

# TRANSIENT RECEPTOR POTENTIAL CHANNELS

The mammalian TRP (transient receptor potential) ion channels are named after the role of the channels in *Drosophila* phototransduction. They are encoded by at least 21 channel subunit genes. The TRP channel primary structures predict six transmembrane domains (6TM) with a pore domain between the fifth (S5) and sixth (S6) segments, and both C- and N-termini located intracellularly. This architecture is a common theme

for hundreds of ion channels present in life forms ranging from bacteria to mammals. The mammalian TRP channel family – comprising three subfamilies TRPC, TRPV and TRPM – is united primarily by structural homology within the transmembrane domains (Figure 1), but overall sequence identities between members can be as low as 25%. Other features include a 25-amino acid (aa) motif (the TRP domain) containing a

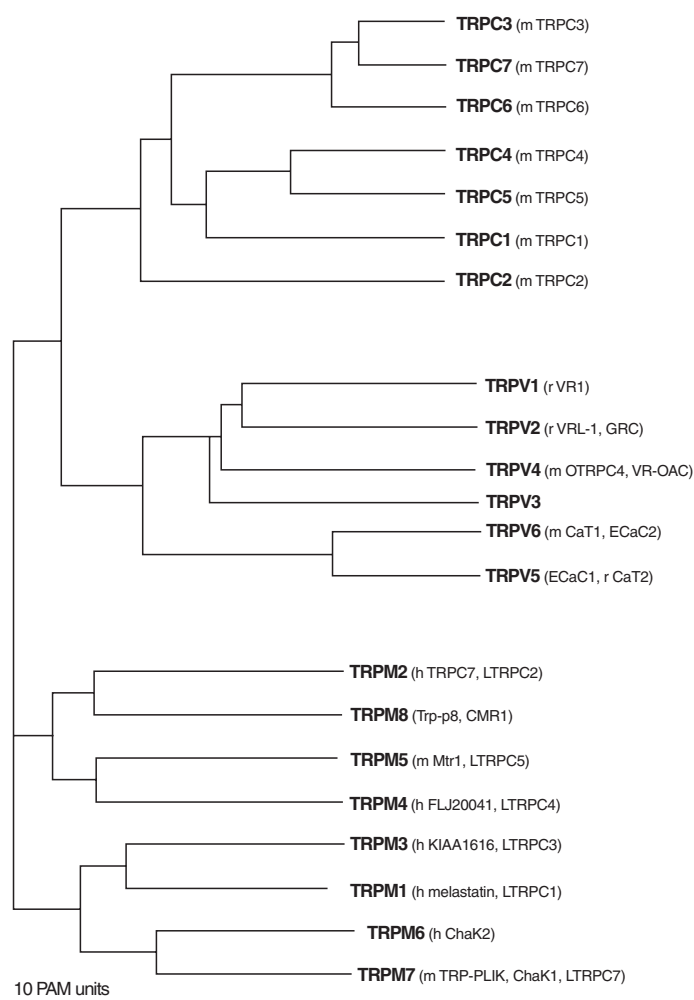


Figure 1. **Phylogenetic relationship in the TRP protein family.** The evolutionary tree was calculated by the neighbour-joining method<sup>53</sup>. The evolutionary distance is shown by the total branch lengths in point accepted mutations (PAM) units, which is the mean number of substitutions per 100 residues. The recommended nomenclature is in bold, with alternative or previous nomenclature between parentheses. The corresponding GenBank accession numbers for the proteins are: melastatin (NM\_002420), ChaK (NP\_067425), TRP-PLIK (AF375874), KIAA1616 (BAB13442), LTRPC2 (NP\_003298), Mtr1 (AAF98120), r TRPM8, CMR1 (AY072788), FLJ20041 (NP\_060106), TRPC1 $\alpha$  (AAB50622), TRPC2 $\alpha$  (AAG29950), TRPC3 (NP\_06283), TRPC4 (AAC05179), TRPC5 (AAC13550), TRPC6 (AAC06146), TRPC7 (AAD4206), m OTRPC4 (AAG17543), r VR1 (T09054), m VR1-L (NP\_035836), m CaT1 (BAA99538), r ECaC (BAA99541).

