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REVIEW

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Transient receptor potential channels as molecular substrates of receptor-mediated cation entry

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Abstract Calcium is a versatile multitarget intracellular second messenger in eukaryotic cells. In addition to calcium release from intracellular stores and influx via voltage- or ligand-operated channels, agonist-induced calcium entry constitutes one of the main pathways by which cytosolic calcium is elevated. Receptor-stimulated currents are initiated in response to agonist binding to G-protein-coupled receptors and to receptor tyrosine kinases. Within the past few years our knowledge about the molecular identity of receptor-stimulated channels has expanded substantially. *Drosophila melanogaster* visual transduction channels associated with the transient receptor potential (*trp*) and the *trp*-like (*trpl*) mutant visual phenotypes were the first members of this category of channels to be identified at the molecular level. Since then an entire mammalian gene family of TRP homologues has been discovered by homology cloning. Only now are we beginning to fully understand the functional roles of TRP channels in mammalian cells. We review recent findings in TRP channel research and discuss the role of these proteins for receptor-activated cation entry.

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Key words Transient receptor potential · TRP-homologous channel · Receptor-stimulated channel · Store-operated channel · Calcium

Abbreviations *$[Ca^{2+}]_i$:* Intracellular free calcium concentration · *CRAC channel*: Calcium release-activated calcium channel · *DAG:* Diacylglycerol · *$InsP_3$:* Inositol-1,4,5-trisphosphate · *PLC:* Phospholipase C · *SOC:* Store-operated cation channel · *TRP:* Transient receptor potential · *TRPC:* TRP-homologous channel · *TRPL:* Transient receptor potential-like

Introduction

Extracellular messenger molecules such as hormones, neurotransmitters, and growth factors convey information between single cells of a multicellular organism. They bind to membrane proteins such as G-protein-coupled receptors or receptor tyrosine kinases. Consequently, effector enzymes are activated to generate intracellular second messenger molecules. Among such enzymes, adenylyl cyclases converting ATP to cyclic AMP and phospholipase C (PLC) β are well characterized. A different PLC isoenzyme (PLCγ) is activated by receptor tyrosine kinases such as the epithelial growth factor receptor. Metabolism of the membrane phospholipid phosphatidylinositol by PLC results in the production of the

soluble second messenger inositol-1,4,5-trisphosphate ($InsP_3$) and of membrane-confined diacylglycerol (DAG) [1]. $InsP_3$ mediates calcium mobilization from intracellular calcium storage organelles [2], while DAG activates a subset of protein kinases C [3, 4].

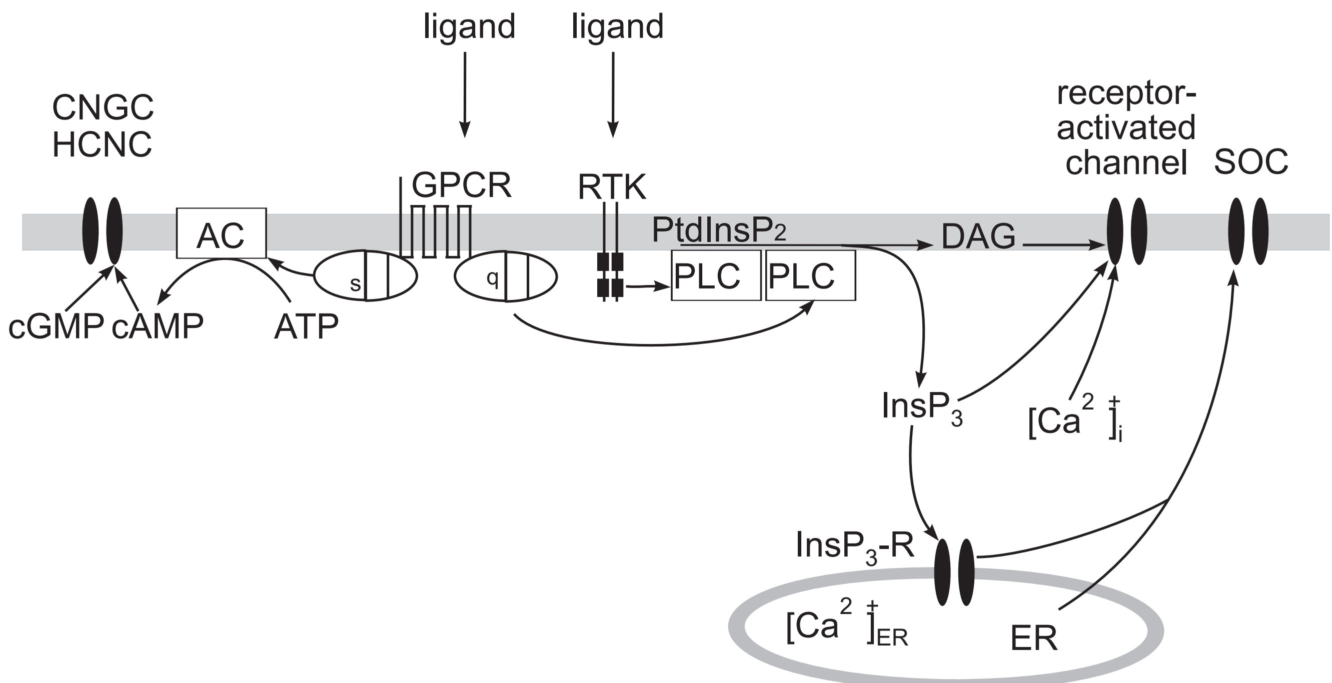
Calcium is an important second messenger endowed with a wide spectrum of target proteins. Increases in the intracellular free calcium concentration ($[Ca^{2+}]_i$) profoundly affect central cellular functions such as metabolism, contraction, secretion, and transcription. $[Ca^{2+}]_i$ is elevated by release of calcium from intracellular calcium stores subsequent to binding of $InsP_3$ to its receptor [2, 5] and by calcium influx through plasma membrane channels; this influx may or may not be regulated by the filling state of the calcium stores. Many excitable cells express voltage-operated calcium channels [6] which translate an action potential into a calcium pulse initiating events such as contraction or secretion. Ligand-operated cation channels are expressed in a variety of neuronal and extraneuronal cells and are directly gated by binding of an extracellular agonist to the channel protein. Neither voltage-operated nor ligand-operated channels are discussed here.

Receptor-mediated cation entry

Receptor-stimulated cation channels are gated in response to agonist-binding to a membrane receptor distinct from the channel protein itself. Thus channel proteins represent integrating effectors receiving inputs from various classes of cell surface receptors. Some receptor-stimulated cation channels have been shown to be activated downstream of tyrosine kinases [7, 8, 9] and others via G protein signaling cascades as inferred from studies with nonhydrolyzable guanine nucleotide analogues. Receptor-stimulated cation channels are expressed in a broad spectrum of both excitable and nonexcitable cells, including smooth muscle [10, 11, 12, 13, 14, 15], mast cells [16], epidermis [7], and renal mesangial cells [8, 9], and differ considerably in such biophysical properties as conductance, ion selectivity, and activation/inactivation kinetics.

Receptor-stimulated cation channels can be further classified according to various mechanisms of channel gating operative downstream of receptor activation (Fig. 1). Channels positively modulated by second messengers are referred to as second messenger-operated channels. Thus, cyclic nucleotides generated by adenylyl and guanylyl cyclases directly activate cation-permeable channels that have been molecularly characterized [17, 18]. Cyclic nucleotide-gated cation channels are predominantly, but not exclusively, expressed in sensory tissues such as the retina and in olfactory/gustatory epithelia, where they contribute to the encoding of receptor potentials. Due to their similar structural and functional characteristics, hyperpolarization-activated cyclic nucleotide-gated channels belong to the same protein superfamily. They are modulated in a receptor-dependent fashion in

Fig. 1 Synopsis of the main activation pathways of receptor- and second-messenger-activated cation channels. *CNGC* Cyclic nucleotide-activated channel; *HCNC* hyperpolarization-activated cyclic nucleotide-binding channel; *AC* adenylyl cyclase; *ATP* adenosine-5'-trisphosphate; *cAMP* cyclic adenosine-3',5'-monophosphate; *cGMP* cyclic guanosine-3',5'-monophosphate; *GPCR* G-protein-coupled receptor; *RTK* receptor tyrosine kinase; $\alpha/\beta/\gamma$ subunits of heterotrimeric G-proteins; *SOC* store-operated channel; *$InsP_3$-R* $InsP_3$ receptor; *ER* endoplasmic reticulum; *$[Ca^{2+}]_{ER}$* free calcium concentration of the ER



that their voltage-activation curve is shifted towards more positive membrane potentials after binding of cyclic nucleotides [19, 20, 21, 22].

For several channels calcium plays the role of an activating second messenger. Apart from calcium-activated potassium and chloride channels, certain receptor-stimulated cation channels in neutrophils [23], smooth muscle [24], and mast cells [25] belong to this functional group. Inositol phosphates generated upon PLC activation constitute another group of second messengers which are known to activate channel proteins. Apart from the intracellular $InsP_3$ receptor, plasma membrane channels responsive to $InsP_3$ have been reported, for instance, in T-lymphocytes, mast cells, and epidermal cells [26, 27, 28, 29, 30]. The biophysical properties of $InsP_3$-responsive second messenger-operated channels differ considerably. Lückhoff and Clapham [31] described a channel in endothelial cells which is directly activated by inositol-1,3,4,5-tetrakisphosphate but is unresponsive to $InsP_3$.

In some cases lipid messengers such as arachidonic acid and its metabolites have been noted to mediate the activation of receptor-stimulated calcium-permeable channels [32, 33, 34]. This raises the possibility that other signaling systems, including those of phospholipase A_2 and DAG lipase, which otherwise have been neglected in this regard, may play a role in channel gating.

Store-operated cation entry

The so-called store-operated cation channels (SOCs), which by convention are not classified as receptor-activated cation channels, constitute a separate functional category. For these channels depletion of the calcium stores represents the activating principle. The archetypical SOC is the so-called CRAC channel from mast cells [35, 36] and T-lymphocytes [37]. I_{crac} is highly calcium selective and exhibits a single-channel conductance smaller than 1 pS under physiological ion conditions. Under divalent ion-free conditions whole-cell CRAC currents are considerably augmented and display a unitary conductance of 36–40 pS with readily resolvable single-channel events [38]. I_{crac} is rapidly counterregulated by elevated $[Ca^{2+}]_i$ [39]. Apart from I_{crac}, further distinct SOC channels are known. In A431 epidermal cells a strictly calcium-selective SOC with a considerably higher unitary conductance has been described [40], whereas from endothelial cells a 11-pS SOC with only moderate calcium selectivity is known [41]. Other SOCs display even larger single-channel conductances and are poorly calcium-selective, for instance, the SOC characterized in pancreatic acinar cells [42]. Because transient receptor potential (TRP) channels have been discussed as candidate SOCs, this literature is reviewed below.

The sequence of signaling events leading to SOC gating are far from clear, and three main hypotheses are currently being discussed. The structural interaction of channel proteins with regulatory proteins of intracellular calcium stores is postulated in analogy to the interaction of ryanodine receptor release channels with L-type voltage-operated calcium channels in skeletal muscle ("*conformation-coupling hypothesis*"). This hypothesis has been proposed [43] as a model best accomodating most findings on the cooperative regulation of calcium release by both the intraluminal free calcium concentration of the store organelle and cytosolic inositol phosphates by assuming that $InsP_3$ receptor calcium release channels contain an intraluminal low-affinity calcium sensor. The sensor allosterically interacts with the $InsP_3$ binding site, and calcium release via $InsP_3$ receptors results as a function of both $InsP_3$ and the calcium filling state of the store. As an extrapolation, SOC channel proteins could adopt "store sensitivity" by direct allosteric interaction with a "store-sensor" $InsP_3$ receptor or similar proteins. However, direct experimental evidence of a conformational coupling-dependent SOC activation pathway is lacking so far. As an alternative hypothesis, low-molecular-weight cytosolic factors such as the calcium influx factor purified from activated Jurkat T-lymphocytes and reported as a phosphorylated compound with a molecular weight smaller than 500 Da have been invoked as store-derived messengers in the activation of SOCs ("*soluble-messenger hypothesis*") [44]. As this view is challenged by recent findings [45], a third hypothesis has been gaining acceptance. This so-called secretion- or *secretion-coupling model* [45, 46, 47] assumes a secretion-like insertion of channel-containing vesicles into the plasma membrane. The role of the cytoskeleton in this process is controversial [45, 47]. Future research must clarify whether the above activation models are sufficient to account for the experimental data obtained, and which mode of activation applies to a given SOC in a particular cell system.

TRP and TRPL: molecularly defined receptor-stimulated cation channels from *Drosophila* eye

The first receptor-stimulated cation channels identified at the molecular level were the TRP and TRP-like (TRPL) visual transduction channels in *Drosophila melanogaster*. In contrast to mammals, visual transduction in the dipterian system is mediated via PLC rather than by cyclic nucleotide dependent processes: PLC-deficient fly mutants are blind. In the *Drosophila* eye activated rhodopsin stimulates a PLCβ-type phospholipase via G proteins of the G_q subfamily. As a consequence, the light-induced conductance is activated and gives rise to a receptor potential accompanied by an elevation of $[Ca^{2+}]_i$. This current is fairly calcium selective (P_{Ca}/P_{Na}=25) with an apparent unitary conductance of 17 pS and is rapidly inactivated in a calcium/calmodulin-dependent fashion [48, 49, 50].

The TRP channel protein has been isolated in a genetic approach by identifying the gene locus associated with the TRP phenotype, a fly mutant whose visual receptor currents are considerably diminished [51]. By its calmodulin-binding, an additional protein with significant

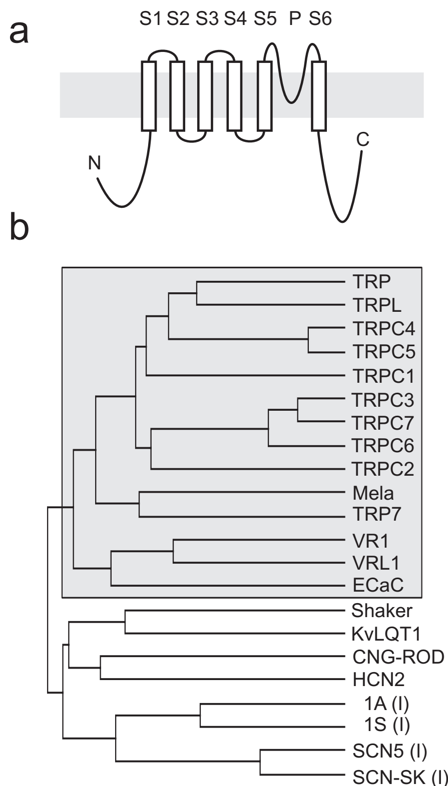


Fig. 2a,b Phylogenetic tree of the six-transmembrane-domain family of cation channels. **a** The generic structure of six-transmembrane-domain channels is depicted schematically. The channels contain intracellular N- and C-termini. *S1–S6* Transmembrane helices; *P* a segment thought to form the channel pore and determining ion selectivity. **b** The amino acid sequences of the transmembrane cores (*S1–S6*) of selected members of the main subfamilies were aligned using the Clustal algorithm. *Shaded box* TRP family. *Mela* Melastatin; *TRP7* melastatin-homologous cDNA; *VR1* vanilloid receptor 1; *VRL1* "vanilloid receptorlike" heat-activated cation channel; *ECaC* rabbit epithelial calcium channel; *Shaker Drosophila* A-type K^+ channel; *KvLQT1* delayed rectifier-type voltage-operated K^+ channel associated with a form of long QT syndrome; *α1a (I)*/*α1s (I)* first of four transmembrane cassettes from the α_1 subunits of P/Q- and L-type voltage-operated calcium channels; *SCN5 (I)*/*SCN-SK (I)* first TMD segments from heart and skeletal muscle voltage-operated sodium channels; *CNG-rod* α-subunit of rat retinal rod cyclic-nucleotide-gated cation channel; *HCN2* mouse hyperpolarization-activated cyclic-nucleotide-binding channel type 2

amino acid homology to TRP (38.8%), thus termed TRPL, was identified in the *Drosophila* eye [52]. TRPL-deficient flies also display impaired light response. Structural homology allocates the TRP/TRPL gene products to the superfamily of six-transmembrane ion channels (Fig. 2) comprising most voltage-operated potassium channels, the cyclic nucleotide gated channel families, and single transmembrane cassettes of voltage-operated calcium and sodium channels. *Trp* and *trpl* encode proteins with six putative transmembrane domains and a slightly hydrophobic pore-forming region between the transmembrane domains 5 and 6. Its fourth transmembrane domain lacks the highly conserved positively charged amino acid residues, conferring voltage-sensitivity to voltage-operated channel proteins. Both termini are thought to be located intracellularly, as shown for one of the TRP channels discussed below [53]. As a further structural hallmark of the TRP family, the N-terminus contains ankyrin homology domains thought to anchor the channel to cytoskeletal proteins.

A number of studies have found both cDNAs to be either expressed alone or coexpressed in insect or mammalian cell systems. In addition, TRP and TRPL have been functionally characterized in native photoreceptor cells from mutant flies lacking one of the two molecular components of the light-induced current. The latter approach allows investigation of the isolated components within their native physiological context.

TRP represents a fairly calcium-selective channel characterized by a unitary conductance of approximately 4 pS. In heterologous expression systems TRP expression results in increased store-operated cation currents [54, 55, 56], and attempts have been made to localize store sensitivity to a proline-rich stretch in the C-terminal cytoplasmic domain [57]. In photoreceptor cells, however, calcium mobilization after light stimulation is negligible [58, 59], and thapsigargin, an inhibitor of Ca^{2+} ATPase-mediated calcium uptake into the calcium store, fails to activate a receptor current. Most importantly, genetic disruption of the singular *Drosophila* $InsP_3$ receptor gene leaves visual responses entirely unaffected [60]. Thus, store depletion should no longer be regarded as the physiological activation mechanism for the light-induced current [61].

TRPL behaves as a nonselective cation channel poorly discriminating between mono- and divalent cations with a single channel conductance of 35 pS [62] as determined by fluctuation analysis of photoreceptor currents. In excised patches under calcium- and magnesium-free conditions, the unitary conductance of TRPL is approximately 115 pS [63, 64]. In heterologous expression systems the channel displays some constitutive activity [64, 65], is inhibited by intracellular divalent cations such as Ca^{2+} and Mg^{2+} [63, 64], and is activated in a receptor/G-protein-dependent fashion [66, 67]. Some authors assign this activation to $InsP_3$ [68], whereas others have not confirmed this finding [69]. Receptor-mediated TRPL activation relies exclusively on membrane-confined signaling steps and is initiated by G proteins of the $G_{q/11}$ subtype as demonstrated by channel activation following the application of constitutively active $G\alpha_{11}$ subunits to excised patches [70]. PLC has been further shown to be a crucial intermediate within this signaling cascade [69]. Recently polyunsaturated fatty acids were introduced as direct activators of TRP and TRPL channels delineating a PLC-dependent signaling pathway not related to store-operated calcium entry [71].

Drosophila visual transduction channels take part in the formation of supramolecular complexes additionally containing signaling molecules such as PLC, protein kinase C, and calmodulin assembled by a scaffolding protein derived from the a mutant fly with an "inactivation no afterpotential D" visual phenotype. This protein inter-

connects the components of the signaling complex [72, 73, 74], resulting in spatially well-organized structures referred to either as "transducisome" or as "signalplex." The former notion was derived from biochemical findings in the *Drosophila* eye, the latter stresses the subcellular complexity and dynamic nature of protein interactions within the signaling complex [75, 78]. The physiological importance of signaling complex formation was recently demonstrated in mutant photoreceptor cells. The mutant allele mentioned above interferes with complex formation and precludes a coordinated light response [76]. Supramolecular assembly of the mammalian counterparts of TRP/TRPL (see below) has not been demonstrated yet.

Several studies addressing the mutual relationship of TRP and TRPL postulate heteromultimer formation based on coimmunoprecipitation and electrophysiological data obtained under heterologous coexpression conditions [56, 77]. However, a detailed analysis of activation kinetics, ion selectivity, and block in wild-type photoreceptors compared to *trp* and *trpl* mutants suggests an independent and additive contribution of TRP and TRPL to the light-induced current [62].

In summary, the *Drosophila* phototransduction proteins TRP and TRPL can be regarded as the first molecularly defined receptor-stimulated cation channels. They most probably form homomultimeric, biophysically distinct channels that are parts of supramolecular signaling complexes. The activation of both TRP and TRPL depends on PLC activity and is most likely mediated by fatty acid second messengers generated by an as yet unidentified pathway in a store depletion-independent manner.

Cloning of mammalian TRP-homologous channels

Based on structural information obtained from TRP and TRPL, numerous efforts have been undertaken to identify homologues in other species. A close TRP homologue was identified in the eye of the blowfly *Calliphora vicina* [72]. A distant TRP homologue has been biochemically purified and cloned from squid [79]. Proteins more distantly related to TRP such as the vanilloid receptor channels [80, 81] or an additional membrane protein family with extremely long N-termini [82, 83] are not discussed in this review.

Searching expressed sequence tag databases, a cDNA fragment corresponding to human TRP-homologous channel (TRPC) 1 was identified, and the corresponding full-length cDNA was cloned [84, 85, 86]. TRPC1 shares the assumed membrane topology with TRP and TRPL including structural motifs such as the three N-terminal ankyrin homology repeats. As inferred from glycosylation scanning [53], TRPC proteins possess an additional nontransmembrane hydrophobic segment N-terminally of the six transmembrane domains. This S0 segment is conserved in TRPL and squid TRP, but not in TRP and distantly related TRP homologues such as the vanilloid receptors. The functional role of this domain remains elusive. The C-terminal domain is considerably shorter than that of the *Drosophila* proteins. From the cDNAs reported, hTRPC1A is alternatively spliced and lacks the first of three ankyrin homology domains. A rat TRPC1A orthologue has been shown to be correctly inserted into the plasma membrane [87]. Whereas in humans no further splice variants have been identified, a mouse insulinoma cell line expresses as many as four different N-terminal splice variants [88]. TRPC1 orthologues are widely expressed in mammals, and their amino acid sequence is highly conserved (>99%) throughout species. Even a more distantly related species such as the claw frog *Xenopus laevis* expresses a TRPC1 orthologue sharing 89% amino acid homology with its mammalian counterparts [89].

Expressed sequence tag databases contained two additional human TRP-homologous cDNA sequences. In accord with the nomenclature proposed by Montell and colleagues [84], they are referred to as trpc2 and trpc3. Human trpc2 most probably is a pseudogene because the open reading frames of all known trpc2 sequence tags contain multiple premature stop codons. Bovine trpc2 mRNA is expressed in a restricted number of tissues, for example, testis (see Table 1), encoding a protein which is truncated at its N-terminus, i.e., it comprises only the last four transmembrane domains and the cytoplasmic C-terminus. The functional role of this or other truncated TRPCs remains unclear. Full-length TRPC2 proteins are expressed in rodents. Mouse TRPC2 is reported to contain a substantially longer N-terminus than its rat orthologue or any other TRPC. It has been reported as possible SOC predominantly expressed in testis [90]. Conversely, rat TRPC2 expression is restricted to the vomeronasal organ, a sensory apparatus involved in pheromone perception [91]. Similar to *Drosophila* photoreceptor rhabdomeres, vomeronasal receptor cells contain a specialized microvillar signaling compartment which by itself does not contain calcium stores. Rat TRPC2 is exclusively expressed in this compartment, and future research will have to evaluate the functional role of this protein.

Full-length human trpc3 cDNA encodes an 873 amino acid protein [92, 93] with closely related orthologues in rodents. It is expressed mainly but not exclusively in brain. By means of homology searches, the family of mammalian TRP homologues has been shown to contain at least seven members, TRPC1–TRPC7 [92, 94]. TRPC3–TRPC7 form two structural subfamilies within the TRPC family, as shown in the phylogenetic tree in Fig. 2. Full-length cDNAs of mouse and rat trpc6 have been isolated from brain [95, 96, 97], and human trpc6 from placenta [98]. Murine TRPC6 is expressed as two splice variants, the shorter lacking a 54 amino acid sequence at the extreme N-terminus in comparison with the rat and human protein. In contrast to TRPC3, TRPC6 appears to be more widely expressed in extraneural tissues, for instance, in lung (Fig. 3, Table 1). Very recently a new TRPC3-like channel was cloned which is predominantly expressed in mouse heart, lung, and eye, and

Table 1 Tissue distribution of TRP channels (*MTN* multiple tissue northern blotting, *RT-PCR* reverse transcriptase polymerase chain reaction, *ISH* in situ hybridization)

Subtype	Species	Method	Tissue distribution	Reference
TRPC1	Human	MTN	Heart, brain, testis, ovary, intestine	[84]
		MTN	Ubiquitous	[85]
	Rat	RT-PCR	Ubiquitous	[111]
		RT-PCR	Heart, brain, kidney, intestine, adrenal gland	[97]
TRPC2	Cattle	MTN	Testis, liver, spleen	[112]
		ISH	Late spermatogenic cells	[112]
	Mouse	MTN	Testis, cerebrum, cerebellum, heart	[90]
	Rat	ISH	Vomeronasal organ sensory epithelium	[91]
TRPC3	Human	MTN	Brain	[92]
	Mouse	ISH	Cerebellar Purkinje cells	[113]
		MTN	Brain	[113]
	Rat	RT-PCR	Ubiquitous	[111]
		RT-PCR	Cerebellum, midbrain, olfactory bulb, cortex	[97]
TRPC4	Cattle	MTN	Adrenal gland, testis, retina, heart, brain	[99]
	Mouse	MTN	Cerebrum, adrenal gland, cerebellum, ubiquitous	[113]
		ISH	Dentate gyrus granule cells, ca1 pyramidal neurons, cortex	[113]
	Rat	RT-PCR	Olfactory bulb, hippocampus, cortex	[97]
		RT-PCR	Cerebrum, nodose ganglion, testis, ovary, heart, lung	[111]
TRPC5	Mouse	MTN	Brain	[101]
		RT-PCR	Brain, testis, kidney, uterus	[101]
	Rat	RT-PCR	Cerebrum, cerebellum, ovary, adrenal gland, testis, ovary, nodose ganglion, kidney	[111]
	Rabbit	MTN	Brain	[102]
TRPC6	Human	MTN	Lung, placenta, ovary, spleen, ubiquitous	This report
	Mouse	MTN	Lung, brain	[95]
	Rat	RT-PCR	Lung, cerebrum, ovary, ubiquitous	[111]
		RT-PCR	Cerebral cortex, hippocampus, heart, kidney, lung, adrenal gland	[97]
		ISH	Dentate gyrus granule cells, cerebral cortical neurons	[97]
TRPC7	Mouse	MTN	Heart, lung, eye, hindbrain, spleen, testis	[94]
		ISH	Cerebellar Purkinje cells, olfactory bulb, hippocampus	[94]

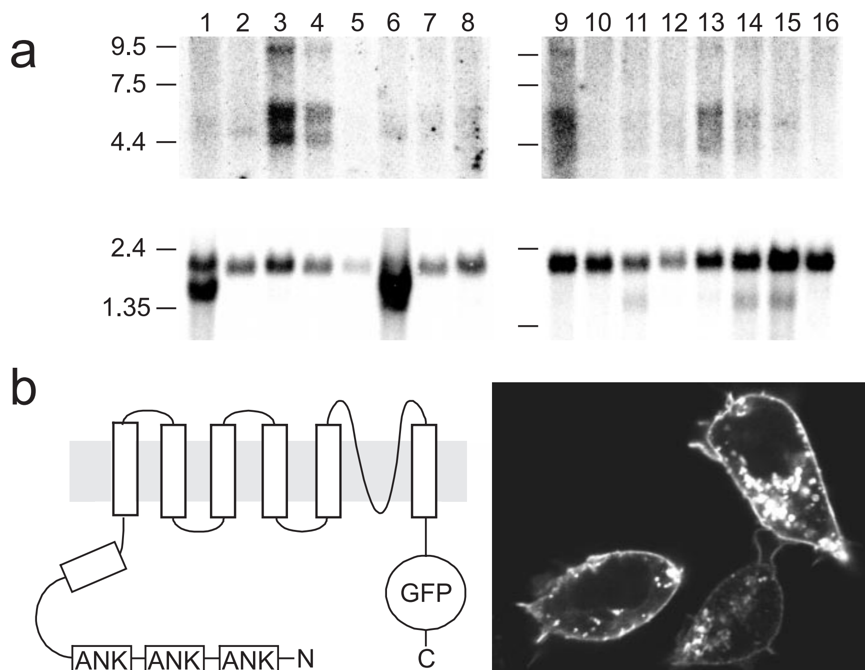


Fig. 3a,b Tissue distribution and subcellular localization of human TRPC6. **a** Multiple-tissue Northern blot. The blots were probed with a fragment comprising nucleotides 393–1546 and washed under highly stringent conditions. Blots were reprobed with human β-actin (below). The lane loading is as follows: *1* heart; *2* brain; *3* placenta; *4* lung; *5* liver; *6* skeletal muscle; *7* kidney; *8* pancreas; *9* spleen; *10* thymus; *11* prostate; *12* testis; *13* ovary; *14* small intestine; *15* colon; *16* peripheral blood leukocytes. **b** Subcellular localization of human TRPC6 in a heterologous expression system. A chimera of TRPC6 and green fluorescent protein (*GFP*), as depicted schematically, was transiently expressed in human embryonic kidney 293 cells. Cells were examined by confocal laser microscopy

which is classified as mTRP7 [94]. The logical nomenclature chosen by these authors is at odds with the previous classification of a very distantly *trp*-related, melastatin-like cDNA as trpc7 [81]. Therefore we refer to the latter gene as "trp7" because it is not yet clear whether it indeed encodes a channel (Fig. 2).

Trpc4 full-length cDNA was cloned from bovine adrenal gland [99]. In mice TRPC4 is expressed as two C-terminal splice variants, TRPC4A and TRPC4B, with TRPC4B being 80 amino acids shorter than the bovine protein and a rat orthologue. Interestingly, rat TRPC4 lacks 23 amino acids corresponding to the highly conserved putative second transmembrane domain [100]. Whether this protein shares the membrane topology of other TRPCs [53], and whether this deletion has also functional consequences, remains to be addressed. TRPC5, a close homologue of TRPC4, is known from mouse and rabbit [101, 102]. TRPC5 expression appears to be more restricted to neuronal and endocrine tissues than is TRPC4 (see Table 1).

For most TRPCs orthologues are highly conserved among species (>90%). Functional characterization is currently under way, and in many cases different orthologues are used by different workers. This may account for some of the partly conflicting functional results reviewed below.

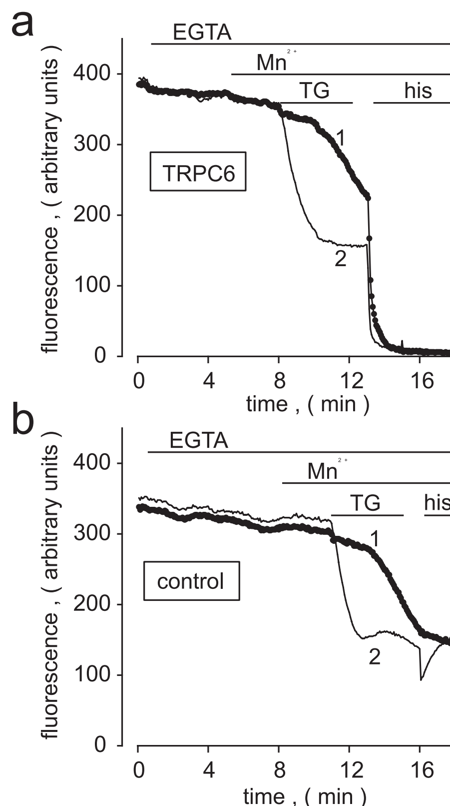


Fig. 4a,b Store depletion-independent activation of human TRPC6. Chinese hamster ovary cells were microinjected with expression constructs coding for the histamine H_1 receptor and either TRPC6 or empty vector (*control*). Cells were loaded with the fluorescent dye fura-2, which senses intracellular Ca^{2+} by a specific spectral shift and manganese cations (Mn^{2+}) by an overall decrease in fluorescence. Both TRPC6 and control cells were superfused with a Ca^{2+}-free medium containing the Ca^{2+} chelator ethylene-glycol-*O*,*O*'-bis-*N*, *N*,*N*',*N*'-tetraacetic acid (*EGTA*) to remove trace amounts of calcium. Mn^{2+} was present for the time indicated. The time course at two different excitation wavelengths in one single cell either TRPC6-positive (**a**) or control (**b**) is depicted. *Dotted line* fluorescence at the isosbestic wavelength (1,360 nm), which is unaffected by Ca^{2+}-induced spectral shifts. Fluorescence decreases at wavelength 2 (380 nm, thin line) relative to wavelength 1 indicates an increase in the intracellular free calcium concentration. In both cases, thapsigargin (*TG*), an inhibitor of calcium ATPases, passively releases calcium into the cytosol as sensed at wavelength 2 and induces a small and delayed decline at wavelength 1, representing influx through endogenous Mn^{2+}-permeable store-operated channels. Subsequent stimulation of the coexpressed H_1 histamine receptor with 100 µM histamine (*his*) induces a Mn^{2+} influx exclusively in TRPC6-expressing cells

Store-operated cation entry via TRP channels

According to the hypothesis of TRP being a SOC, it was inferred that its mammalian homologues would represent store-operated calcium entry channels. The observation that stable expression of a mixture of trpc1–trpc6 partial cDNAs in antisense direction resulted in diminished calcium influx after receptor stimulation was interpreted as a hint that one or more TRPCs are SOCs [92]. In the same report, recalcification transients were enhanced either after activation of a G_q-coupling receptor or after passive store depletion with the calcium ATPase inhibitor thapsigargin in cells overexpressing human TRPC3. It was concluded that TRPC3 might be a molecularly defined mammalian SOC. Subsequently other authors have reported store-operated cation entry via TRPC3 [103, 104].

Irrespective of the activation mechanism, it seems that TRPC3 cannot be the molecular substrate of I_{crac} or of any other higher conductance, calcium-selective SOCs because TRPC3 is a nonselective cation channel positively regulated by $[Ca^{2+}]_i$ with a high unitary conductance of 66 pS [93].

A recent report [105] supports the hypothesis of TRPC3 being a store-operated calcium-permeable channel by describing low-level stable expression of TRPC3 in the HEK293 cell line giving rise to currents which can be activated exclusively under conditions under which both $InsP_3$ bound to its receptor and depleted calcium store organelles are present. In excised membrane patches, TRPC3 currents activated under these conditions were reported to be abolished after extensive washing and restituted by addition of brain microsomal membranes. From these findings a conformational coupling model of channel activation was deduced for TRPC3. For TRPC6, a close structural relative of TRPC3, available data argue against a store-operated activation mechanism. A representative experiment shown in Fig. 4 further illustrates findings published previously [95, 98]. Thapsigargin treatment transiently elevates $[Ca^{2+}]_i$ and induces a moderate store depletion-induced Mn^{2+} influx in both TRPC6-expressing and control cells, whereas

subsequent stimulation of the G_q-coupling H_1 histamine receptor elicits a prominent and immediate Mn^{2+} influx exclusively in TRPC6-expressing cells. This observation strongly suggests a store depletion-independent mechanism of receptor-stimulated activation of this channel.

Bovine TRPC4 was reported to form a nonselective cation channel activated by dialysis of nonhydrolysable guanine nucleotide analogues into cells, indicating a G-protein-dependent mechanism. Because a similar current was triggered by $InsP_3$ dialysis or by thapsigargin treatment, TRPC4 was considered as a SOC. In addition, channel activation has been shown to depend on the external calcium concentration [99]. The closest structural relative of TRPC4, TRPC5, has been characterized as a SOC mainly by means of recalcification experiments monitoring changes in fura-2 fluorescence. Similarly, TRPC5 activation depends on extracellular calcium [102]. An alternative hypothesis for TRPC5 activation is discussed below.

For TRPC1, functional data are very scarse. Human TRPC1A encodes a 16-pS (by fluctuation analysis) nonselective cation channel. Thapsigargin treatment of TRPC1 expressing cells is reported to result in augmented whole cell currents and elevated calcium transients in recalcification experiments [86]. Therefore TRPC1 is considered a SOC. However, others [106] reported that the same splice variant of TRPC1 is poorly store-operated when expressed in the baculovirus/Sf9 cell system, but rather behaves as a constitutively active calcium entry pathway.

Thus for nearly every known TRPC a store-operated gating mechanism has been discussed by at least one group. For many TRP channels, however, evidence in favor of noncapacitative activation hypotheses is accumulating.

Receptor-activated cation entry via TRP channels

Although TRPC4 is thought to be a SOC by some investigators, there is convincing evidence that at least its closest structural relative, TRPC5, is activated independently of store depletion [101]. Expression of this protein gives rise to a nonselective cation current, with some calcium selectivity and a current-voltage relationship very similar to rabbit TRPC5 [102]. However, expression of mouse TRPC5 does not enhance recalcification transients after thapsigargin treatment, and subsequent receptor stimulation induces an additional elevation in $[Ca^{2+}]_i$. These observations suggest a noncapacitative, rather than a store-operated mechanism of TRPC5 activation.

In the case of TRPC3 there is convincing evidence that store-operated activation does not, or at least not fully, explain its activation mechanism. TRPC3 is activated downstream of receptor stimulation [107, 108] and PLC activation [109]. In most instances, common protocols that activate SOCs fail to activate TRPC3. TRPC3 expression poorly augments recalcification-induced calcium transients following thapsigargin treatment [107], and $InsP_3$ dialysis into TRPC3-expressing cells evokes no currents [93]. However, a steep positive calcium dependence of TRPC3 function is observed and is discussed as a possible mechanism of noncapacitative, receptor-mediated activation. For TRPC6, the structurally closest relative of TRPC3, a similar noncapacitative, receptor-stimulated activation mechanism has been established [95, 98].

The inhibition of receptor-mediated TRPC6 activation by a PLC inhibitor indicates that the activity of this enzyme may be a crucial intermediate within this signaling pathway. This observation could also be relevant for SOCs, because $InsP_3$ production resulting from PLC activity activates calcium release and store depletion. However, $InsP_3$ dialyzed into TRPC6-expressing cells did not alter membrane conductance, thus ruling out both SOC activation and direct activation of a channel by $InsP_3$ [98]. Interestingly, external application of membrane-permeable DAG analogues gives rise to TRPC6 currents as well as Ca^{2+} and Mn^{2+} influx. Application of endogenously occurring DAGs to the cytosolic face of excised membrane patches evokes TRPC6 single-channel activity. The precursor of this membrane lipid messenger, phosphatidylinositol-4,5-bisphosphate, and monoacylglycerols fail to activate TRPC6. A possible contribution of protein kinase C to TRPC6 activation has been excluded [98]. DAG activates TRPC6 in a membrane-delimited fashion. TRPC3 is activated by DAG in a similar manner, while other TRP channels are unresponsive to this lipid messenger. The recently discovered mouse TRPC7, a very close homologue of TRPC3 [94] (Fig. 2) is activated by DAG in a similar fashion. Thus TRPC3/6/7-type TRPCs form a structural and functional subfamily of second messenger-operated cation channels coupling receptor-PLC signaling pathways to calcium entry. Receptor-stimulated cation channels displaying similar activation characteristics in a native cellular environment are known from rabbit portal vein myocytes [110]. It is tempting to speculate that this or one of the other known receptor-stimulated cation channels rather than SOCs represents the native correlate of the TRPC3/6/7 family.

Conclusions

Although receptor-mediated cation entry is observed in a large number of cell types, its role in cellular calcium homeostasis is only partly understood. While in smooth-muscle cells, receptor-activated cation channels are known to contribute directly to cellular effects such as contraction, a major contribution to the overall rise in $[Ca^{2+}]_i$ appears unlikely in many other cell types, mainly due to the low expression level of these channel proteins [27, 28]. Numerous cellular functions (e.g., several signaling pathways responsible for cellular differentiation), however, rely upon the temporospatial pattern of cellular calcium concentration rather than its mere amplitude.

There is evidence that receptor-activated cation channels are involved in the fine-tuning of calcium dynamics, for example, as frequency modulators of calcium oscillations by providing localized "trigger calcium" [33]. As a first hint to the functional relevance of receptor-activated TRPC channels cellular differentiation, the development of neuroendocrine tumors in the mouse model has been found to be associated with down-regulation of TRPC6 [96].

The limited knowledge about the molecular identity of the channels involved in either store-operated or receptor-activated cation currents precludes a systematic approach to these physiological effects in the majority of cases. In this context, the TRPC family provides the first structural knowledge about candidate receptor-activated cation channels. At this point, it can be stated that TRPC proteins represent more or less nonselective cation channels which are activated after receptor stimulation (Fig. 4). Some of them may contribute to store-operated cation entry but none of them matches the biophysical features of known SOCs, especially I_{crac}. Most of them are more likely to represent receptor- or second messenger operated channels rather than SOCs. However, we are just beginning to understand the exact activation mechanisms of TRPCs. With the identification of lipid messengers as activators of TRPC3, TRPC6, TRPC7, TRP, and TRPL gating, novel physiological roles for these second messengers have been defined. Until now most functional data on TRPC channels have been derived from in vitro models. The analysis of tissue expression pattern alone fails to relate these results unequivocally to the large body of literature on native receptor-activated channels. The identification of direct cellular activators such as DAG, which are specific for a single subfamily of TRPCs, offers novel experimental approaches to examine the molecular identity of the channels underlying these currents in native cell systems. In addition, the availability of transgenic techniques may be instrumental in evaluating the role of single members of the widely expressed TRPC family in vivo and may thus help to resolve the bewildering complexity of receptor-activated calcium entry.

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References

1. Divecha N, Irvine RF (1995) Phospholipid signaling. Cell 80:269–278
2. Streb H, Irvine RF, Berridge MJ, Schulz I (1983) Release of Ca^{2+} from a nonmitochondrial intracellular store in pancreatic acinar cells by inositol-(1,4,5)-trisphosphate. Nature 306:67–69
3. Kishimoto A, Takai Y, Mori T, Kikkawa U, Nishizuka Y (1980) Activation of calcium and phospholipid-dependent protein kinase by diacylglycerol, its possible relation to phosphatidylinositol turnover. J Biol Chem 255:2273–2276
4. Mellor H, Parker PJ (1998) The extended protein kinase C superfamily. Biochem J 332:281–292
5. Berridge MJ (1993) Inositol trisphosphate and calcium signalling. Nature 361:315–325
6. Jones WJ (1998) Overview of voltage-operated calcium channels. J Bioenerg Biomemb 30:299–312
7. Mozhayeva GN, Naumov AP, Kuryshev YA (1989) Epidermal growth factor activates calcium-permeable channels in A431 cells. Biophys Acta 1011:171–175
8. Ma H, Matsunaga H, Li B, Marrero MB, Ling BN (1996) Regulation of PDGF-β receptor-operated Ca^{2+} channels by phospholipase C-γ1 in glomerular mesangial cells. Am J Physiol 271:F994–F1003
9. Marrero MB, Venema RC, Ma H, Ling BN, Eaton DC (1998) Erythropoietin receptor-operated Ca^{2+} channels: activation by phospholipase Cγ. Kidney Int 53:1259–1268
10. Benham CD, Bolton TB, Lang RJ (1985) Acetylcholine activates an inward current in single mammalian smooth muscle cells. Nature 316:345–347
11. Benham CD, Tsien RW (1987) A novel receptor-operated Ca^{2+}-permeable channel activated by ATP in smooth muscle. Nature 328:275–278
12. Benham CD, Tsien RW (1988) Noradrenaline modulation of calcium channels in single smooth muscle cells from rabbit ear artery. J Physiol (Lond) 404:767–784
13. Van Renterghem C, Romey G, Lazdunski M (1988) Vasopressin modulates the spontaneous electrical activity in aortic cells (line A7r5) by acting on three different types of ionic channels. Proc Natl Acad Sci USA 85:9365–9369
14. Kuriyama H, Kitamura K, Nabata H (1995) Pharmacological and physiological significance of ion channels and factors that modulate them in vascular tissues. Pharmacol Rev 47:387–573
15. Kuriyama H, Kitamura K, Itoh T, Inoue R (1998) Physiological features of visceral smooth muscle cells, with special reference to receptors and ion channels. Physiol Rev 78:811–920
16. Fasolato C, Hoth M, Matthews G, Penner R (1993) Ca^{2+} and Mn^{2+} influx through receptor-mediated activation of nonspecific cation channels in mast cells. Proc Natl Acad Sci USA 90:3068–3072
17. Kaupp UB, Niidome T, Tanabe T, Terada S, Bonigk W, Stuhmer W, Cook NJ, Kangawa K, Matsuo H, Hirose T, et al (1989) Primary structure and functional expression from complementary DNA of the rod photoreceptor cyclic GMP-gated channel. Nature 342:762–766
18. Biel M, Zong X, Ludwig A, Sautter A, Hofmann F (1999) Structure and function of cyclic nucleotide-gated channels. Rev Physiol Biochem Pharmacol 135:151–171
19. DiFrancesco D, Tortora P (1991) Direct activation of cardiac pacemaker channels by intracellular cyclic AMP. Nature 351: 145–147
20. Gauss R, Seifert R, Kaupp UB (1998) Molecular identification of a hyperpolarization-activated channel in sea urchin sperm. Nature 393:583–587
21. Ludwig A, Zong X, Jeglitsch M, Hofmann F, Biel M (1998) A family of hyperpolarization-activated mammalian cation channels. Nature 393:587–591
22. Santoro B, Liu DT, Yao H, Bartsch D, Kandel ER, Siegelbaum SA, Tibbs GR (1998) Identification of a gene encoding a hyperpolarization-activated pacemaker channel of brain. Cell 93:717–729
23. von Tscharner V, Prod'hom B, Baggiolini M, Reuter H (1986) Ion channels in human neutrophils activated by a rise in free cytosolic calcium concentration. Nature 324:369–372
24. Loirand G, Pacaud P, Baron A, Mironneau C, Mironneau J (1991) Large conductance calcium-activated non-selective cation channel in smooth muscle cells isolated from rat portal vein. J Physiol (Lond) 437:461–475
25. Obukhov AG, Jones SVP, Degtiar VE, Lückhoff A, Schultz G, Hescheler J (1995) Ca^{2+}-permeable large-conductance non-selective cation channels in rat basophilic leukemia cells. Am J Physiol 269:C1119–C1125
26. Kuno M, Gardner P (1987) Ion channels activated by inositol-(1,4,5)-trisphosphate in plasma membrane of human T-lymphocytes. Nature 326:301–304

27. Matthews G, Neher E, Penner R (1989) Second-messenger-activated calcium influx in rat peritoneal mast cells. J Physiol (Lond) 418:105–130
28. Penner R, Matthews G, Neher E (1988) Regulation of calcium influx by second messengers in rat mast cells. Nature 334:499–504
29. Kiselyov KI, Mamin AG, Semyonova SB, Mozhayeva GN (1997) Low-conductance high selective inositol-(1,4,5)-trisphosphate activated Ca^{2+} channels in plasma membrane of A431 carcinoma cells. FEBS Lett 407:309–312
30. Kiselyov KI, Semyonova SB, Mamin AG, Mozhayeva GN (1999) Miniature Ca^{2+} channels in excised plasma-membrane patches: activation by IP_3. Pflügers Arch 437:305–314
31. Lückhoff A, Clapham DE (1992) Inositol-(1,3,4,5)-tetrakisphosphate activates an endothelial Ca^{2+}-permeable channel. Nature 355:356–358
32. Peppelenbosch MP, Tertoolen LG, den Hertog J, de Laat SW (1992) Epidermal growth factor activates calcium channels by phospholipase A_2/5-lipoxygenase-mediated leukotriene C_4 production. Cell 69:295–303
33. Shuttleworth TJ, Thompson JL (1998) Muscarinic receptor activation of arachidonate-mediated Ca^{2+} entry in HEK293 cells is independent of phospholipase C. J Biol Chem 273:32636–32643
34. Broad LM, Cannon TR, Taylor CW (1999) A non-capacitative pathway activated by arachidonic acid is the major Ca^{2+} entry mechanism in rat A7r5 smooth muscle cells stimulated with low concentrations of vasopressin. J Physiol (Lond) 517:121–134
35. Hoth M, Penner R (1992) Depletion of intracellular calcium stores activates a calcium current in mast cells. Nature 355: 353–356
36. Hoth M, Penner R (1993) Calcium release-activated calcium current in rat mast cells. J Physiol (Lond) 465:359–386
37. Zweifach A, Lewis RS (1993) Mitogen-regulated Ca^{2+} current of T lymphocytes is activated by depletion of intracellular Ca^{2+} stores. Proc Natl Acad Sci USA 90:6296–6299
38. Kerschbaum HH, Cahalan MD (1999) Single-channel recording of a store-operated Ca^{2+} channel in Jurkat T-lymphocytes. Science 283:836–839
39. Zweifach A, Lewis (1995) Rapid inactivation of depletion-activated calcium current (I_{crac}) due to local calcium feedback. J Gen Physiol 105:209–226
40. Lückhoff A, Clapham DE (1994) Calcium channels activated by depletion of internal calcium stores in A431 cells. Biophys J 67:177–182
41. Vaca L, Kunze DL (1994) Depletion of intracellular Ca^{2+} stores activates a Ca^{2+}-selective channel in vascular endothelium. Am J Physiol 267:C920–C925
42. Krause E, Pfeiffer F, Schmid A, Schulz I (1996) Depletion of intracellular calcium stores activates a calcium conducting cation current in mouse pancreatic acinar cells. J Biol Chem 271:32523–32528
43. Irvine RF (1990) 'Quantal' Ca^{2+} release and the control of Ca^{2+} entry by inositol phosphates – a possible mechanism. FEBS Lett 263:5–9
44. Randriamampita C, Tsien RY (1993) Emptying of intracellular Ca^{2+} stores releases a small messenger that stimulates Ca^{2+} influx. Nature 364:809–814
45. Yao Y, Ferrer-Montiel AV, Montal M, Tsien RY (1999) Activation of store-operated Ca^{2+} current in *Xenopus* oocytes requires SNAP-25 but not a diffusible messenger. Cell 98:475–485
46. Patterson RL, van Rossum DB, Gill DL (1999) Store-operated Ca^{2+} enty: evidence for a secretion-like coupling model. Cell 98:487–499
47. Holda JR, Blatter LA (1997) Capacitative calcium entry is inhibited in vascular endothelial cells by disruption of cytoskeletal microfilaments. FEBS Lett 403:191–196
48. Hardie RC (1991) Whole-cell recordings of the light induced current in dissociated *Drosophila* photoreceptors: evidence for feedback by calcium permeating the light-sensitive channels. Proc R Soc Lond B Biol Sci 245:203–210
49. Hardie RC (1996) INDO-1 measurements of absolute resting and light-induced Ca^{2+} concentration in *Drosophila* photoreceptors. J Neurosci 16:2924–2933
50. Scott K, Sun Y, Beckingham K, Zuker CS (1997) Calmodulin regulation of *Drosophila* light-activated channels and receptor function mediated termination of the light response in vivo. Cell 91:375–383
51. Montell C, Rubin MR (1989) Molecular characterization of the *Drosophila trp* locus: a putative integral membrane protein required for phototransduction. Neuron 2:1313–1323
52. Phillips AM, Bull A, Kelly LE (1992) Identification of a *Drosophila* gene encoding a calmodulin-binding protein with homology to the *trp* phototransduction gene. Neuron 8:631–642
53. Vannier B, Zhu X, Brown D, Birnbaumer L (1998) The membrane topology of human transient receptor potential 3 as inferred from glycosylation-scanning mutagenesis and epitope immunocytochemistry. J Biol Chem 293:8675–8679
54. Vaca L, Sinkins WG, Hu Y, Kunze DL, Schilling WP (1994) Activation of recombinant TRP by thapsigargin in Sf9 cells. Am J Physiol 267:C1501–C1505
55. Petersen CCH, Berridge MJ, Borgese MF, Bennett DL (1995) Putative capacitive calcium entry channels: expression of *Drosophila* TRP and evidence for the existence of vertebrate homologues. Biochem J 311:41–44
56. Xu XZS, Li HS, Guggino WB, Montell C (1997) Coassembly of TRP and TRPL produces a distinct store-operated conductance. Cell 89:1155–1164
57. Sinkins WG, Vaca L, Hu Y, Kunze DL, Schilling WP (1996) The COOH-terminal domain of *Drosophila* TRP channels confers thapsigargin sensitivity. J Biol Chem 271:2955–2960
58. Peretz A, Suss-Toby E, Rom-Glas A, Arnon A, Payne R, Minke B (1994) The light response of *Drosophila* photoreceptors is accompanied by an increase in cellular calcium: effects of specific mutations. Neuron 12:1257–1267
59. Ranganathan R, Bacskai BJ, Tsien RY, Zuker CS (1994) Cytosolic calcium transients: spatial localization and role in *Drosophila* photoreceptor cell function. Neuron 13:837–848
60. Acharya JK, Jalink K, Hardy RW, Hartenstein V, Zuker CS (1997) $InsP_3$ receptor is essential for growth and differentiation but not for vision in *Drosophila*. Neuron 18:881–887
61. Scott K, Zuker CS (1998) TRP, TRPL and trouble in photoreceptor cells. Curr Opin Neurobiol 8:383–388
62. Reuss H, Mojet MH, Chyb S, Hardie RC (1997) In vivo analysis of the *Drosophila* light-sensitive channels, TRP and TRPL. Neuron 19:1249–1259
63. Obukhov AG, Schultz G, Lückhoff A (1998) Regulation of heterologously expressed transient receptor potential-like channels by calcium ions. Neuroscience 85:487–495
64. Kunze DL, Sinkins WG, Vaca L, Schilling WP (1997) Properties of single TRPL channels expressed in Sf9 insect cells. Am J Physiol 272:C27–C34
65. Hu Y, Vaca L, Zhu X, Birnbaumer L, Kunze DL, Schilling WP (1994) Appearance of a novel Ca^{2+} influx pathway in Sf9 insect cells following expression of the transient receptor potential-like (TRPL) protein of *Drosophila.* Biochem Biophys Res Commun 201:1050–1056
66. Hu Y, Schilling WP (1995) Receptor-mediated activation of recombinant TRPL expressed in Sf9 insect cells. Biochem J 305:605–611
67. Harteneck C, Obukhov AG, Zobel A, Kalkbrenner F, Schultz G (1995) The *Drosophila* cation channel TRPL expressed in insect Sf9 cells is stimulated by agonists of G-protein-coupled receptors. FEBS Lett 358:297–300
68. Dong Y, Kunze DL, Vaca L, Schilling WP (1995) Ins $(1,4,5)P_3$ activates *Drosophila* cation channel TRPL in recombinant baculovirus-infected Sf9 insect cells. Am J Physiol 269: C1332–C1339
69. Hardie RC, Raghu P (1998) Activation of heterogenously expressed Drosophila TRPL channels: Ca^{2+} is not required and $InsP_3$ is not sufficient. Cell Calcium 24:153–163
70. Obukhov AG, Harteneck C, Zobel A, Harhammer R, Kalkbrenner F, Leopoldt D, Lückhoff A, Nürnberg B, Schultz G

(1996) Direct activation of TRPL cation channels by $G\alpha_{11}$ subunits. EMBO J 15:5833–5838
71. Chyb S, Raghu P, Hardie RC (1999) Polyunsaturated fatty acids activate the *Drosophila* light-sensitive channels TRP and TRPL. Nature 397:255–259
72. Huber A, Sander P, Gobert A, Bähner M, Hermann R, Paulsen R (1996) The transient receptor potential protein (TRP), a putative store-operated Ca^{2+} channel essential for phosphoinositide-mediated photoreception, forms a signaling complex with NorpA, InaC and InaD. EMBO J 15:7036–7045
73. Chevesich J, Kreuz AJ, Montell C (1997) Requirement for the PDZ domain protein, INAD, for localization of the TRP store-operated channel to a signaling complex. Neuron 18:95–105
74. Huber A, Sander P, Bähner M, Paulsen R (1998) The TRP Ca^{2+} channel assembled in a signaling complex by the PDZ domain protein INAD is phosphorylated through the interaction with protein kinase C (ePKC). FEBS Lett 425:317–322
75. Montell C (1998) TRP trapped in fly signaling web. Curr Opin Neurobiol 8:389–397
76. Scott K, Zuker CS (1998) Assembly of the *Drosophila* phototransduction cascade into a signalling complex shapes elementary responses. Nature 395:805–808
77. Gillo B, Chorna I, Cohen H, Cook B, Manistersky I, Chorev M, Arnon A, Pollock JA, Selinger Z, Minke B (1996) Coexpression of *Drosophila* TRP and TRP-like proteins in *Xenopus* oocytes reconstitutes capacitative Ca^{2+} entry. Proc Natl Acad Sci USA 93:14146–14151
78. Tsunoda S, Sierralta J, Sun Y, Bodner R, Suzuki E, Becker A, Socolich M, Zuker CS (1997) A multivalent PDZ-domain protein assembles signaling complexes in a G-protein-coupled cascade. Nature 388:243–249
79. Monk PD, Carne A, Liu SH, Ford JW, Keen JN, Findlay JB (1996) Isolation, cloning, and characterisation of a TRP homologue from squid (*Loligo forbesi*) photoreceptor membranes. J Neurochem 67:2227–2235
80. Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389:816–824
81. Caterina MJ, Rosen TA, Tominaga M, Brake AJ, Julius D (1999) A capsaicin-receptor homologue with a high threshold for noxious heat. Nature 398:436–441
82. Hunter JJ, Shao J, Smutko JS, Dussault BJ, Nagle DL, Woolf EA, Holmgren LM, Moore KJ, Shyjan AW (1998) Chromosomal localization and genomic characterization of the mouse melastatin gene (Mlsn1). Genomics 54:116–123
83. Nagamine K, Kudoh J, Minoshima S, Kawasaki K, Asakawa S, Ito F, Shimizu N (1998) Molecular cloning of a novel putative Ca^{2+} channel protein (TRPC7) highly expressed in brain. Genomics 54:124–131
84. Wes P, Chevesich J, Jeromin A, Rosenberg C, Stetten G, Montell C (1995) TRPC1, a human homolog of a *Drosophila* store-operated channel. Proc Natl Acad Sci USA 92:9652–9656
85. Zhu X, Chu PB, Peyton M, Birnbaumer L (1995) Molecular cloning of a widely expressed human homologue for the *Drosophila trp* gene. FEBS Lett 373:193–198
86. Zitt C, Zobel A, Obukhov AG, Harteneck C, Kalkbrenner F, Lückhoff A, Schultz G (1996) Cloning and functional expression of a human Ca^{2+}-permeable cation channel activated by store depletion. Neuron 16:1189–1196
87. Wang W, O'Connell B, Dykeman R, Sakai T, Delporte C, Swaim W, Zhu X, Birnbaumer L, Ambudkar IS (1999) Cloning of Trp1β isoform from rat brain: immunodetection and localization of the endogenous Trp1 protein. Am J Physiol 276:C969–C979
88. Sakura H, Ashcroft FM (1997) Identification of four trp1 gene variants murine pancreatic beta-cells. Diabetologia 40:528–532
89. Bobanovic LK, Laine M, Petersen CCH, Bennett DL, Berridge MJ, Lipp P, Ripley SJ, Bootman MD (1999) Molecular cloning and immunolocalization of a novel vertebrate *trp* homologue from *Xenopus*. Biochem J 340:593–599
90. Vannier B, Peyton M, Boulay G, Brown D, Qin N, Jiang M Zhu X, Birnbaumer L (1999) Mouse TRP2, the homologue of the human TRPC2 pseudogene, encodes mTRP2, a store depletion-activated capacitative Ca^{2+} entry channel. Proc Natl Acad Sci USA 96:2060–2064
91. Liman ER, Corey DP, Dulac C (1999) TRP2: a candidate transduction channel for mammalian pheromone sensory signaling. Proc Natl Acad Sci USA 96:5791–5796
92. Zhu X, Jiang M, Peyton M, Boulay G, Hurst R, Stefani E, Birnbaumer L (1996) *trp*, a novel mammalian gene family essential for agonist-activated capacitive Ca^{2+} entry. Cell 85: 661–671
93. Zitt C, Obukhov AG, Strübing C, Zobel A, Kalkbrenner F, Lückhoff A, Schultz G (1997) 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
94. Okada T, Inoue R, Yamazaki K, Maeda A, Kurosaki T, Yamakuni T, Tanaka I, Shimizu S, Ikenaka K, Imoto K, Mori Y (1999) Molecular and functional characterization of a novel mouse transient receptor potential protein homologue TRP7. J Biol Chem 274:27359–27370
95. Boulay G, Zhu X, Peyton M, Jiang M, Hurst R, Stefani E, Birnbaumer L (1997) Cloning and expression of a novel mammalian homolog of *Drosophila* transient receptor potential (*Trp*) involved in calcium entry secondary to activation of receptors coupled by the G_q class of G protein. J Biol Chem 272:29672–29680
96. Buess M, Engler O, Hirsch HH, Moroni C (1999) Search for oncogenic regulators in an autocrine tumor model using differential display PCR: identification of novel candidate genes including the calcium channel mtrp6. Oncogene 18:1487–1494
97. Mizuno N, Kitayama S, Saishin Y, Shimada S, Morita K, Mitsuhata C, Kurihara H, Dohi T (1999) Molecular cloning and characterization of rat TRP homologues from brain. Brain Res Mol Brain Res 64:41–51
98. Hofmann T, Obukhov AG, Schaefer M, Harteneck C, Gudermann T, Schultz G (1999) Direct activation of human TRPC6 and TRPC3 channels by diacylglycerol. Nature 397:259–263
99. Philipp S, Cavalie A, Freichel M, Wissenbach U, Zimmer S, Trost C, Marquart A, Murakami M, Flockerzi V (1996) A mammalian capacitive calcium entry channel homolgous to *Drosophila* TRP and TRPL. EMBO J 15:6166–6171
100. Funayama M, Goto K, Kondo H (1996) Cloning and expression localization of a cDNA for a rat homolog of the TRP protein, a possible store-operated calcium (Ca^{2+}) channel. Brain Res Mol Brain Res 43:259–266
101. Okada T, Shimizu S, Wakamori M, Maeda A, Kurosaki T Takada N, Imoto K, Mori Y (1998) Molecular cloning and functional characterization of a novel receptor-activated TRP Ca^{2+} channel from mouse brain. J Biol Chem 273:10279–10287
102. Philipp S, Hambrecht J, Braslavski L, Schroth G, Freichel M, Murakami M, Cavalie A, Flockerzi V (1998) A novel capacitive calcium entry channel expressed in excitable cells. EMBO J 17:4274–4282
103. Preuß KD, Nöller JK, Krause E, Göbel A, Schulz I (1997) Expression and characterization of a TRPL homolog from rat. Biochem Biophys Res Commun 240:167–172
104. Groschner K, Hingel S, Lintschinger B, Balzer M, Romanin C, Zhu X, Schreibmayer W (1998) TRP proteins form store-operated cation channels in human vascular endothelial cells. FEBS Lett 437:101–106
105. Kiselyov K, Xu X, Mozhayeva G, Kuo T, Pessah I, Mignery G, Zhu X, Birnbaumer L, Muallem S (1998) Functional interaction between $InsP_3$ receptors and store-operated Htrp3 channels. Nature 396:478–482
106. Sinkins WG, Estacion M, Schilling WP (1998) Functional expression of TRPC1: a human homologue of the *Drosophila* TRP channel. Biochem J 331:331–339

107. Zhu X, Jiang M, Birnbaumer L (1998) Receptor-activated Ca^{2+} influx via human Trp3 stably expressed in human embryonic kidney (HEK)293 cells. J Biol Chem 273:133–142
108. Hurst RS, Zhu X, