwardly rectifying, have relatively low selectivity for Ca^{2+} over Na^+ , are sensitive to intracellular Ca^{2+} , and are activated by DAG^{19,27}. Their relatively high expres-

sion levels in smooth muscle and heart cells make them promising candidates for the as yet unidentified non-selective cationic channels in these muscle cells. The finding that TRPC6 is an essential part of the α_1 -adrenoreceptor-activated cation channel in rabbit portal vein myocytes²⁸ supports this idea.

Less information is available about TRPC2, which shares ~30% sequence identity with the TRPC3, TRPC6 and TRPC7 subfamilies. Full-length TRPC2 messenger RNA (mRNA) and several amino terminal

Table 1 | TRP channel properties

Name	Proposed name	Selectivity ($P_{\text{Na}}/P_{\text{Ca}}$)	Single-channel conductance (pS)	Proposed regulation	Tissue distribution	References
TRPC						
TRPC1	TRPC1	Non-selective	N.D. (16 pS by noise analysis)	Receptor-operated (heteromer), store depletion?	Widely expressed	14–16, 31,58
TRPC2	TRPC2	N.D.	N.D.	Store depletion?	VNO, testis, heart, brain, sperm	29,30, 32,33
TRPC3	TRPC3	1/1.5	66	DAG, InsP_3 R	Brain, placenta, heart, muscle	19,23,26, 58–60
TRPC4, CCE1	TRPC4	1/1.1* 1/7* (bCCE)	41	Receptor-operated, store depletion?	Brain, testis, placenta, adrenal gland, endothelial cells	18,21, 61,62
TRPC5, CCE2	TRPC5	1/9 [§] 1/1.8*	63	Receptor-operated, store depletion?	Brain	17,18,22
TRPC6	TRPC6	1/5	35	DAG	Lung, brain, muscle	19,28,63
TRPC7	TRPC7	1/2	N.D.	DAG	Heart, muscle, lung, eye, brain	27,64
TRPV						
VR1	TRPV1	1/3	110 (Ca^{2+} -free)	Capsaicin, heat, PKC	Brain, spinal cord, peripheral sensory neurons	36,65
VRL-1, GRC	TRPV2	1/3	N.D.	Heat, growth factors	Brain, spinal cord, spleen, lung, peripheral sensory neurons	37,66
OTRPC4, VR-OAC	TRPV4	1/6	90	Osmolarity	Brain, liver, kidney, fat, heart, testis, salivary gland, trachea	38,39
ECaC1, CaT2	TRPV5	1/107	75 (Ca^{2+} -free)	Low intracellular Ca^{2+}	Intestine, kidney, placenta	40–42,67
CaT1, ECaC2	TRPV6	1/130	42–58 (Ca^{2+} -free)	Store depletion, low intracellular Ca^{2+}	Intestine, placenta, kidney, prostate, salivary gland	45,46,68
TRPM						
Melastatin, LTRPC1	TRPM1	N.D.	N.D.	N.D.	Eye (melanocytes)	52,54
TRPC7, LTRPC2	TRPM2	N.D.	N.D.	ADP-ribose	Fetal and adult brain, placenta	48,49
KIAA1616, LTRPC3	TRPM3	N.D.	N.D.	N.D.	N.D.	
FLJ20041, LTRPC4	TRPM4	N.D.	N.D.	N.D.	N.D.	
Mtr1, LTRPC5	TRPM5	N.D.	N.D.	N.D.	Widely expressed	57,69
ChaK(1), TRP-PLIK	TRPM7	3/1	105	Phosphorylation	Kidney, heart, liver, spleen, lung, brain	47
ChaK2	TRPM6	N.D.	N.D.	N.D.	N.D.	(AF350881)
Trp-p8	TRPM8	N.D.	N.D.	N.D.	N.D.	51

Estimates of selectivity from *REF. 18, [†]REF. 61 and [§]REF. 17. ^{||}GenBank accession number. (N.D., not determined; VNO, vomeronasal organ; DAG, diacylglycerol; InsP_3 R, inositol-1,4,5-trisphosphate receptor; PKC, protein kinase C; ADP-ribose, adenosine diphosphoribose.)

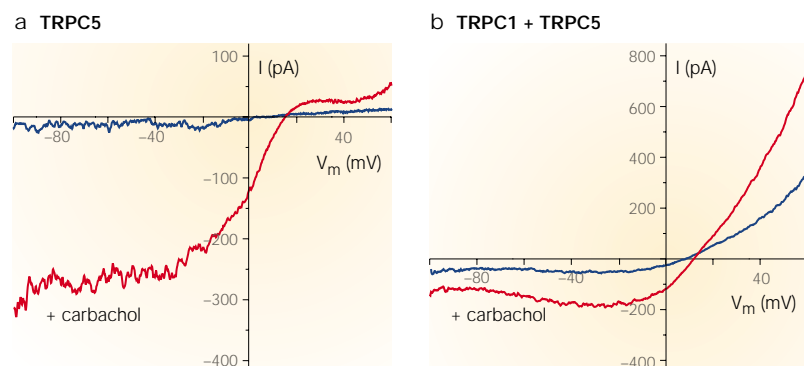


Figure 4 | Current-voltage relation of homomeric TRPC5 and heteromeric TRPC1/TRPC5 channels. **a** | TRPC5 and **b** | TRPC1/TRPC5 whole-cell currents were recorded from HEK-293 cells stably transfected with the muscarinic M1 receptor and transiently transfected with TRPC5 or TRPC1 plus TRPC5 complementary DNAs before (blue) and after (red) the application of 20 μ M carbachol. Voltage ramps (1 mV ms⁻¹) were applied from a holding potential of -60 mV. The pipette solution contained (in mM): 120 CsOH, 120 gluconic acid, 2 MgCl₂, 3 CaCl₂, 5 Cs₄-BAPTA, 10 HEPES; pH 7.3. The free intracellular Ca²⁺ concentration was calculated to be ~200 nM. Cells were bathed in (mM): 140 NaCl, 5.4 KCl, 2 CaCl₂, 1 MgCl₂, 10 glucose, 10 HEPES; pH 7.4. (Data reproduced with permission from REF. 16; © (2001) Elsevier Science.)

splice variants have been found in mouse and rat tissue, but TRPC2 seems to be a PSEUDOGENE in humans^{29–32}. TRPC2 protein has been localized to neuronal microvilli in rat vomeronasal organ²⁹ and in the head of mouse sperm³³. THAPSIGARGIN-induced Ca²⁺ influx and purine-receptor-induced currents are elevated in cells that express the longer TRPC2 (TRP2A, TRP2B) splice variants, which contain a unique extended amino terminus^{30,33}. But expression of two shorter TRPC2 isoforms has failed to yield measurable ion currents or increases in thapsigargin-induced Ca²⁺ influx³². Some of the first insights into the function of TRPC2 came from a recent study showing that TRPC2 is responsible for the sustained Ca²⁺ influx into mouse sperm during fertilization³³. Interestingly, PLC- δ 4 has recently been found to be required for the zona-pellucida-induced acrosome reaction³⁴.

The TRPV family

At present, the TRPV family has five members grouped into three subfamilies. TRPV1 and TRPV2 are the vanilloid receptors **VR-1** and **VRL-1**. TRPV4 is the osm-9-like **OTRPC4**, and TRPV5 and TRPV6 are the Ca²⁺-selective channels **ECaC1/CaT2** (epithelial calcium channel/calcium transporter) and **ECaC2** (also called CaT1).

The vanilloid receptors are the best-understood ion channels in this class³⁵. VR-1 (TRPV1), was isolated by expression cloning from a rat dorsal-root-ganglion library using the hot pepper compound capsaicin³⁶. The expressed capsaicin receptor shows the same properties as capsaicin receptors in sensory neurons: it is a relatively Ca²⁺-selective ion channel with an outwardly rectifying CURRENT-VOLTAGE RELATIONSHIP and exhibits Ca²⁺-dependent desensitization. Thapsigargin did not activate expressed TRPV1 channels, whereas capsaicin and the capsaicin analogue resiniferatoxin do. On the other hand, the antagonist capsazepine and ruthenium red block its activation. Endogenous

