TRP channels as cellular sensors

David E. Clapham

Howard Hughes Medical Institute, Pediatric Cardiology, Children's Hospital of Boston, Department of Neurobiology, Harvard Medical School, Enders 1309, 320 Longwood Avenue, Children's Hospital, Boston, Massachusetts 02115, USA

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TRP channels are the vanguard of our sensory systems, responding to temperature, touch, pain, osmolarity, pheromones, taste and other stimuli. But their role is much broader than classical sensory transduction. They are an ancient sensory apparatus for the cell, not just the multicellular organism, and they have been adapted to respond to all manner of stimuli, from both within and outside the cell.

The human genome encodes hundreds of channels that broker the passage of charged ions across impermeable lipid bilayers[1]. While energy-requiring pumps labour to build charge and concentration gradients across the membrane, ion channels spend this stored energy, much as a switch releases the electrical energy of a battery. Small conformational changes cause channels to open, allowing over ten million ions to flow per second through each channel. Ca^{2+} ions are particularly important in cellular homeostasis and activity, and the surface of each cell holds thousands of channels that precisely control the timing and entry of Ca^{2+} ions.

Transient receptor potential (TRP) channels were first described in *Drosophila*, where photoreceptors carrying *trp* gene mutations exhibited a transient voltage response to continuous light[2,3]. Unlike most ion channels, TRP channels are identified by their homology rather than by ligand function or selectivity, because their functions are disparate and often unknown. They have been called store-operated channels (SOCs), but this description is theoretical and related to a poorly understood phenomenon.

The known functions are diverse. Yeast use a TRP channel to perceive and respond to hypertonicity[4,5]. Nematodes use TRP channels at the tips of neuronal dendrites in their 'noses' to detect and avoid noxious chemicals[6]. Male mice use a pheromone-sensing TRP channel to tell males from females[7]. Humans use TRP channels to appreciate sweet, bitter and umami (amino acid) tastes[8], and to discriminate warmth, heat and cold. In each of these cases, TRPs mediate sensory transduction, not only in a classical sense, for the entire multicellular organism, but also at the level of single cells. Almost all mammalian TRP channel genes are now known. Here I summarize the common characteristics of the diverse mechanisms of TRP channel activation, highlighting major questions that remain to be answered. More details can be found in other reviews[9–15].

What are TRP channels?

Mammalian TRP channels comprise six related protein families with sequence identity as low as 20% (Fig. 1). All TRP channels are putative six-transmembrane (6TM) polypeptide subunits that assemble as tetramers to form cation-permeable pores (Box 1). In general, they are almost ubiquitously expressed and most have splice variants. So most cells have a number of TRP channel proteins.
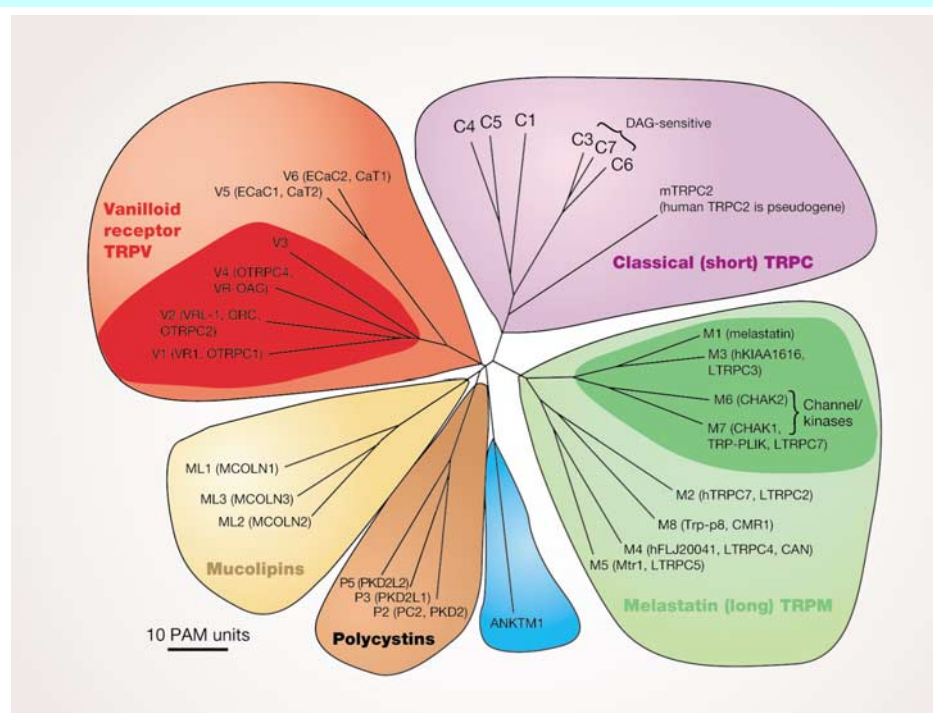
Figure 1 Mammalian TRP family tree. The evolutionary distance is shown by the total branch lengths in point accepted mutations (PAM) units, which is the mean number of substitutions per 100 residues.

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It will take time to elucidate all of the diverse functions of TRP proteins. To date, the most informative approaches have been ligand-specific expression cloning, and targeted (global) gene inactivation in mice. In the near future, further information is likely to be gleaned from tissue-specific and developmental-stage-specific gene targeting.

The TRPC (canonical TRP) subfamily

All mammalian TRPC proteins appear to be analogous to the TRP involved in *Drosophila* phototransduction, in that they function as receptor-operated channels. They are activated by stimulation of G-protein-coupled receptors (GPCRs) and receptor tyrosine kinases (Box 2).

TRPC1, the first mammalian TRP reported[16], forms heteromeric channels with TRPC4 and/or TRPC5 (ref. 10). The properties of the heteromultimers are distinct from those of TRPC4 and TRPC5 homomultimers (see Table 1 and Fig. 2). TRPC5, but not TRPC1, is present in hippocampal growth cones and modulates neurite

extension[17]. Mice lacking TRPC4 have defects in agonist-induced vasoregulation and lung microvascular permeability[18,19].

TRPC3, TRPC6 and TRPC7 proteins share ~75% identity, have relatively low selectivity for Ca^{2+} over Na^+, and are sensitive to the intracellular concentration of Ca^{2+} ($[Ca^{2+}]_i$). Diacylglycerol (DAG) analogues[20] potentiate their activity, but not through protein kinase C activation. TRPC3 has been investigated extensively as a putative inositol-1,4,5-trisphosphate ($InsP_3$) receptor (IP_3R)-binding SOC, with conflicting results[21]. All of the TPRC3, TRPC6 and TRPC7 subfamily are highly expressed in smooth and cardiac muscle cells, making them candidates for the receptor-activated nonselective cation channels known to exist in these sites. In support of this idea, TRPC6 is an essential part of the α_1-adrenoreceptor-activated cation channel in rabbit portal vein myocytes[22]. They may also have roles in the regulation of vascular tone, airway resistance and cardiac function.

TRPC2 appears to be a pseudogene in humans, but its rat
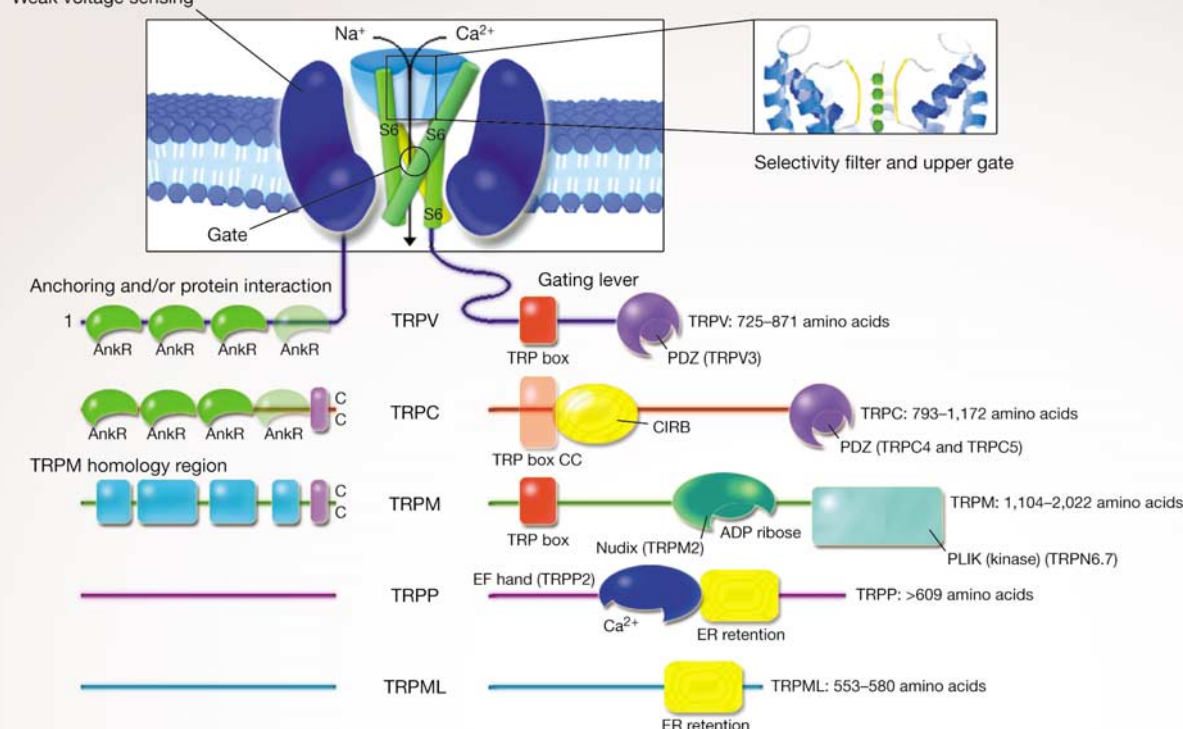

Box 1
TRP channel architecture

Life-forms, from bacteria to all higher plants and animals, use 6TM (S1–S6)-containing ion channels to sense and respond to stimuli. The 6TM element is one of four similar or identical subunits presumed, by analogy to K^+ channels[97], to surround the central pore. The gate and selectivity filter are formed by the four 2TM elements (S5–pore loop–S6) facing the centre of the channel. Cations are selected for permeation by the extracellular-facing pore loop, held in place by the S5 and S6 α-helices. All TRP channels are nonselective with $P_{Ca}/P_{Na} \leq 10$, with the exception of the monovalent-selective TRPM4 and TRPM5, and the Ca^{2+}-selective TRPV5 and TRPV6.

The cytoplasmic ends of the S6 helices form the lower gate, which opens and closes to regulate cation entry into the channel. The selectivity filter itself may also gate. The S1–S4 domain may flex relative to S5–S6 in response to stimuli, but the paucity of positively charged arginines in TRP S4 helices indicates weak voltage sensitivity. All elements outside the S5–S6 region provide means of either subunit

association or act as linkers to elements that control gating.

Box 1 figure emphasizes the diversity of TRP cytoplasmic domains. The selectivity filter (light blue and inset) is formed by amino acids that dip into the bilayer (pore loops), one contributed from each of the four subunits. S5 has been removed to emphasize the link between the S6 gating helix and the TRP C-terminal polypeptide chain. The TRP box is EWKFAR in TRPC, but is less conserved in TRPV and TRPM. CC indicates a coiled-coil domain. Ankyrin repeats (AnkR) range from 0 to 14 in number (3 or 4 in TRPV and TRPC, 14 in ANKTM; not shown). Numbers on the right indicate range in length. CIRB, putative calmodulin- and IP_3R-binding domain; EF hand, canonical helix–loop–helix Ca^{2+}-binding domain; PDZ, amino acids binding PDZ domains; PLIK, phospholipase-C-interacting kinase, an atypical protein kinase intrinsic to the TRPM6 and TRPM7 polypeptide chains; Nudix, NUDT9 hydrolase protein homologue binding ADP ribose. The function of the TRPM homology region is not known. Domains not drawn to scale.

S1–S4 transmembrane domains
Weak voltage sensing

Selectivity filter and upper gate

Anchoring and/or protein interaction

Gating lever

TRPV: 725–871 amino acids
TRPC: 793–1,172 amino acids
TRPM: 1,104–2,022 amino acids
TRPP: >609 amino acids
TRPML: 553–580 amino acids

orthologue encodes an important sensor localized to neuronal microvilli in the vomeronasal organ[23]. Trpc2-deficient mice display abnormal mating behaviour, consistent with a role for this channel in pheromone signalling[7].

The TRPV (vanilloid receptor, osm9-like) subfamily

TRPV1 was identified by expression cloning using the 'hot' pepper-derived vanilloid compound capsaicin as a ligand. TRPV1 is a Ca^{2+}-permeant channel[24,25] that is potentiated by heat ($>43\,°C$) and decreased pH, and inhibited by intracellular phosphatidylinositol-4,5-bisphosphate (PIP_2)[24,26]. Its thermal sensitivity is enhanced by bradykinin and nerve growth factor, which appear to act via phospholipase C (PLC) to hydrolyse PIP_2, releasing inhibition of the channel[26]. $Trpv1^{-/-}$ mice are defective in nociceptive, inflammatory and hypothermic responses to vanilloid compounds, supporting the interpretation that TRPV1 contributes to acute thermal nociception and hyperalgesia after tissue injury[27]. TRPV1 also participates in mechanically evoked purinergic signalling by the bladder urothelium[28].

TRPV2, which is 50% identical to TRPV1 (ref. 29), may mediate high-threshold ($>52\,°C$) noxious heat sensation, perhaps through lightly myelinated Aδ nociceptors. Interestingly, TRPV2 translocates from intracellular pools upon insulin growth factor stimulation of transfected cells[30]. Stretch reportedly increases TRPV2

translocation, and cardiac-specific transgene expression of TRPV2 results in Ca^{2+}-overload-induced cardiomyopathy[31]. But it is not surprising that overexpression of a Ca^{2+}-permeant channel induces cardiomyopathy, because such TRP channels are deleterious to many cells, including neurons. In fact, the mechanism of pain-relieving topical capsaicin is due, in part, to neuronal cell death.

Increased temperature also activates TRPV3 ($>31\,°C$)[32–34] and TRPV4 ($>25\,°C$)[35]. The neuronal distribution of TRPV3 overlaps with TRPV1, raising the interesting possibility that they may heteromultimerize[32]. TRPV3 is also highly expressed in skin, tongue and the nervous system, possibly explaining the activity of 'warm-sensitive' neurons. The effect of temperature on rates of biological processes is expressed as the $10\,°C$ temperature coefficient[1]: $Q_{10} = rate(T + 10\,°C)/rate(T)$. Most ion channels and enzymes have gating Q_{10} values of 3–5, but the Q_{10} of TRPV3 gating is >20 (ref. 33), and for TRPV1 and TRPV4 it is estimated to be ~10–20 (ref. 36). TRPV4 current is potentiated by hypotonicity (cell swelling)[37,38]. $Trpv4^{-/-}$ mice have a marginally impaired renal response to hypertonicity, probably due to abnormal central control of antidiuretic hormone secretion[39]. Hypotonicity increases TRPV4-mediated current in primary afferent nociceptive nerve fibres, an effect that is enhanced by the hyperalgesic inflammatory mediator prostaglandin E_2 (ref. 40). Expressed TRPV4 may be gated by epoxyeicosatrienoic acids[41].

Box 2
Canonical TRP signal transduction

TRP channels are activated primarily by signal transduction pathways. A common pathway that is well established for *Drosophila* TRP activation is outlined in the Box 2 figure (for a review, see ref. 11). In mammalian cells, a GPCR (for example, muscarinic M1 acetylcholine receptor) catalyses G protein nucleotide exchange to form active Gα and Gβ-γ subunits, in turn activating PLCβ. Alternatively, tyrosine kinase (TK) receptors activate PLCγ. PLC hydrolyses an abundant membrane component, PIP_2, into soluble and lipophilic messengers. Diacylglycerol, one product of PIP_2 hydrolysis, remains in the membrane. Soluble $InsP_3$ activates the IP_3R on the endoplasmic reticulum to release intracellular Ca^{2+}. The inset to the Box 2 figure shows PIP_2 (yellow) with its charged head group protruding above the bilayer (cytoplasmic side shown). A hydrophobic peptide (green) with interspersed basic amino acid residues sequesters PIP_2. The $+25\,mV$ electrostatic potential for the peptide (blue lines) and the $-25\,mV$ electrostatic potential for PIP_2 (red lines) are shown[82] (inset courtesy of S. McLaughlin, SUNY Stony Brook, USA).

For a cell at rest, $[Ca^{2+}]$ is $\sim$20,000 times lower in the cytoplasm than outside the cell. It is maintained at 50–100 nM concentrations by transporters and a wealth of binding proteins. Readily bound by proteins, Ca^{2+} is inherently a localized second messenger. Ca^{2+} escapes the grasp of negatively charged domains for only microseconds before being rebound, dramatically decreasing its effective diffusion coefficient. It is likely that it modulates, either directly or indirectly, all TRP channels. DAG, a diester of glycerol and two fatty acids, is best known for its anchoring and activation of protein kinase C, but it also binds and translocates other proteins (for example, RasGRPs, Munc13 s and DAG kinase γ). DAG kinase (DAGK) phosphorylates DAG to form phosphatidic acid. Several TRP channels are activated by DAG and *Drosophila* TRP channels are constitutively active in DAGK-defective mutants[98]. DAG is also converted into arachidonic acid by DAG lipase. Arachidonic acid, itself a second messenger, is the wellspring of a large cascade of active molecules.

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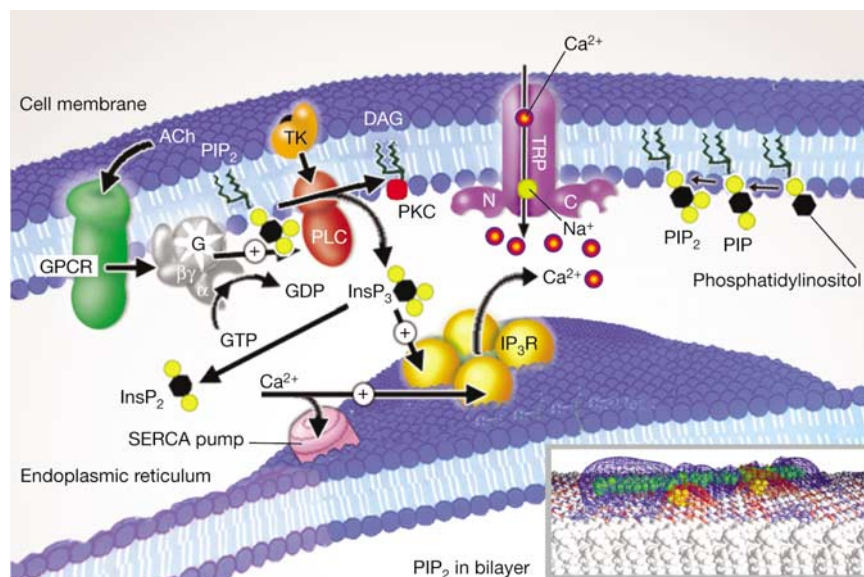
TRPV5 and TRPV6 comprise a distinct subfamily of homomeric and heteromeric channels found in transporting epithelia of the kidney and intestine. They show strong inwardly rectifying currents and are the most Ca^{2+}-selective TRP channels (permeability ratio $P_{Ca}/P_{Na} > 100$)[42,43], suggesting that they mediate Ca^{2+} uptake. Both are inactivated by $[Ca^{2+}]_i$ (ref. 44); TRPV6 shows voltage-dependent intracellular Mg^{2+} blockade[45].

The TRPM (melastatin) subfamily and TRPA

TRPM1 (melastatin) was initially identified as a transcript that showed decreased expression in highly metastatic versus non-metastatic melanoma cells[46]. It is widely expressed in normal tissues, but its function and electrophysiological properties have not been described.

TRPM2 (ref. 47) forms a Ca^{2+}-permeant channel[48] that is gated by binding of ADP ribose ($EC_{50} \approx 100\,\mu M$) and nicotinamide adenine dinucleotide (NAD; $\sim 1\,mM$)[48,49] to a carboxy-terminal NUDT9 Nudix hydrolase domain. ADP ribose is a breakdown product of NAD, CD38, cyclic ADP ribose (a Ca^{2+}-release messenger) and protein de-acetylation (O-acetylated ADP ribose[50]), but the TRPM2 domain itself is an ineffective hydrolase[51]. The channel is regulated by signalling pathways responsive to H_2O_2 and tumour-necrosis factor-α, suggesting that it may act as a sensor of intracellular oxidation/reduction[52], possibly during the oxidative burst of neutrophils[53].

TRPM3 (refs 54, 55) forms a Ca^{2+}-permeant nonselective channel that is constitutively active when heterologously expressed. Its activity is increased by hypotonicity (200 mOsm per litre), but there is little homology to TRPV4 that might suggest a common mechanism of activation[54]. TRPM4 is expressed primarily in kidney distal-collecting-duct epithelium and in the central nervous system.

TRPM4 and TRPM5 are the only monovalent-selective ion channels of the TRP family. They are widely distributed and may account for observed Ca^{2+}-activated ~ 20–30 pS nonselective channel activities[56,57]. They are activated through GPCRs coupled to PLC-dependent endoplasmic reticular Ca^{2+} release, perhaps by direct Ca^{2+} binding to the channel. However, relatively high $[Ca^{2+}]_i$ is required to activate these channels[56,57], suggesting that they localize close to sites of Ca^{2+} release or that other modulators are important. Although their instantaneous I–V relationships are linear, the fraction of open channels increases at positive potentials. This voltage dependence is not mediated by divalent cation binding, suggesting an intrinsic voltage-sensing mechanism[57,58].

TRPM5 is found in cells expressing taste receptors[59]. In an in vivo study in TrpM5$^{-/-}$ mice, it was shown that taste receptors T1R and T2R share a common signalling pathway involving PLCβ2 and TRPM5, to produce sweet, umami and bitter taste sensations[8]. The authors concluded that InsP$_3$, Ca^{2+} and thapsigargin-mediated store depletion did not activate TRPM5. However, it is possible that PIP$_2$ or other molecules modulate its sensitivity to $[Ca^{2+}]_i$.

TRPM6 and TRPM7 are unique among ion channels because they also contain functional kinase domains[60]. TRPM7 passes little inward current under physiological conditions, is permeant to both Ca^{2+} and Mg^{2+}, and is inhibited by ~ 0.6 mM intracellular free Mg^{2+} (refs 61, 62). In contrast to other GPCR-activated TRP channels, TRPM7 current increases slowly under whole-cell recording conditions and is inactivated by PIP$_2$ hydrolysis by PLCβ or PLCγ[62]. The function of the kinase domain is poorly understood and its substrates have not been identified. In contrast to our original

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Table 1 Identifying properties of TRP channel subunits

TRP	Functions	Interacting proteins	Activation	P_{Ca}/P_{Na}
C1 250687	Central nervous system (CNS)	TRPC4; TRPC5; TRPC3 (embryo); calmodulin; IP$_3$R; caveolin-1; G$\alpha_{q/11}$; PMCA	GPCR (G$_q$-PLCβ); TKR-PLCγ?	<10
C2 *602343	Pheromone sensing		GPCR (G$_q$-PLCβ); TKR-PLCγ?	
C3 150981	Vasoregulation (resistance arteries); airway regulation; CNS	TRPC1 (embryo); PLCβ; IP$_3$R; ryanodine receptor; SERCA; caveolin-1; calmodulin	GPCR (G$_q$-PLCβ); DAG; TKR-PLCγ; $[Ca^{2+}]_i$?	1.6
C4 262960	Vasoregulation; microvascular permeability?; CNS; gastrointestinal motility?	TRPC1; TRPC5; PLCβ; NHERF via PDZ-binding domain; IP$_3$R; calmodulin	GPCR (G$_q$-PLCβ); TKR-PLCγ?; GTPγS; La^{3+}; calmidazolium	~ 1
C5 247868	CNS; growth cone morphology	TRPC1; TRPC4; calmodulin; PLCβ; stathmin	GPCR (G$_q$-PLCβ); GTPγS; La^{3+}	~ 1
C6 159003	Vasoregulation (resistance arteries); airway regulation; smooth muscle?	TRPC3; TRPC7; calmodulin	GPCR (G$_q$-PLCβ); DAG; TKR-PLCγ?; $[Ca^{2+}]_i$; AlF$_4$; flufenamate	5
C7 283104		TRPC3; TRPC6; calmodulin	GPCR (G$_q$-PLCβ); DAG; TKR-PLCγ?; $[Ca^{2+}]_i$	~ 0.5
V1 (VR1) 283010	'Hot' pepper and heat sensation; inflammatory thermal hyperalgesia; bladder distension sensation	TRPV3; calmodulin	$T > 43\,°C$; capsaicin; resiniferatoxin; anandamide; H$^+$	10 ($P_{Mg}/P_{Na} \approx 5$)
V2 (VRL1; GRC) 279746	Noxious heat sensing; muscle degeneration?; other pain pathways		$T > 53\,°C$; insulin growth factor-1	3
V3 375034	Warm sensing?; pain?	TRPV1	$T > 30\,°C$	~ 10
V4 (OTRPC4; VR-OAC) 287776	CNS osmotic sensing; pressure sensing (dorsal root ganglion); nociception; warm sensing	Calmodulin; Src family kinases	Hypo-osmolarity; $T > 24\,°C$; phorbol esters; anandamide; arachidonic acid; epoxyeicosatrienoic acid	6
V5 (ECaC1; CaT2) 283369	Ca^{2+} uptake in kidney; intestine?	TRPV6; S100A10/annexin 2	Constitutive	>100
V6 (ECaC2; CaT1) 302740	Ca^{2+} uptake in intestine?	TRPV5; S100A10/annexin 2	Constitutive	>100
M1 (MLSN) 43265				
M2 133517	Oxidant stress sensor?		ADP ribose; βNAD; H$_2$O$_2$?	~ 1 (estimate)
M3 288911	Ca^{2+} uptake in kidney?		Constitutive; hypo-osmolarity	1.6
M4 31608			$[Ca^{2+}]_i$	<0.05
M5 (Mtr1) 272287	Taste (sweet; bitter; umami)		T1R; T2R–G$_{gus}$–PLCβ2; $[Ca^{2+}]_i$	<0.05
M6 (CHAK2) 272225	Mg^{2+} uptake in kidney and intestine?			
M7 (TRP-PLIK) 33819	Cellular Mg^{2+} homeostasis?	PLCβ1; PLCβ2; PLCβ3; PLCγ1	PIP$_2$; tyrosine phosphate	0.3
M8 (CMR1) 366053	Noxious cold sensing; nociception; cancer?		$T < 25\,°C$; menthol and icilin potentiate	1–3
A1 (ANKTM1) 186329	Noxious cold sensing		$T < 18\,°C$; icilin potentiates	1.4 ($P_{Ca} \approx P_{Mg}$)
P2 (PC2; PKD2) 82001	Mechanosensing in cilia?; fertility?	PC1; Hax-1; cortactin	Mechanical stress?; $[Ca^{2+}]_i$?	~ 1–5
P3 (PKD2L1) 159241	Kidney and retinal development?		$[Ca^{2+}]_i$	~ 4
P5 (PKD2L2) 272418			$[Ca^{2+}]_i$?	~ 1–5 ($P_{Mg} \approx 0$)