TRP box (EWKFAR) just C-terminal to S6. The TRP domain and box are present in all TRPC channel genes, but not in all TRP channel genes. The N-terminal cytoplasmic domains of TRPC and TRPV channels contain ankyrin repeats, while those of the TRPC and TRPM channels contain proline-rich sequences in the region just C-terminal to the predicted S6 (Figure 2). At present, no one feature other than overall 6TM architecture and homology defines the TRP family. Thus, it is predicted that the definition of TRP channels will evolve as functions and structures are clarified.

Genes for the TRP ion channel subunits were first defined in the *Drosophila* visual system. In the *trp* mutation, the light response (receptor potential) decays during prolonged exposure to light. TRP-deficient flies are blinded by intense light because sustained  $\text{Ca}^{2+}$  entry via TRP ion channels and subsequent  $\text{Ca}^{2+}$ -dependent adaptation is disrupted. Three genes (*TRP*, *TRPL*, *TRP $\gamma$* ) in *Drosophila* encode TRP channels that mediate fly vision and other unknown functions. Genetic approaches in flies have not resolved the mechanism of TRP activation, but confirm the importance of phospholipase C $\beta$  (PLC $\beta$ ) and other components of the phosphatidylinositol pathway<sup>1-4</sup>.

### Structural features

The high resolution structure of a 6TM channel is not yet available. However, the 2TM structure of a bacterial  $\text{K}^+$  channel (KcsA) is analogous to the S5 and S6 domains joined by a short pore  $\alpha$  helix of the 6TM architecture<sup>5</sup>. The KcsA channel is a tetramer of 2TM  $\alpha$  helices. The helices corresponding to S5 face the lipid membrane while the helices corresponding to S6 line the pore. At both inner and outer membrane faces, layers of aromatic amino acids form a cuff around the

pore. In KcsA the selectivity filter is a narrow region near the outer face of the membrane lined by the carbonyl backbone of five conserved amino acids. These amino acids are not present as a group in the largely non-selective TRP channels. In KcsA, rings of carbonyl oxygens act as surrogate waters to coordinate the dehydrated  $\text{K}^+$  ions in the channel. The rest of the S5 and S6 spanning regions are likely to be analogous to KcsA, in which the narrow channel in the selectivity filter rapidly broadens in hourglass fashion. The four short pore  $\alpha$  helices focus their helix dipole negative electrostatic fields on the cavity to shield the cation from the hydrophobic lipid environment. The S6 base lines the rest of the channel on its way to the cytoplasm. The S6 segment and the C-terminal amino acids extending into the cytoplasm, are where the interesting gating features of TRP channels are likely to emerge. The most conserved regions between the three families are in the S6 domain.

The detailed structure of the S1–S4 segments of the 6TM channels is not available, but mutagenesis data provide some clues about their functions. The S4 segment in voltage-sensitive channels contains at least four charged arginine or lysine residues that convey voltage changes across the membrane into movement of the helix, somehow gating the pore by twisting this S4 helix. The TRP channels are very weakly voltage-dependent and lack the full complement of charged amino acids in the S4 domain.

### Functional features

Of the functionally expressed clones so far (19 of the 21), only TRPV5 (also called CaT2, ECaC1) and TRPV6 (also called CaT1, ECaC2) are relatively  $\text{Ca}^{2+}$ -selective. To date, no currents have been reported from TRPM3 and TRPM5 expression in heterologous systems.

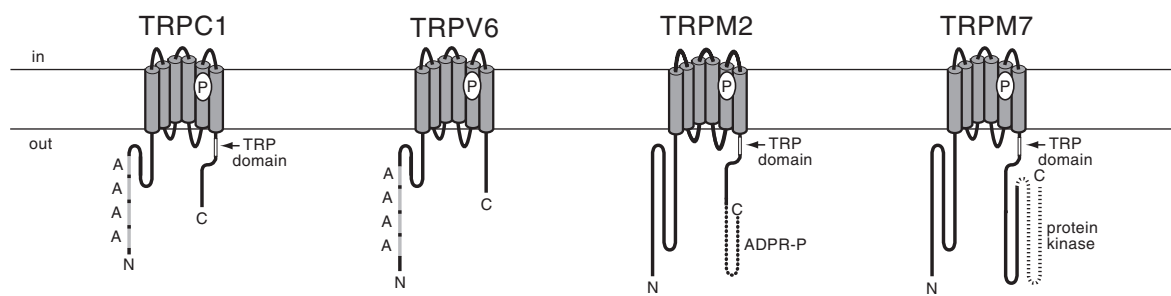


Figure 2. **Architecture of TRP channels.** Schematic diagram showing the common structure with the broad class of 6-transmembrane domain ion channels. S1–S6, transmembrane segments.

The TRP channels do not have the sharp voltage-sensitivity of the characterised channels in the  $\text{Ca}_v$  and  $\text{Na}_v$  families (Figure 3). Of the functionally expressed TRP channels, all but TRPV5 and TRPV6 are relatively non-selective to cations, but TRPM4 is selective to monovalent cations. Thus, upon opening, they depolarise cells from their resting membrane potentials (approximately  $-70\text{mV}$  in most mammalian cells) to around  $0\text{mV}$ . In short, they depolarise cells and raise intracellular  $\text{Na}^+$  and, usually,  $\text{Ca}^{2+}$ .

Two common signal transduction pathways that regulate the release of intracellular  $\text{Ca}^{2+}$  are the G protein-coupled and the tyrosine kinase activation of phospholipase C (PLC). PLC hydrolyses phosphatidylinositol 4,5 bisphosphate ( $\text{PIP}_2$ ) to form inositol (1,4,5) trisphosphate ( $\text{IP}_3$ ) that opens the  $\text{IP}_3$  receptor, and liberates  $\text{Ca}^{2+}$  from the endoplasmic reticulum (ER)<sup>6</sup>. Accompanying these chains of events, and not necessarily linked to  $\text{Ca}^{2+}$  store (ER) depletion, is activation of the TRP channels. The details of these mechanisms are incompletely understood at present. The strongest associations between

the phosphatidylinositol pathway and TRP channels involve  $\text{PLC}\beta$  and  $\text{PIP}_2$ . Based on *Drosophila* TRP channels, elements of these signal transduction pathways are linked by scaffolding proteins<sup>7</sup>.

It has been proposed that emptied  $\text{Ca}^{2+}$  stores (ER) somehow gate  $\text{Ca}^{2+}$  entry of external  $\text{Ca}^{2+}$  in order to replenish the deficit<sup>8</sup>. The physiological hallmark of the store-operated,  $\text{Ca}^{2+}$  entry process is a large receptor-mediated transient  $[\text{Ca}^{2+}]_i$  increase followed by a prolonged high  $[\text{Ca}^{2+}]_i$  plateau phase dependent on  $[\text{Ca}^{2+}]_o$ . A very specific and highly  $\text{Ca}^{2+}$ -selective current (the calcium-release activated current;  $I_{\text{CRAC}}$ ) is activated by a variety of store-depletion protocols in whole-cell recordings from single blood cells<sup>9,10</sup>, but store-operated entry may not be solely through  $I_{\text{CRAC}}$ . From their early identification, TRP channels have been the major suspects for the store-operated channel(s), including mediation of  $I_{\text{CRAC}}$ . At odds with this supposition is the high  $\text{Ca}^{2+}$ -selectivity of  $I_{\text{CRAC}}$  compared to the cationic non-selectivity of most of the TRP channel family.