cannabinoid receptor ligands such as anandamide are potential TRPV1 agonists. TRPV1 is expressed throughout most unmyelinated small- to medium-diameter peptide- and non-peptide-releasing neurons in dorsal root, trigeminal and nodose sensory ganglia. In spinal cord, this channel is present in laminae I and II of the dorsal horn. TRPV1 or its homologues might also be widely distributed in spinal cord sensory neurons and in brain. Cell lines made to express TRPV1, as well as native neuronal pain fibres, die after several hours of continuous exposure to capsaicin. The exact mechanism of TRPV1 activation is not completely understood, but the channel is sensitive to heat (>43°C) and lipids in a membrane-delimited fashion. The size of the current is increased by acidic (low) pH and is modulated by intracellular PtdIns(4,5)P₂. Experiments with TRPV1-knockout mice confirm that it is essential for transducing the nociceptive, inflammatory and hypothermic effects of vanilloid compounds. There seem to be a number of alternatively spliced TRPV1 transcripts, some of which are insensitive to heat and capsaicin, including a putative stretch-inhibitable channel³⁵.

The vanilloid-receptor-like channel (VRL-1, TRPV2) is 50% identical to TRPV1, is insensitive to capsaicin, but is inhibited in a non-competitive manner by ruthenium red³⁷. Like TRPV1, TRPV2 is more permeable to Ca²⁺ than to Na⁺ ($P_{Ca}/P_{Na} = 3/1$) and is outwardly rectifying. It is expressed in medium- to large-diameter neurons of sensory ganglia, but is also present in brain, spinal cord, spleen and lung. It has been proposed to mediate high-threshold (>52°C) noxious heat sensation, perhaps in the lightly myelinated A δ nociceptors, but its presence in non-sensory tissue indicates additional functions.

TRPV4 (OTRPC4, VR-OAC) is ~40% identical to TRPV1 and TRPV2^{38,39}. When expressed in mammalian cells, it forms a moderately selective cationic channel ($P_{Ca}/P_{Na} = 6/1$), with a gently outwardly rectifying current-voltage relationship similar to that of TRPV1. Its single-channel conductance is the same as for TRPV1 (~90 pS in the outward direction³⁸; but see REF. 39). Ruthenium red (10 μ M) and Gd³⁺ block more potently than La³⁺, but unlike TRPV1, TRPV4 is not activated by capsaicin or heat. It is present in liver, kidney and heart. In the nervous system, it is present in ependymal cells of the lateral ventricle, in neurons found throughout the brain, in auditory hair cells, and abundantly in sensory cells of the trigeminal ganglion. In the kidney, it is localized to the inner cortex, more specifically to the distal convoluted tubule. TRPV4 is not known to be activated by a G-protein-coupled receptor, but downstream components of the phosphatidylinositol pathway have not been ruled out. In isotonic media, TRPV4 is active, but its current was found to be further increased by reducing the extracellular osmolarity (leading to cell swelling), with 50% activation by 270 mosmol l⁻¹ (physiological media = 290 mosmol l⁻¹). Hypertonic media (causing cell shrinkage) decreased current activation. Deletion of the ankyrin-repeat domains blunted the response to low osmolar solutions³⁹. Store depletion with thapsigargin did not activate the channel.

PSEUDOGENE

A sequence in DNA that is related to a functional gene but cannot be transcribed due to mutational changes or the lack of regulatory sequences.

THAPSIGARGIN

Plant diterpene that blocks smooth endoplasmic Ca²⁺-ATPase pumps and depletes Ca²⁺ stores.

CURRENT-VOLTAGE RELATIONSHIP

A plot of the changes in ionic current as a function of membrane voltage.