Numbers in TRP column are Unigene designation (human, except for *mouse). GPCR (G$_q$-PLCβ) indicates the canonical TRP signalling cascade. For complete table and tissue distribution, see http://clapham.tch.harvard.edu/.

report, it is not required for channel activation[62,63]. The catalytic core of the kinase domain is similar to that of other eukaryotic protein kinases and to enzymes with ATP-grasp domains[64]. The sensitivity of TRPM7 to physiological Mg–ATP levels has been suggested to have a central role in metabolic sensing[61] or to serve as a mechanism to adjust cellular Mg^{2+} homeostasis[63]. But a spontaneous human mutation in TRPM6 results in familial hypomagnesaemia with secondary hypocalcaemia, suggesting that TRPM6 may be important for Mg^{2+} uptake in the kidney and intestine.

TRPM8 was identified as a messenger RNA that was upregulated in prostatic and other cancers. Its sensory role was recognized when it was isolated by expression cloning of a menthol receptor from trigeminal neurons[65,66]. TRPM8 is a nonselective, outwardly rectifying channel that can be activated by cold (8–28 °C) and enhanced by 'cooling' compounds such as menthol and icilin. TRPM8 is widely expressed, but thought to function specifically as a thermosensor in TrkA$^+$, small-diameter primary sensory neurons.

ANKTM1, a Ca^{2+}-permeant, nonselective channel homologous to *Drosophila painless*[67], is distinguished by ~14 amino-terminal ankyrin repeats. It is activated by noxious cold temperature (<15 °C) but bears little similarity to menthol-sensitive TRPM8 (ref. 68). It is found in a subset of nociceptive sensory dorsal root ganglion neurons, in the company of capsaicin-sensitive TRPV1, but not TRPM8. Interestingly, the *Drosophila* orthologue of ANKTM1 responds to warming (>27 °C) rather than to cooling when expressed in *Xenopus* oocytes[69]. These observations are consistent with sensitivity to the surrounding membrane environment, but might also be reconciled if the lowest energy state of the mammalian channel is the open configuration. Other TRP channels have not been systematically tested for temperature sensitivity, but such a comparison would clarify this issue.

The TRPP (polycystin) and TRPML (mucolipin) subfamilies

Polycystic kidney disease proteins PKD2, PKD2L1 and PKD2L2 are 6TM Ca^{2+}-permeant channels called TRPP2, TRPP3 and TRPP5, respectively. The much larger TRPP1, polycystin-REJ and polycystin-1L1 proteins are 11TM proteins that contain a C-terminal 6TM TRP-like channel domain. TRPP1 is not known to form a channel by itself, but it complexes with TRPP2 to form a Ca^{2+}-permeable nonselective cation channel[70]. Autosomal dominant polycystic kidney disease is caused by mutations in TRPP1 or TRPP2, leading to alterations in the polarization and function of cyst-lining epithelial cells. Trpp1$^{-/-}$ and Trpp2$^{-/-}$ mice die *in utero* with cardiac septal defects and cystic changes in nephrons and pancreatic

ducts[71,72]. The mouse orthologue of TRPP3 is deleted in *krd* mice, resulting in defects in the kidney and retina[73].

TRPP proteins have another role in development. Normal body asymmetry appears to arise from leftward extracellular flow generated by motor-protein-dependent rotation of monocilia on the ventral surface of the embryonic node. Motile monocilia generate nodal flow, and non-motile TRPP2-containing cilia sense nodal flow, initiating an asymmetric Ca^{2+} signal at the left nodal border[74]. TRPP1 and TRPP2 both appear to be targeted to primary cilia cells of renal epithelia, where the channel complex is gated by fluid flow[75].

The mucolipins (MCOLN1, MCOLN2 and MCOLN3) are 6TM channels that are probably restricted to intracellular vesicles. Mutations in MCOLN1 (TRPML1) are associated with mucolipidosis type IV, a neurodegenerative lysosomal storage disorder[76]. The defect appears to be in sorting or transport in the late endocytic pathway. Mutations in a *Caenorhabditis elegans* TRPML1 homologue, *cup-5*, cause excess lysosome formation and apoptosis in all cell types[77]. TRPML3 is present in the cytoplasm of hair cells and the plasma membrane of stereocilia. TRPML3 is mutated in the *varitint-waddler* mouse, resulting in deafness and pigmentation defects[78].

Theories of TRP channel gating

The gnawing mystery of TRP channels is their elusive mechanism of gating. Many TRP channels show some constitutive activity in overexpression systems, but only a few have been studied in their native environment or at physiological temperatures. Our poor understanding of TRP channel gating may reflect our ignorance of potential intra- or extracellular ligands. But a more interesting possibility is that there might be a common TRP gating mechanism. Attempts to understand TRP channel activation have given rise to several theories, discussed below.

Receptor-operated theory

This is the most likely mechanism for TRPC channels and the *Drosophila* photoreceptor TRP (Box 2). All mammalian TRPC channels can be activated by GPCRs. These include muscarinic type 1 (TRPC1, TRPC4, TRPC5 heteromers, or presumed TRPC4, TRPC5 homomers), histaminergic type 1 (TRPC3, TRPC6) and purinergic receptors (TRPC7). GPCRs are often attached to multimolecular complexes by scaffolding proteins, adding an extra level of complexity. To date, investigators have identified only the proteins that interact prior to PLC activation. Unfortunately, PLC activation generates DAG and free cytoplasmic Ca^{2+} (via InsP$_3$), both of which can unleash legions of active signal transduction molecules. The major challenge of the receptor-operated theory is to find a receptor-activated messenger that directly binds and specifically activates a TRP channel. To establish this theory for a subset of TRP channels, it will be necessary to identify the native receptors that activate a particular channel, the downstream messengers mediating activation, and the physiological roles of these proteins and messengers in the context of the tissues that normally express them. One potential messenger, PIP$_2$, is described in more detail.

PIP$_2$ comprises ~1% of anionic phospholipids in cells and is much more abundant than its signalling relative PIP$_3$. PIP$_2$ regulates at least seven types of ion channel and transporter[79]. Because several TRPs (TRPC3, TRPC4, TRPM7) are known to bind PLCβ and/or PLCγ, PLC in part determines the PIP$_2$ concentration that TRPs encounter. PIP$_2$ inhibits *Drosophila* TRP and TRPL[80] and mammalian TRPV1 (ref. 26), but increasing PIP$_2$ lowers the temperature activation threshold of TRPV1 (ref. 81) and probably other TRP channels. Constitutive TRPM7 activity is increased by PIP$_2$ and inactivated by PIP$_2$ hydrolysis at 22 °C (ref. 62). These results suggest that PIP$_2$ commonly interacts with positively charged regions of ion channels to alter the energy required for gating. Is PIP$_2$ a common denominator of TRP activation?

The head group of PIP$_2$, carrying about four negative charges, may
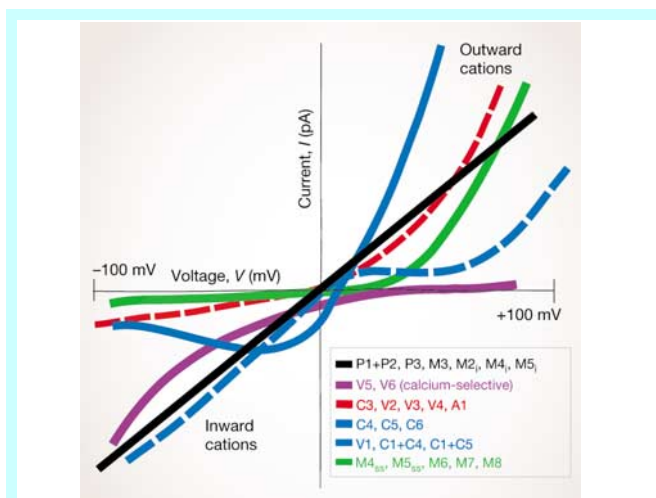
Figure 2 Representative transmembrane currents flowing in response to a set voltage (*I–V* relation) of various TRP channels. Inward cation flow is described as negative current. ss, steady state; i, instantaneous.

'snorkel' into the aqueous phase above other phospholipids (inset to Box 2 figure). Positively charged peptides containing basic residues can hug the bilayer if they contain aromatic amino acids (particularly Phe and Trp). If the peptide also contains clusters of basic residues (≥ 4), it can electrostatically fence in PIP_2, forming basins of concentrated PIP_2 (ref. 82). Indeed, such a cluster in the mid-C terminus is required for inhibition of TRPV1 by PIP_2 (ref. 81), but is missing in the PIP_2-insensitive TRPV3 (S. Ramsey, unpublished work). Interestingly, all TRPs contain a Trp/Phe segment with basic lysine and arginine residues (TRP box) just distal to the S6 gating helix/cytoplasmic junction, but there may not be enough positive charge in this region to sequester PIP_2. Considering the data to date, PIP_2 is likely to modulate gating of some TRP channels, but it is not a unifying mechanism of TRP channel activation.

Store-operated calcium entry hypothesis

Putney proposed that emptied Ca^{2+} stores (primarily in the endoplasmic reticulum) somehow gate the entry of external Ca^{2+} to replenish the deficit (for a review, see ref. 83). This mechanism is called capacitative Ca^{2+} entry, or store-operated Ca^{2+} entry (SOCE). The physiological hallmark of SOCE is a large, receptor-mediated, transient $[Ca^{2+}]_i$ increase followed by a prolonged high $[Ca^{2+}]_i$ plateau that is dependent on $[Ca^{2+}]_o$ (ref. 84). Thapsigargin, an inhibitor of smooth endoplasmic reticulum Ca^{2+}-ATPase (SERCA) pumps, is often used to examine this phenomenon, with the assumption that the drug is specific for SERCAs. The SOCE hypothesis has intrigued many scientists and has led to the search for an ion channel and a mechanism that could link store depletion with Ca^{2+} entry. I_{CRAC} (calcium-release-activated current) is the best candidate for a store-depletion-responsive current, but it does not appear to be mediated by any TRP protein. None of the TRP channels exhibits the requisite high Ca^{2+} selectivity ($P_{Ca}/P_{Na} \approx 1,000$), low single-channel conductance (<0.1 pS), and pharmacological enhancement by 1–5 µM 2-APB[85]. The three main theories for SOCE are a direct coupling mechanism, a diffusible messenger, and store-depletion-mediated fusion of a vesicle containing a Ca^{2+}-permeant channel (Fig. 3).

Although dozens of papers tout the link between SOCE and TRP channels, it is worth re-examining the data. The most common
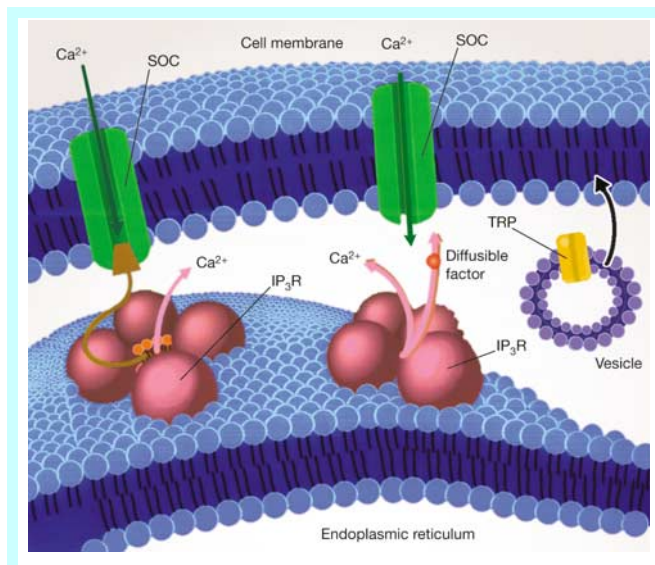
Figure 3 Three theories of store-operated Ca^{2+} entry. Depletion of Ca^{2+} in the endoplasmic reticulum causes opening of the SOC. Left, a direct link between the IP_3R opens such a channel, in analogy to the link between ryanodine receptors and Ca_V channels. Middle, a diffusible second messenger released from Ca^{2+}-depleted endoplasmic reticulum activates the SOC. Right, Ca^{2+} depletion results in fusion of vesicles containing SOCs.

standard of proof has been to show, either by Ca^{2+} imaging or by electrophysiology, that a particular heterologously expressed TRP channel enhances store-depletion-mediated entry. However, a better standard for this activity would be total abrogation of the phenomenon by elimination of a protein or gene, either *in vivo* or in a faithful model of the native system.

Nilius and colleagues[18] studied $Trpc4^{-/-}$ mice and concluded that TRPC4 was an essential component of SOCE channel activation. However, electrophysiological characterization suggested that the defect resulted from a decrease in I_{CRAC}, and TRPC4 alone has few properties characteristic of CRAC channels. Our laboratory suggested that the Ca^{2+}-selective TRPV6 might be a component of a CRAC channel[42], and a dominant-negative TRPV6 subunit in Jurkat cells was interpreted as suppressing endogenous I_{CRAC}[86]. But TRPV6 alone does not account for several properties of I_{CRAC}[42,87]. In a careful Ca^{2+} imaging and electrophysiological study using double-stranded RNA to target endogenous TRPC1-containing channels in Chinese hamster ovary cells, it was concluded that TRPC1 is an essential component of SOCE[88]. Despite the use of knockout or knockdown methods, none of these proteins is universally accepted as a store-operated Ca^{2+} channel. In my opinion, the major molecules comprising CRAC remain unknown.

Almost every publication concluding that a TRP is a SOC is countered by a paper stating that the same TRP is not a SOC. Most of the data based on Ca^{2+} imaging are pro-SOCE, whereas most of the electrophysiological data are con-SOCE. Ca^{2+} imaging is prone to false-positives and electrophysiology is prone to false-negatives. Ca^{2+} imaging has an advantage in that cells can be kept in a fairly normal environment. But Ca^{2+} imaging is a very indirect assay of channel function because it reflects accumulated free $[Ca^{2+}]_i$, regardless of the source. Addition of thapsigargin without accounting for constitutive Ca^{2+} entry leads to falsely high $[Ca^{2+}]_i$. SERCA pump inhibition can also artificially increase the Ca^{2+} signal by eliminating the endoplasmic reticulum as a Ca^{2+} buffer and reducing the effective Ca^{2+} volume of the cell. Ca^{2+} entry into the unbuffered cytoplasm may induce Ca^{2+}-activated channels, Ca^{2+} transporters, or other unrelated Ca^{2+}-dependent processes that affect the results (this problem can be reduced by substitution of extracellular Ca^{2+} with Ba^{2+}). Most importantly, voltage levels, the driving force for Ca^{2+} entry into the cytoplasm, are uncontrolled.

Patch clamp is a direct assay of channel activity that overcomes most of these limitations. In patch clamp, the channel can be bathed in defined solutions both inside and outside the cell, and voltage is controlled. Excellent time and current resolution allows the dissection of independent processes. Test compounds and proteins can readily be applied to either side of the channel. However, in standard patch-clamp recordings, intracellular contents are perfused, rapidly replacing small molecules and diffusible proteins. This problem can be circumvented by perforated patch recording, in which molecules larger than ions are restricted. Unfortunately, although it is not difficult to perform, it has rarely been used for these studies. Finally, electrophysiologists routinely record at 22 °C, and the few TRP channels that have been tested are highly sensitive to temperature. These problems could easily be rectified, and if they are, electrophysiology will most probably become the standard for SOC identification.

In my opinion, Ca^{2+}-permeant TRPs, like all Ca^{2+}-permeant channels, contribute to $[Ca^{2+}]_i$ and thus affect the SOCE process. But they have not been proved to be SOCs in any direct sense. The SOCE hypothesis for TRPs will not be settled until there is a consensus on the molecular identity of SOCs and the endoplasmic-reticulum-dependent signal that activates them. In the interim, the use of the term 'store-operated channel' for TRPs is confusing and should be avoided.

Vesicle fusion hypothesis

A Ca^{2+} entry channel was proposed to fuse with the plasma membrane to mediate SOCE, but neither the channel mediating

this event nor the mechanism was identified[89,90]. Independent of the SOCE theory, do TRPs rapidly translocate to the plasma membrane in response to stimuli? The mucolipins (TRPML) are involved in intravesicular trafficking, but little more is known of their function or whether they can also be present in the plasma membrane of native cells. Interestingly, TRPC1 and TRPC3–TRPC6 are all present in rat brain synaptosomes[91]. Recently, *C. elegans* sperm TRP (TRP-3), required for sperm–egg interaction, was localized in intracellular vesicles until fertilization competence required TRP-3 translocation from the vesicles to the plasma membrane[92].

These observations raise the question of whether some TRP channels are held in reserve and are then rapidly placed in the cell membrane in response or in adaptation to a stimulus. If so, what mechanism links the stimulus to the translocation of TRP-containing vesicles? And are the TRP channels in vesicles required for vesicle trafficking, swelling and/or the fusion process itself?

Cell sensory hypothesis

Block[93] beautifully encapsulated the underlying physical principles of sensory transduction. At the heart of sensation is the ability to distinguish input from a photon (vision, heat, electromagnetic force), phonon (sound), chemical (for example, odorant), or mechanical force (stretch, osmolarity, gravity) from background thermal energy or extraneous inputs (noise). Evolution has enhanced these abilities not just by sensitive detection, but also by amplification and signal processing. Built into almost all TRPs is the ability to conduct Ca^{2+} ions in a parsimonious manner; their I–V relations, nonselective character and low density dictate that they admit relatively little Ca^{2+} per second compared with, for example, a voltage-gated Ca^{2+} channel (Ca_V). Second, they are active at resting membrane potentials where cells spend most of their time. These characteristics can be harmful to a cell if TRPs are overexpressed, as is commonly observed in expression systems or in some cancer cells. As for most Ca^{2+}-permeant channels, the majority of TRPs are inherently self-inactivating by virtue of their $[Ca^{2+}]_i$ sensitivity. These properties provide a first level of signal processing common to sensory mechanisms, but what about detection?

Sensation requires the detection of force. The primary mechanisms for TRP activation involve mechanical force, intracellular ligand binding (signal transduction molecules) and temperature. How do these forces translate into channel gating? The Boltzmann equation gives the equilibrium distribution of channels in the open and closed states; the fraction of open channels is $[1 + \exp(\Delta G/Nk_BT)]^{-1}$, where ΔG is the free energy of transition between the closed and open state, k_B is Boltzmann's constant, N is Avogadro's number and T is the absolute temperature. The free energy, ΔG, is equal to w–ψ, where w is the conformational energy increase upon gating the channel and ψ represents the sum of energies transduced to the channel from external forces. Voltage-sensitive channels are usually held in a higher-energy, closed state at resting membrane potentials; removal of this energy (depolarization to 0 mV) allows the channel protein to relax into its lower-energy structural configuration. To sense this electromotive energy, voltage-sensitive ion channels have charged amino acid 'solenoids' that drive conformational changes. Most TRP channels lack these attributes and at the resting membrane potential are contentedly immune to the transmembrane field. What then is the ψ for TRPs?

The detection limit for an open TRP channel is imposed by thermal energy ($\sim$0.6 kcal mol^{-1} at 37 °C). This energy is tiny, less than the energy of a single photon of visible light per molecule. But other cellular noise sources must be overcome, probably requiring channel gating for the cell to record a significant stimulus. The gating energy for the membrane-tension-sensitive bacterial channel in a liposome is $\sim$10 kcal mol^{-1} (ref. 94), only 20 times the thermal limit. Mechanosensation—the basis of hearing, osmolar sensing, stretch and flow sensing—easily provides these levels of energy, especially if the mechanical advantage provided by cilia is taken into

account[95]. TRPC2 (ref. 23), OSM-9 (ref. 6), TRPP2 (PKD)[75] and *Nan*[96] are all found in ciliated structures. TRPs are also common within ciliated cells that sense flow in kidney epithelia, vascular endothelia, lung and intestine, and in the hearing and vestibular apparatus, taste cells and odorant-sensing cells. Anchoring TRPs to mechanical forces is not without its risks to the cell. Prolonged Ca^{2+} entry due to abnormal membrane tension, such as occurs with loss of membrane-stabilizing proteins in muscular dystrophy, may result in muscle degeneration. Prolonged stretch in cardiac muscle leads to Ca^{2+} overload, hypertrophy and cardiac failure.

For signal-transduction-gated TRPs, the energies imparted by intracellular ligand binding are also sufficient for gating, depending on the binding affinity of the specific interaction (dissociation constant values of 1 μM imply $\sim$1–10 kcal mol^{-1}). But the most obscure mechanism for activation of TRPs is temperature. Oddly, all TRP recordings published to date fail to reach steady state and do not saturate within the range of practical recordings. This prevents estimates of gating energy, but temperature-sensitive TRP channels have estimated Q_{10} values up to ten times larger than is typical for enzymes or channels. Temperature-dependent TRPV3 current is reversible upon cooling, but exhibits a pronounced hysteresis; the current increases with increasing temperature but abruptly collapses when cooling begins[33]. Perhaps heat induces lipid bilayer rearrangements to alter membrane tension. Alternatively, the protein may denature (melt) and rapidly refold when energy is removed. Finally, TRPs could be gated by temperature-dependent cooperative binding by second messengers.

What's next?

The large number of TRP subtypes, their overlapping electrophysiological characteristics, broad expression patterns, heteromultimerization, lack of specific blockers, and poorly understood mechanisms of activation have made their study difficult for TRP channel researchers. But the field is now progressing beyond simple overexpression and Ca^{2+} imaging, and more specific tools are being used. In the near future, a wealth of data from genetically altered animals will emerge, as well as useful molecular tools and assays. The next few years should determine whether any of the TRPs are indeed SOCs, or whether this term has simply substituted for a poorly understood activation mechanism. TRPs are sensory molecules, but not just in the usual organismal definition of sensation. TRP channels are intrinsic sensors of the cellular environment. □