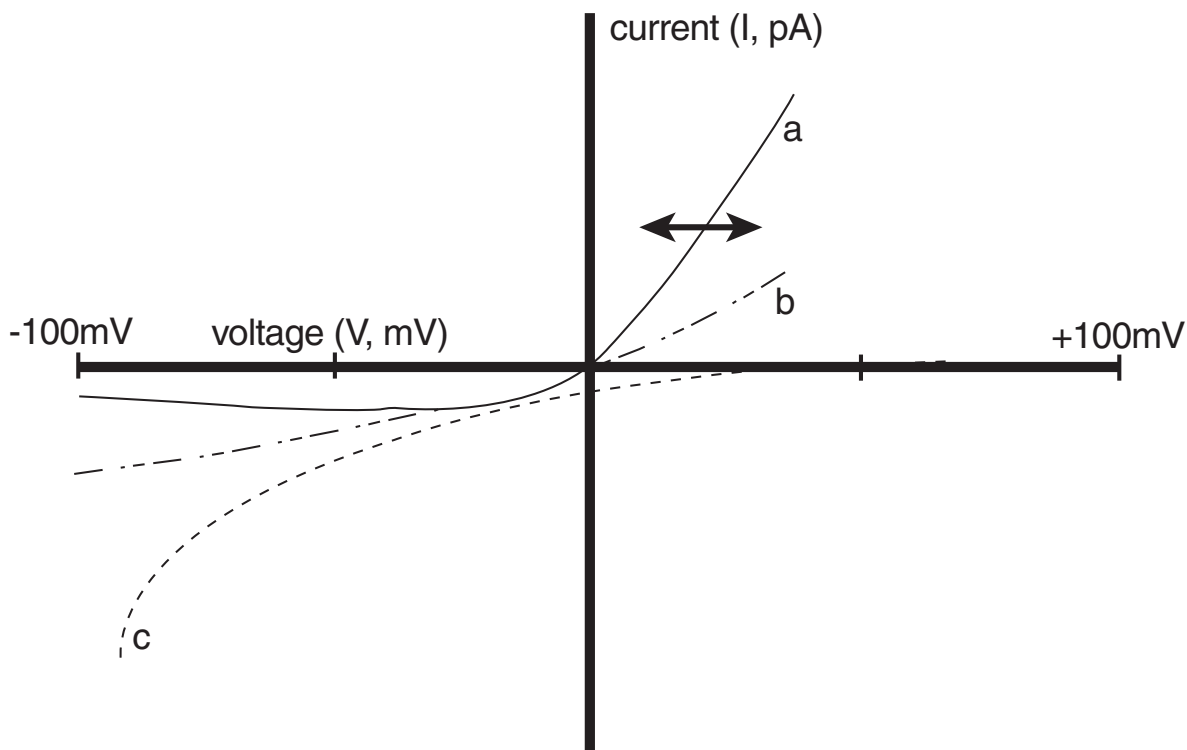


Figure 3. **Current-voltage (I-V) relationships of TRP channels.** The different I-V relationships are shown on the three curves. a, TRPC1, TRPC4, TRPC5, TRPM7, TRPM8; b, TRPC3, TRPC6, TRPV1, TRPV4; c, TRPV5, TRPV6.

### Classification and nomenclature

In this section of the compendium, the focus is on the TRP channels encoded by mammalian genes and the rational, but preliminary, nomenclature system adopted by a number of workers in the field<sup>3,4,11</sup>. The three subfamilies of the TRP channels are named TRPC1 etc. (corresponding to the earlier short, C, denomination), TRPV1 etc. (from the vanilloid, osm-9-like channels), and TRPM1 etc. (for the melastatin, long family).

### The TRPC channels

The TRPC family can be divided into four subgroups: TRPC1; TRPC4 and TRPC5; TRPC3, TRPC6, and TRPC7; and TRPC2 – by sequence homology as well as functional similarities. TRPC1 was the first member of the mammalian TRP family purported to form an ion channel<sup>12</sup>. Given the widespread expression of TRPC1 and its ability to coassemble with other TRPC subunits<sup>13–15</sup>, TRPC1 might be a component of different heteromeric TRP complexes. Whether TRPC1 can form functional channels in the absence of other TRP subunits is not established.