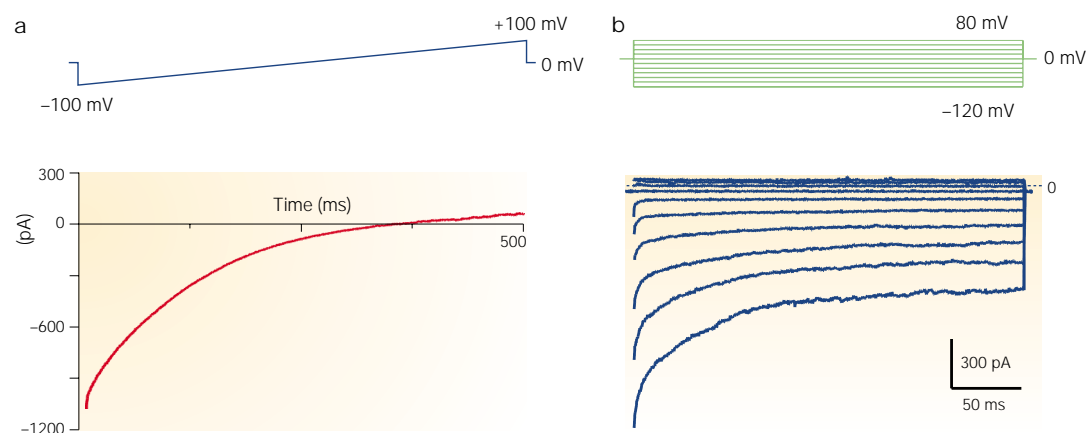


Figure 5 | Current–voltage relationship of the TRPV6 (CaT1/ECaC2) channel expressed in CHO-K1 cells. **a** | Current elicited by a 500 ms voltage ramp from -100 to $+100$ mV. **b** | Current recorded in response to 250 ms voltage steps ranging from -120 to $+80$ mV. Holding potential = 0 mV. The internal pipette solution contained (in mM): 145 Cs-methanesulphonate, 8 NaCl, 1 MgCl₂, 10 HEPES, 10 EGTA; pH 7.2. The extracellular solution contained (in mM): 145 NaCl, 10 CaCl₂, 5 CsCl, 1 MgCl₂, 10 HEPES and 10 glucose; pH = 7.4. (Data reproduced with permission from REF. 46; © (2001) Macmillan Magazines Ltd.)

TRPV5 (ECaC1, CaT2) was isolated by expression cloning from rabbit kidney cell lines as a 730-amino-acid protein⁴⁰. It is only 30% identical to TRPV1, but is similar (66% identical) to TRPV6; indeed, many of its electrophysiological properties are indistinguishable from it. TRPV5 is present in kidney, small intestine and placenta. The expressed channel shows strong inward rectification, is highly Ca²⁺-selective ($P_{\text{Ca}}/P_{\text{Na}} > 100$), but exhibits a slightly different divalent selectivity profile, with Mn²⁺ being more permeant than Ba²⁺ or Sr²⁺ (REFS 41,42). In the absence of divalent cations, the conductance of TRPV5 increases; single channels with a conductance of ~ 75 pS are detected. Mg²⁺ and Ca²⁺ block the open channel in a fashion similar to TRPV6. These properties are consistent with proposed mechanisms for Ca²⁺-selective channel function in which negatively charged glutamic or aspartic acid residues provide a binding site for divalent cations within the pore⁴³. Indeed, mutation of a single amino acid — D542 — abolished Ca²⁺ permeation, Ca²⁺-dependent current decay and blockade by extracellular Mg²⁺ in TRPV5. Substitution by glutamic acid lowered the permeability of this channel, suggesting that both the charge and the side-chain length are important for Ca²⁺ binding. Two other charged residues in the pore do not have such effects, indicating that the single aspartic acid residue is the important binding site⁴⁴. The absence of this amino acid residue in TRPV1 might explain the ~ 20 -fold lower selectivity of the vanilloid receptor channel for Ca²⁺. The presence of two putative binding sites in voltage-gated Ca²⁺ channels may account for their even higher Ca²⁺ selectivity. Co-localization of TRPV5 with 1,25 dihydroxyvitamin D₃-dependent calbindin-D_{28K} in apical membranes of Ca²⁺-transporting epithelia suggests that this channel might be responsible for vitamin-D-responsive Ca²⁺ uptake in these cells. In patch-clamp recordings, TRPV5 appears to be constitutively active. Store-dependent activation of this channel has not been reported and its mechanism of activation is unknown.