1. Hille, B. *Ion Channels of Excitable Membranes* 3rd edn, 663–723 (Sinauer, Sunderland, MA, 2001).
2. Minke, B. *Drosophila* mutant with a transducer defect. *Biophys. Struct. Mech.* **3**, 59–64 (1977).
3. Montell, C., Jones, K., Hafen, E. & Rubin, G. Rescue of the *Drosophila* phototransduction mutation trp by germline transformation. *Science* **230**, 1040–1043 (1985).
4. Denis, V. & Cyert, M. S. Internal Ca^{2+} release in yeast is triggered by hypertonic shock and mediated by a TRP channel homologue. *J. Cell Biol.* **156**, 29–34 (2002).
5. Zhou, X. L. *et al.* The transient receptor potential channel on the yeast vacuole is mechanosensitive. *Proc. Natl Acad. Sci. USA* **100**, 7105–7110 (2003).
6. de Bono, M., Tobin, D. M., Davis, M. W., Avery, L. & Bargmann, C. I. Social feeding in *Caenorhabditis elegans* is induced by neurons that detect aversive stimuli. *Nature* **419**, 899–903 (2002).
7. Stowers, L., Holy, T. E., Meister, M., Dulac, C. & Koentges, G. Loss of sex discrimination and male–male aggression in mice deficient for TRP2. *Science* **295**, 1493–1500 (2002).
8. Zhang, Y. *et al.* Coding of sweet, bitter, and umami tastes: different receptor cells sharing similar signaling pathways. *Cell* **112**, 293–301 (2003).
9. Harteneck, C., Plant, T. D. & Schultz, G. From worm to man: three subfamilies of TRP channels. *Trends Neurosci.* **23**, 159–166 (2000).
10. Clapham, D. E., Runnels, L. W. & Struebing, C. The TRP ion channel family. *Nature Rev. Neurosci.* **2**, 387–396 (2001).
11. Minke, B. & Cook, B. TRP channel proteins and signal transduction. *Physiol. Rev.* **82**, 429–472 (2002).
12. Montell, C., Birnbaumer, L. & Flockerzi, V. The TRP channels, a remarkably functional family. *Cell* **108**, 595–598 (2002).
13. Birnbaumer, L., Yidirim, E. & Abramowitz, J. A comparison of the genes coding for canonical TRP channels and their M, V and P relatives. *Cell Calcium* **33**, 419–432 (2003).
14. Nilius, B. TRP Channels: facts, fiction, challenges. *Cell Calcium* **14**, 33–34 (2003).
15. Corey, D. New TRP channels in hearing and mechanosensation. *Neuron* **39**, 585–588 (2003).
16. Wes, P. D. *et al.* TRPC1, a human homolog of a *Drosophila* store-operated channel. *Proc. Natl Acad. Sci. USA* **92**, 9652–9656 (1995).
17. Greka, A., Navarro, B., Oancea, E., Duggan, A. & Clapham, D. E. TRPC5 is a regulator of hippocampal

 523

neurite length and growth cone morphology. *Nature Neurosci.* **6,** 837–845 (2003).

18. Freichel, M. *et al.* Lack of an endothelial store-operated Ca²⁺ current impairs agonist-dependent vasorelaxation in TRP4⁻/⁻ mice. *Nature Cell Biol.* **3,** 121–127 (2001).

19. Tiruppathi, C. *et al.* Impairment of store-operated Ca²⁺ entry in TRPC4⁻/⁻ mice interferes with increase in lung microvascular permeability. *Circ. Res.* **91,** 70–76 (2002).

20. Zitt, C. *et al.* Expression of TRPC3 in Chinese hamster ovary cells results in calcium-activated cation currents not related to store depletion. *J. Cell Biol.* **138,** 1333–1341 (1997).

21. Trebak, M., Vazquez, G., Bird, G. S. & Putney, J. W. The TRPC3/6/7 subfamily of cation channels. *Cell Calcium* **33,** 451–461 (2003).

22. Inoue, R. *et al.* The transient receptor potential protein homologue TRP6 is the essential component of vascular α₁-adrenoceptor-activated Ca²⁺-permeable cation channel. *Circ. Res.* **88,** 325–332 (2001).

23. Liman, E. R., Corey, D. P. & Dulac, C. TRP2: a candidate transduction channel for mammalian pheromone sensory signaling. *Proc. Natl Acad. Sci. USA* **96,** 5791–5796 (1999).

24. Caterina, M. J. *et al.* The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature* **389,** 816–824 (1997).

25. Jordt, S. E. & Julius, D. Molecular basis for species-specific sensitivity to "hot" chili peppers. *Cell* **108,** 421–430 (2002).

26. Chuang, H. H. *et al.* Bradykinin and nerve growth factor release the capsaicin receptor from PtdIns(4,5)P₂-mediated inhibition. *Nature* **411,** 957–962 (2001).

27. Caterina, M. J. *et al.* Impaired nociception and pain sensation in mice lacking the capsaicin receptor. *Science* **288,** 306–313 (2000).

28. Birder, L. A. *et al.* Altered urinary bladder function in mice lacking the vanilloid receptor TRPV1. *Nature Neurosci.* **5,** 856–860 (2002).

29. Caterina, M. J., Rosen, T. A., Tominaga, M., Brake, A. J. & Julius, D. A capsaicin-receptor homologue with a high threshold for noxious heat. *Nature* **398,** 436–441 (1999).

30. Kanzaki, M. *et al.* Translocation of a calcium-permeable cation channel induced by insulin-like growth factor-I. *Nature Cell Biol.* **1,** 165–170 (1999).

31. Iwata, Y. *et al.* A novel mechanism of myocyte degeneration involving the Ca²⁺-permeable growth factor-regulated channel. *J. Cell Biol.* **161,** 957–967 (2003).

32. Smith, G. D. *et al.* TRPV3 is a temperature-sensitive vanilloid receptor-like protein. *Nature* **418,** 186–190 (2002).

33. Xu, H. *et al.* TRPV3 is a calcium-permeable temperature-sensitive cation channel. *Nature* **418,** 181–186 (2002).

34. Peier, A. M. *et al.* A heat-sensitive TRP channel expressed in keratinocytes. *Science* **296,** 2046–2049 (2002).

35. Guler, A. D. *et al.* Heat-evoked activation of the ion channel, TRPV4. *J. Neurosci.* **22,** 6408–6414 (2002).

36. Benham, C. D., Gunthorpe, M. J. & Davis, J. B. TRPV channels as temperature sensors. *Cell Calcium* **33,** 479–487 (2003).

37. Strotmann, R., Harteneck, C., Nunnenmacher, K., Schultz, G. & Plant, T. D. OTRPC4, a nonselective cation channel that confers sensitivity to extracellular osmolarity. *Nature Cell Biol.* **2,** 695–702 (2000).

38. Liedtke, W. *et al.* Vanilloid receptor-related osmotically activated channel (VR-OAC), a candidate vertebrate osmoreceptor. *Cell* **103,** 525–535 (2000).

39. Mizuno, A., Matsumoto, N., Imai, M. & Suzuki, M. Impaired osmotic sensation in mice lacking TRPV4. *Am. J. Physiol. Cell Physiol.* **285,** C96–C101 (2003).

40. Alessandri-Haber, N. *et al.* Hypotonicity induces TRPV4-mediated nociception in rat. *Neuron* **39,** 497–511 (2003).

41. Watanabe, H. *et al.* Anandamide and arachidonic acid use epoxyeicosatrienoic acids to activate TRPV4 channels. *Nature* **424,** 434–438 (2003).

42. Yue, L., Peng, J. B., Hediger, M. A. & Clapham, D. E. CaT1 manifests the pore properties of the calcium-release-activated calcium channel. *Nature* **410,** 705–709 (2001).

43. den Dekker, E., Hoenderop, J. G., Nilius, B. & Bindels, R. J. The epithelial calcium channels, TRPV5 and TRPV6: from identification towards regulation. *Cell Calcium* **33,** 497–507 (2003).

44. Hoenderop, J. G. *et al.* Homo- and heterotetrameric architecture of the epithelial Ca²⁺ channels TRPV5 and TRPV6. *EMBO J.* **22,** 776–785 (2003).

45. Voets, T., Janssens, A., Prenen, J., Droogmans, G. & Nilius, B. Mg²⁺-dependent gating and strong inward rectification of the channel TRPV6. *J. Gen. Physiol.* **121,** 245–260 (2003).

46. Duncan, L. M. *et al.* Down-regulation of the novel gene melastatin correlates with potential for melanoma metastasis. *Cancer Res.* **58,** 1515–1520 (1998).

47. Nagamine, K. *et al.* Molecular cloning of a novel putative Ca²⁺ channel protein (TRPC7) highly expressed in brain. *Genomics* **54,** 124–131 (1998).

48. Perraud, A. L. *et al.* ADP-ribose gating of the calcium-permeable LTRPC2 channel revealed by Nudix motif homology. *Nature* **411,** 595–599 (2001).

49. Sano, Y. *et al.* Immunocyte Ca²⁺ influx system mediated by LTRPC2. *Science* **293,** 1327–1330 (2001).

50. Rafty, L. A., Schmidt, M. T., Perraud, A. L., Scharenberg, A. M. & Denu, J. M. Analysis of O-acetyl-ADP-ribose as a target for Nudix ADP-ribose hydrolases. *J. Biol. Chem.* **277,** 47114–47122 (2002).

51. Perraud, A. L., Schmitz, C. & Scharenberg, A. M. TRPM2 Ca²⁺ permeable cation channels: from gene to biological function. *Cell Calcium* **33,** 519–531 (2003).

52. Hara, Y. *et al.* LTRPC2 Ca²⁺-permeable channel activated by changes in redox status confers susceptibility to cell death. *Mol. Cell* **9,** 163–173 (2002).

53. Heiner, I., Eisfeld, J. & Luckhoff, A. Role and regulation of TRP channels in neutrophil granulocytes. *Cell Calcium* **33,** 533–540 (2003).