The second TRPC subgroup most closely related to TRPC1 is comprised of TRPC4 and TRPC5. Murine TRPC4 and TRPC5 can form homomeric cation channels that are activated following stimulation of G<sub>q</sub>-coupled receptors<sup>16,17</sup> as well as receptor tyrosine kinases (RTKs)<sup>17</sup>. Co-expression of TRPC1 and TRPC4 or TRPC5 results in a novel non-selective cation channel with a voltage-dependence similar to NMDA receptor channels, but unlike that of any reported TRP channel. The details of the activation mechanism remain elusive but the two primary products of PLC enzyme activity, IP<sub>3</sub> and DAG, do not activate TRPC4 and TRPC5 (refs. 17,18). Both TRPC4 and TRPC5 contain a C-terminal PDZ-binding motif (VTTRL) not present in other TRP channels. PDZ domain scaffolding proteins such as the Na<sup>+</sup>/H<sup>+</sup> exchanger regulatory factor (NHERF) as well as signalling molecules such as PLCβ1, co-immunoprecipitate with TRPC4 and TRPC5 (ref. 19), indicating that the channels may be part of multimolecular signalling complexes similar to the signalplex or transducisome of *Drosophila* photoreceptors.

TRPC3, TRPC6 and TRPC7 are ~75% identical and when expressed constitute a non-selective cation current that rectifies in both the inward (negative voltages) and outward (positive voltages) directions. Similar to TRPC3, TRPC6 and TRPC7 are inwardly and outwardly rectifying, have relatively low selectivity for Ca<sup>2+</sup> over Na<sup>+</sup>,

are sensitive to intracellular Ca<sup>2+</sup>, and are activated by DAG (refs. 18,20). Their relatively high expression levels in smooth muscle and heart cells make them promising candidates for the as yet molecularly undefined non-selective cation channels in these muscle cells. In support of this idea is the finding that TRPC6 is an essential part of the α<sub>1</sub>-adrenoreceptor-activated cation channel in rabbit portal vein myocytes<sup>21</sup>.

Less information is available about TRPC2, which shares about 30% sequence identity with the TRPC3, TRPC6, and TRPC7 subfamilies. Full length TRPC2 mRNA and several N-terminal splice variants have been found in mouse and rat tissue, but TRPC2 appears to be a pseudogene in humans<sup>22–25</sup>. TRPC2 protein was localised to neuronal micovilli in rat vomeronasal organ<sup>22</sup> and in the head of mouse sperm<sup>26</sup>. TRPC2-deficient mice display abnormal mating behaviour, consistent with a role for this channel in pheromone signalling.

### The TRPV channels

Currently the TRPV channel subfamily has six members divided into three groups. TRPV1 and TRPV2 are the vanilloid receptors and vanilloid-like receptors, VR-1 and VRL-1, respectively. TRPV4 is the osm-9 like OTRPC4, and TRPV5 and TRPV6 are the Ca<sup>2+</sup>-selective channels, ECaC1 or CaT2 (from epithelial calcium channel or calcium transporter), and ECaC2 (also called CaT1).

The vanilloid receptors are the best understood ion channels in this class<sup>27</sup>. The TRPV1 channel is activated by the 'hot' pepper-derived, vanilloid compound capsaicin<sup>28</sup>, but is not activated by store depletion. The expressed capsaicin receptor is a relatively Ca<sup>2+</sup>-selective ion channel with an outwardly rectifying current-voltage (I-V) relation and exhibits Ca<sup>2+</sup>-dependent desensitisation. Endogenous cannabinoid receptor ligands, such as anandamide, are potential TRPV1 agonists. The exact mechanism of TRPV1 activation is not completely understood, but it is sensitive to temperature (≥ 43°C) and lipids in a membrane-delimited fashion. The size of its current is increased by acid pH and is modulated by intracellular PIP<sub>2</sub>, which appears to inhibit the channel<sup>29</sup>. Experiments using TRPV1 knock-out mice confirm that it is essential for transducing the nociceptive, inflammatory, and hypothermic effects of vanilloid compounds and contributes to acute thermal nociception and thermal hyperalgesia following tissue injury.