TRPV6 (CaT1, ECaC2) was identified by expression cloning from rat duodenum using a Ca²⁺ screen⁴⁵. TRPV6 has a widespread tissue distribution and was found by polymerase chain reaction in a rat basophilic leukaemia mast cell line and in the human Jurkat T lymphocyte cell line. When expressed in mammalian cells, TRPV6 exhibited the unique biophysical properties of I_{CRAC}⁴⁶. Only voltage-gated Ca²⁺ channels and I_{CRAC} are selective enough to admit only a few monovalent cations per thousand Ca²⁺. Like I_{CRAC}, expressed CaT1 is highly Ca²⁺-selective ($P_{\text{Ca}}/P_{\text{Na}} > 100$), is activated by low [Ca²⁺]_i and inactivated by higher [Ca²⁺]_i. Unlike many other TRPs, TRPV6 gives a steeply inwardly rectifying current–voltage relationship identical to that of I_{CRAC}, passing most of its current at hyperpolarized potentials (FIG. 5). The channel was indistinguishable from I_{CRAC} in its relative selectivity to divalent cations, ANOMALOUS MOLE-FRACTION EFFECT, whole-cell current kinetics, blockade by low (10 μ M) concentrations of La³⁺, loss of selectivity in the absence of divalent cations, and single-channel conductance to Na⁺ in divalent-free conditions (~ 60 pS). Initial reports of TRPV6 did not reveal activation by store depletion. However, Yue *et al.*⁴⁶ assumed that TRPV6 did not house the signal transduction mechanism that senses store depletion itself, and that TRPV6 overexpression might not be matched by complete upregulation of the signal transduction apparatus. When TRPV6 was recorded at early times after transfection to limit the number of available channels, they found that approximately half the TRPV6 current was activated by Ins(1,4,5)P₃- and thapsigargin-mediated store depletion in the presence of a weak intracellular Ca²⁺ buffer. Yue and colleagues concluded that TRPV6 is likely to comprise part or the entire I_{CRAC} pore, but that other unidentified elements in the I_{CRAC} pathway remain to be identified. At present, it is fair to say that no TRP channel homologue is universally accepted as being the I_{CRAC} channel.

ANOMALOUS MOLE-FRACTION BEHAVIOUR

When two or more ions simultaneously reside inside a channel, their movement through the pore is dependent on each other. When channel conductance is measured as a function of the concentration ratio of two different ionic species and the conductance goes through a minimum rather than changing linearly as the ratio changes, then it is said to show anomalous mole-fraction behaviour.

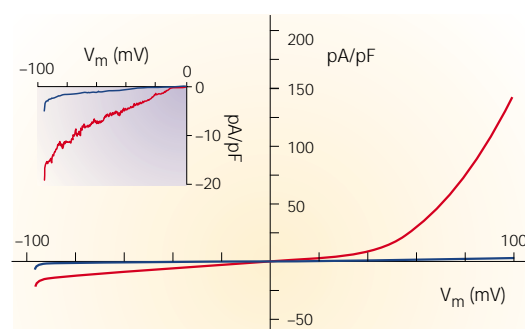


Figure 6 | Current–voltage relationship of the TRPM7 (ChaK1, TRP-PLIK) channel expressed in CHO-K1 cells. A 500 ms voltage ramp from -100 to $+100$ mV evoked the current in cells expressing TRPM7 (red), but not in control cells (blue). Inset shows expanded scale for the region negative to 0 mV. Holding potential = 0 mV. The internal pipette solution contained (in mM): 145 Cs-methanesulphonate, 8 NaCl, 5 ATP, 1 $MgCl_2$, 10 EGTA, 4.1 $CaCl_2$, 10 HEPES; pH 7.2. The extracellular solution contained (in mM): 140 NaCl, 5 CsCl, 2.8 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES and 10 glucose; pH 7.4. (See REF. 47.)

The TRPM family

The TRPM (long TRP, melastatin) family has eight members divided into four groups. Constituents of the first subgroup include the founding member TRPM1 (melastatin or LTRPC1) and TRPM3 (KIAA1616 or LTRPC3). TRPM7 (TRP-PLIK, ChaK(1)) and TRPM6 (ChaK2) belong to the second subgroup. TRPM2 (TRPC7 or LTRPC2) and TRPM8 (Trp-p8) form the third, and TRPM5 (Mtr1 or LTRPC5) and TRPM4 (FLJ20041 or LTRPC4) make up the fourth.

TRPM7 (transient receptor potential-phospholipase interacting kinase (TRP-PLIK)) has 1863 amino acid residues. It is expressed in brain, as well as in heart, kidney, liver and lung. Identified in a yeast two-hybrid screen as a protein interacting with PLC- β_1 , it is the first member of the TRPM group to be expressed as a functional ion channel⁴⁷ (FIG. 6). Like most TRP channels, TRPM7 expression yields a non-selective whole-cell current. Unique among ion channels, it is controlled by the activity of its own carboxy terminal kinase. This kinase is homologous to an unusual α -helical kinase (myosin heavy chain kinase B from *Dictyostelium discoideum*). When mutations designed to disrupt kinase activity were introduced into TRPM7, whole-cell currents were markedly decreased compared with controls. The role of the kinase domain in channel activation is supported by the dependence of the current on cytoplasmic Mg^{2+} and ATP levels. Hydrolysis-resistant analogues of ATP (ATP- γS and AMP-PNP) failed to elicit currents, establishing a role for ATP hydrolysis in controlling the channel (D.E.C. *et al.*, unpublished data). TRPM7 exhibits a steeply outwardly rectifying conductance when expressed in mammalian cells, permitting Na^+ and Ca^{2+} to pass through its pore ($P_{Na}/P_{Ca} = 3/1$). The channel is not blocked by 1 mM Ba^{2+} , 1 mM tetraethylammonium ion (TEA) or 0.2 mM Zn^{2+} , but La^{3+} (2 mM) blocked inward and outward currents by 97% and 37%, respectively. The single-channel slope conductance was 105 ± 8 pS at positive potentials.

Whereas TRPM7 is known to be controlled by its kinase domain, presumably through autophosphorylation, intriguing mysteries remain. Except for the association of the channel with PLC- β , the receptor or signal transduction pathway that activates the channel has not been identified. Perhaps most provocative is the possibility that the kinase domain might have additional intracellular substrates that link it to other signalling pathways. The identification of an alternatively spliced transcript of TRPM7 that contains the kinase domain alone (PLIK) raises the possibility that PLIK might regulate other TRP family members as well. With 2011 amino acid residues, TRPM6 or ChaK2 is the longest member of the TRP family. TRPM6 shares 60% sequence identity with TRPM7 and is predicted to be a transmembrane protein that also contains a putative α -kinase domain.

It is tempting to speculate that the long carboxy tail of the TRPM family imbues them with more than one function. TRPM2 (TRPC7 or LTRPC2) is a 1503-amino-acid protein that is highly expressed in fetal and adult brain⁴⁸. The channel is non-selective, with a linear current–voltage relationship and a single-channel slope conductance of 60 pS. A NUDT9 Nudix hydrolase family domain within the TRPM2 sequence indicates that the channel might be regulated by nucleoside diphosphates and, indeed, when HEK-293 cells expressing TRPM2 were perfused with 100 μM adenosine diphosphoribose (ADP-ribose), the cationic current increased⁴⁹. TRPM2 is also a bifunctional protein with a carboxy terminal NUDT9 domain conferring ADP-ribose pyrophosphatase activity. Nudix domains appear to monitor the concentrations of nucleoside diphosphate derivatives in cells⁵⁰. The discovery that ADP-ribose acts as a second messenger through its ability to gate TRPM2, leading to Ca^{2+} entry, raises interesting questions about the interplay between ADP-ribose and cyclic-ADP-ribose in Ca^{2+} signalling. The related TRPM8 or Trp-p8 is a protein with 41% sequence identity to TRPM2, without an apparent Nudix hydrolase motif⁵¹. It is a protein, the expression of which is normally restricted to the prostate, but which seems to be upregulated in prostate cancer and in other non-prostatic primary neoplasms.

TRPM1 has the distinction of being the first member of the TRP family whose gene product — melastatin — is trademarked. Differential mRNA analysis was employed to predict a 542-amino-acid soluble protein. It is expressed in the eye, and, to varying degrees, in metastatic murine B16 melanoma cell lines⁵². However, melastatin was not recognized as a member of the TRP family until cloning of the human homologue revealed a larger complementary DNA that encoded a protein product of 1533 amino acids with predicted transmembrane domains⁵³. Subsequent studies revealed that multiple short transcripts of human melastatin were present in human melanocytes and melanoma cell lines, whereas the full-length 5.4 kb mRNA transcript encoding TRPM1 was detected only in normal melanocytes⁵⁴. Duncan and her colleagues have pursued the link between TRPM1 and cancer,

BECKWITH-WIEDEMANN SYNDROME

Syndrome of unknown aetiology characterized by the presence of macroglossia (large tongue), visceromegaly (large organs), macrosomia (large body size) and hypoglycemia. Patients show an increased susceptibility to tumour development.