54. Grimm, C., Kraft, R., Sauerbruch, S., Schultz, G. & Harteneck, C. Molecular and functional characterization of the melastatin-related cation channel TRPM3. *J. Biol. Chem.* **278,** 21493–21501 (2003).

55. Lee, N. *et al.* Expression and characterization of human transient receptor potential melastatin 3 (hTRPM3). *J. Biol. Chem.* **278,** 20890–20897 (2003).

56. Launay, P. *et al.* TRPM4 is a Ca²⁺-activated nonselective cation channel mediating cell membrane depolarization. *Cell* **109,** 397–407 (2002).

57. Hofmann, T., Chubanov, V., Gudermann, T. & Montell, C. TRPM5 is a voltage-modulated and Ca²⁺-activated monovalent selective cation channel. *Curr. Biol.* **13,** 1153–1158 (2003).

58. Nilius, B. *et al.* Voltage dependence of the Ca²⁺ activated cation channel TRPM4. *J. Biol. Chem.* **33,** 30813–30820 (2003).

59. Perez, C. A. *et al.* A transient receptor potential channel expressed in taste receptor cells. *Nature Neurosci.* **5,** 1169–1176 (2002).

60. Runnels, L. W., Yue, L. & Clapham, D. E. TRP-PLIK, a bifunctional protein with kinase and ion channel activities. *Science* **291,** 1043–1047 (2001).

61. Nadler, M. J. *et al.* LTRPC7 is a Mg-ATP-regulated divalent cation channel required for cell viability. *Nature* **411,** 590–595 (2001).

62. Runnels, L. W., Yue, L. & Clapham, D. E. The TRPM7 channel is inactivated by PIP₂ hydrolysis. *Nature Cell Biol.* **4,** 329–336 (2002).

63. Schmitz, C. *et al.* Regulation of vertebrate cellular Mg²⁺ homeostasis by TRPM7. *Cell* **114,** 191–200 (2003).

64. Yamaguchi, H., Matsushita, M., Nairn, A. C. & Kuriyan, J. Crystal structure of the atypical protein kinase domain of a TRP channel with phosphotransferase activity. *Mol. Cell* **7,** 1047–1057 (2001).

65. McKemy, D. D., Neuhausser, W. M. & Julius, D. Identification of a cold receptor reveals a general role for TRP channels in thermosensation. *Nature* **416,** 52–58 (2002).

66. Peier, A. M. *et al.* A TRP channel that senses cold stimuli and menthol. *Cell* **108,** 705–715 (2002).

67. Tracey, W. D. Jr, Wilson, R. I., Laurent, G. & Benzer, S. *painless,* a *Drosophila* gene essential for nociception. *Cell* **113,** 261–273 (2003).

68. Story, G. M. *et al.* ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. *Cell* **112,** 819–829 (2003).

69. Viswanath, V. *et al.* Opposite thermosensor in fruitfly and mouse. *Nature* **423,** 822–823 (2003).

70. Hanaoka, K. *et al.* Co-assembly of polycystin-1 and -2 produces unique cation-permeable currents. *Nature* **408,** 990–994 (2000).

71. Wu, G. *et al.* Somatic inactivation of Pkd2 results in polycystic kidney disease. *Cell* **93,** 177–188 (1998).

72. Boulter, C. *et al.* Cardiovascular, skeletal, and renal defects in mice with a targeted disruption of the *Pkd1* gene. *Proc. Natl Acad. Sci. USA* **98,** 12174–12179 (2001).

73. Nomura, H. *et al.* Identification of PKDL, a novel polycystic kidney disease 2-like gene whose murine homologue is deleted in mice with kidney and retinal defects. *J. Biol. Chem.* **273,** 25967–25973 (1998).

74. Nonaka, S., Shiratori, H., Saijoh, Y. & Hamada, H. Determination of left–right patterning of the mouse embryo by artificial nodal flow. *Nature* **418,** 96–99 (2002).

75. Nauli, S. M. *et al.* Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. *Nature Genet.* **33,** 129–137 (2003).

76. Sun, M. *et al.* Mucolipidosis type IV is caused by mutations in a gene encoding a novel transient receptor potential channel. *Hum. Mol. Genet.* **9,** 2471–2478 (2000).

77. Hersh, B. M., Hartwieg, E. & Horvitz, H. R. The *Caenorhabditis elegans* mucolipin-like gene *cup-5* is essential for viability and regulates lysosomes in multiple cell types. *Proc. Natl Acad. Sci. USA* **99,** 4355–4360 (2002).

78. Di Palma, F. *et al.* Mutations in Mcoln3 associated with deafness and pigmentation defects in varitint-waddler (Va) mice. *Proc. Natl Acad. Sci. USA* **99,** 14994–14999 (2002).

79. Hilgemann, D. W., Feng, S. & Nasuhoglu, C. The complex and intriguing lives of PIP₂ with ion channels and transporters. *Sci. STKE* **2001,** RE19 (2001).

80. Hardie, R. C. Regulation of TRP channels via lipid second messengers. *Annu. Rev. Physiol.* **65,** 735–759 (2003).

81. Prescott, E. D. & Julius, D. A modular PIP₂ binding site as a determinant of capsaicin receptor sensitivity. *Science* **300,** 1284–1288 (2003).

82. McLaughlin, S., Wang, J., Gambhir, A. & Murray, D. PIP₂ and proteins: interactions, organization, and information flow. *Annu. Rev. Biophys. Biomol. Struct.* **31,** 151–175 (2002).

83. Putney, J. W. Jr & McKay, R. R. Capacitative calcium entry channels. *Bioessays* **21,** 38–46 (1999).

84. Putney, J. W. Jr TRP, inositol 1,4,5-trisphosphate receptors, and capacitative calcium entry. *Proc. Natl Acad. Sci. USA* **96,** 14669–14671 (1999).

85. Prakriya, M. & Lewis, R. S. CRAC channels: activation, permeation, and the search for a molecular identity. *Cell Calcium* **33,** 311–321 (2003).

86. Cui, J., Bian, J. S., Kagan, A. & McDonald, T. V. CaT1 contributes to the stores-operated calcium current in Jurkat T-lymphocytes. *J. Biol. Chem.* **277,** 47175–47183 (2002).

87. Voets, T. *et al.* CaT1 and the calcium release-activated calcium channel manifest distinct pore properties. *J. Biol. Chem.* **276,** 47767–47770 (2001).

88. Vaca, L. & Sampieri, A. Calmodulin modulates the delay period between release of calcium from internal stores and activation of calcium influx via endogenous TRP1 channels. *J. Biol. Chem.* **277,** 42178–42187 (2002).

89. Patterson, R. L., van Rossum, D. B. & Gill, D. L. Store-operated Ca²⁺ entry: evidence for a secretion-like coupling model. *Cell* **98,** 487–499 (1999).

90. Yao, Y., Ferrer-Montiel, A. V., Montal, M. & Tsien, R. Y. Activation of store-operated Ca²⁺ current in *Xenopus* oocytes requires SNAP-25 but not a diffusible messenger. *Cell* **98,** 475–485 (1999).

91. Goel, M., Sinkins, W. G. & Schilling, W. P. Selective association of TRPC channel subunits in rat brain synaptosomes. *J. Biol. Chem.* **277,** 48303–48310 (2002).

92. Xu, X. Z. & Sternberg, P. W. A *C. elegans* sperm TRP protein required for sperm–egg interactions during fertilization. *Cell* **114,** 285–297 (2003).

93. Block, S. M. Biophysical principles of sensory transduction. *Soc. Gen. Physiol. Ser.* **47,** 1–17 (1992).

94. Sukharev, S. I., Sigurdson, W. J., Kung, C. & Sachs, F. Energetic and spatial parameters for gating of the bacterial large conductance mechanosensitive channel, MscL. *J. Gen. Physiol.* **113,** 525–540 (1999).

95. Howard, J., Roberts, W. M. & Hudspeth, A. J. Mechanoelectrical transduction by hair cells. *Annu. Rev. Biophys. Biophys. Chem.* **17,** 99–124 (1988).

96. Kim, J. *et al.* A TRPV family ion channel required for hearing in *Drosophila. Nature* **424,** 81–84 (2003).

97. Yellen, G. The voltage-gated potassium channels and their relatives. *Nature* **419,** 35–42 (2002).

98. Hardie, R. C. *et al.* Molecular basis of amplification in *Drosophila* phototransduction: roles for G protein, phospholipase C, and diacylglycerol kinase. *Neuron* **36,** 689–701 (2002).