The vanilloid receptor-like channel, TRPV2 (VRL-1) is 50% identical to TRPV1, but is

insensitive to capsaicin<sup>30</sup>. Like TRPV1 it is more permeable to Ca<sup>2+</sup> than Na<sup>+</sup> ( $P_{Ca}/P_{Na} = 3/1$ ) and is outwardly rectifying. It has been proposed to mediate high threshold (greater than 52°C) noxious heat sensation, perhaps in the lightly myelinated A $\delta$  nociceptors, but its presence in nonsensory tissue suggests other functions as well.

A new member of the vanilloid channel family, human TRPV3 (hTRPV3) is expressed in skin, tongue, dorsal root ganglion, trigeminal ganglion, spinal cord, and brain. Increasing temperature from 22°C to 40°C in mammalian cells transfected with hTRPV3 elevates intracellular calcium by activating a non-selective cationic conductance. As in sensory neurones, the current is steeply dependent on temperature, sensitises with repeated heating, and displays a striking hysteresis on heating and cooling. Based on these properties, TRPV3 is thermosensitive in the physiological range of temperatures between TRPM8 and TRPV1.

TRPV4 (OTRPC4, VR-OAC) is ~40% identical to TRPV1 and TRPV2 (refs. 31,32). When expressed in mammalian cells it comprises a moderately selective cation channel ( $P_{Ca}/P_{Na} = 6$ ), which – like TRPV1 – displays a gently outwardly rectifying I–V relation. In isotonic media, TRPV4 was active but the current was further increased by reduction of extracellular osmolality (cell swelling) with 50% activation by 270mosmol/l (physiological = 290mosmol/l). Hypertonic media (cell shrinking) decreased current activation. Deletion of the ankyrin repeat domains blunted the response to low osmolar solutions<sup>32</sup>. Store depletion did not activate the channel.

TRPV5 (ECaC, CaT2) is a 730aa protein<sup>33</sup>. It is only 30% identical to TRPV1, but is similar to TRPV6 (66% identical) and indeed many of its electrophysiological properties are indistinguishable. The expressed channel strongly inwardly rectifies, is relatively highly Ca<sup>2+</sup>-selective ( $P_{Ca}/P_{Na} > 100$ )<sup>34,35</sup>. These properties are consistent with proposed mechanisms for Ca<sup>2+</sup>-selective channels in which negatively charged glutamic or aspartic acid residues provide a binding site for divalents within the pore<sup>36</sup>. Store-dependent activation of this channel has not been reported and its mechanism of activation is unknown.

TRPV6 has a wide tissue distribution. Expressed TRPV6 is Ca<sup>2+</sup>-selective ( $P_{Ca}/P_{Na} > 100$ ), is activated by low levels of intracellular Ca<sup>2+</sup>, and inactivated by higher [Ca<sup>2+</sup>]<sub>i</sub>. Unlike many other TRP channels, TRPV6 displays a steeply inwardly rectifying I–V relation, passing most of its current at hyperpolarised potentials<sup>37</sup>.

### The TRPM channels

The TRPM subfamily 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), LTRPC7) and TRPM6 (ChaK2) belong to the second group. TRPM2 (TRPC7 or LTRPC2) and TRPM8 (Trp-p8 or CMR1) form the third group. TRPM5 (Mtr1 or LTRPC5) and TRPM4 (FLJ20041 or LTRPC4) constitute the fourth.

TRPM7, which has 1863 amino acid residues, was identified in a yeast two-hybrid screen as a protein interacting with PLC $\beta_1$ , and is the first member of the TRPM group to be expressed as a functional ion channel<sup>38</sup>. It appears to be ubiquitously expressed. Unique among ion channels, it is also a protein kinase, but besides TRPM7 itself, its substrates are unknown. The structure of the C-terminal kinase domain has been determined<sup>39</sup>. Surprisingly, although the kinase domain for TRPM7 has little sequence similarity to conventional protein kinases, its structure resembles that of many eukaryotic protein kinases (e.g. cAMP-dependent protein kinase) with the notable exception of having its own zinc-finger domain. TRPM7 exhibits a steeply outwardly rectifying conductance when expressed in mammalian cells ( $P_{Ca}/P_{Na} = 3/1$ ). TRPM7 is inhibited by intracellular magnesium (at concentrations above 1mM)<sup>40</sup>. Although the mechanism of activation of TRPM7 is unknown, receptor-mediated activation of PLC by hormones or growth factors inhibits channel activity by hydrolysing and reducing local PIP<sub>2</sub> concentrations<sup>41</sup>. TRPM6 is the longest member (2011aa) of the TRP channel family, and both TRPM7 and TRPM6 are members of the alpha-kinase family<sup>42</sup>. TRPM6 shares 60% sequence identity with TRPM7 and is predicted to be a transmembrane protein that also contains a putative kinase domain.

TRPM2 is a 1503aa protein that is highly expressed in brain<sup>43</sup>. The channel is non-selective and displays a linear I–V relation. A NUDT9 Nudix hydrolase family domain within the TRPM2 sequence suggests that the channel may be regulated by nucleoside diphosphates and indeed, when HEK-293 cells expressing TRPM2 were perfused with adenosine diphosphoribose (ADP-ribose) or  $\beta$ NAD, cationic current increased<sup>44,45</sup>. TRPM2 is also a bifunctional protein whose C-terminal NUDT9 domain confers ADP-ribose hydrolase activity. The channel is regulated by signalling pathways responsive to

H<sub>2</sub>O<sub>2</sub> and TNF- $\alpha$ , suggesting that is physiological role may be as a sensor of redox status in cells<sup>46</sup>.

TRPM8 is a 1104aa protein that does not appear to contain associated enzymatic domains. TRPM8 is non-selective, outwardly rectifying channel that can be activated by cold (8–28°C) or cooling compounds such as menthol. This channel is expressed in small-diameter primary sensory neurones, where it presumably functions as a thermosensor<sup>47,48</sup>. TRPM8 is also expressed in prostate epithelium, where its physiological function is unknown<sup>49</sup>.

TRPM1 is a 1533aa protein<sup>50</sup>. Down-regulation of TRPM1 mRNA in the primary cutaneous tumour is a prognostic marker for metastasis in patients with localised melanoma<sup>51</sup>. TRPM1, as well as TRPM4, has been expressed and shown to promote Ca<sup>2+</sup> influx in heterologous systems<sup>52</sup>. Moreover, TRPM1 has been proposed to be regulated through direct interaction with a cytosolic isoform generated by alternative RNA splicing<sup>52</sup>.

Identified first by sequencing projects, the function of TRPM3 is unknown. TRPM4 is a Ca<sup>2+</sup>-activated, non-selective cation channel that mediates cell membrane depolarisation. TRPM5, which forms the third TRPM group with TRPM4,

was identified during functional analysis of the chromosomal region (11p15.5) associated with loss of heterozygosity in a variety of childhood and adult tumours.

### In summary

The TRP channels are a family of 6TM proteins expressed in low numbers per cell to yield small net inward currents. At this time there is no unifying theme in their function or mechanism for activation. The TRPV subfamily is the most well-characterised of the group and includes ion channels that are certainly involved in neuronal pain pathways, perhaps to sense heat and osmolarity. The TRPM subfamily may well be the most novel, with potential roles in Ca<sup>2+</sup>-dependent signalling, control of cell cycle progression, division or migration, and thermosensation. TRP proteins are common in many cell types, making expression of confirmed monomeric channels difficult. Several TRP channel proteins are known to form heteromultimers and their electrophysiological properties depend on the subunit composition. The multipotent phosphatidylinositol pathway is involved in most TRP regulation, but the details of this regulation are just beginning to be elucidated.

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