showing that melastatin gene expression correlated with cutaneous melanoma tumour progression, thickness and the potential for metastasis in normal skin, benign melanocytic naevi (moles), and primary cutaneous melanoma metastases⁵⁵. Downregulation of TRPM1 mRNA in the primary cutaneous tumour is a prognostic marker for metastasis in patients with localized melanoma⁵⁶. Treatment of pigmented melanoma cells with the differentiation-inducing agent hexamethylene bisacetamide upregulated the full-length transcript and inhibited growth⁵⁴. Whether the other members of the TRPM1 subgroup, TRPM7 (TRP-PLIK) and TRPM3 (KIAA1616) (~ 51% amino acid sequence identity), are also linked to cell growth and differentiation, or to cancer by their ionic conductance, is a compelling issue that remains to be explored.

Identified first by sequencing projects, the functions of TRPM3 (KIAA1616) and TRPM4 (FLJ20041) are unknown. TRPM5 or Mtr1, which forms the fourth TRPM subgroup together with TRPM4 (FLJ20041), was identified during functional analysis of the chromosomal region (11p15.5) associated with loss of heterozygosity in a variety of childhood and adult tumours. It is also associated with the cancer-predisposing BECKWITH-WIEDEMANN SYNDROME⁵⁷. TRPM5 is expressed in fetal heart, brain, liver and kidney, as well as in adult liver, kidney and lung. The human TRPM5 gene is transcribed into two splice forms coding for 872- and 1165-amino-acid proteins. Whether TRPM5 or the other TRPM family members — TRPM1 (melastatin), TRPM3 (KIAA1616), and TRPM4 (FLJ20041) — are ion channels endowed with bifunctional activities of their own remains to be seen.

Summary

TRP channels are a family of proteins that possess six membrane-spanning domains, and are expressed in low numbers per cell to yield small net inward currents. Many of them show no cation selectivity, similar to the nicotinic and glutamate-receptor channels. Evolution has tinkered with the pore to endow the Ca²⁺-selective TRPV5 and TRPV6 subtypes with one Ca²⁺ binding site per pore, but they show lower selectivity than the two-binding-site voltage-gated Ca²⁺ channels.

The TRP family is likely to be full of interesting surprises. At this time there is no unifying theme to their function or mechanism of activation. We propose that the TRPC subfamily comprises receptor-operated channels long recognized by physiologists (in invertebrate vision and agonist-mediated Ca²⁺ entry). Interestingly, heteromers of these channels show a current–voltage relationship that bears many similarities to those of glutamate receptors, allowing Ca²⁺ to enter cells and depolarize them. So far, they have not been identified at synapses, but few investigators have studied this aspect of their localization. The TRPV (osm-9-like/vanilloid TRPC) subfamily is the best characterized member of the group and includes ion channels that are certainly involved in neuronal pain pathways, perhaps to sense heat and osmolarity. The TRPM subfamily might be the most novel, with potential roles in Ca²⁺-dependent signalling, control of cell-cycle progression, cell division or cell migration.

Two issues confound our understanding of TRP channels. The first is that the family of proteins is large and widely distributed across a range of cell types, making it difficult to express confirmed monomeric channels. Several TRPs are known to form heteromultimers and their electrophysiological properties depend on the subunit composition. Second, the multipotent phosphatidylinositol pathway is certainly involved in TRP regulation, but the panoply of messages generated (Gα, Gβγ, inositol phosphates, PtdIns(4,5)P₂, Ca²⁺, DAG, phosphatidic acid, PtdIns(4)P congeners, fatty acids and their metabolites, and so on) and the store-depletion mechanism are closely intertwined and difficult to separate. We suspect that store-operated signalling is not a general mechanism underlying the regulation of TRP channels, but that the process of store depletion intersects with their normal signal transduction control.

Links

DATABASE LINKS [K_v](#) | [CNG](#) | [calmodulin](#) | [phospholipase C](#) | [Ins\(1,4,5\)P₃ receptor](#) | [TRPL](#) | [protein kinase C](#) | [PDGF](#) | [EGF](#) | [TRPC](#) | [VR-1](#) | [VRL-1](#) | [OTRPC4](#) | [ECaC1](#) | [ECaC2](#) | [melastatin](#) | [KIAA1616](#) | [ChaK\(1\)](#) | [ChaK2](#) | [Mtr1](#) | [FLJ20041](#) | [NUDT9](#)

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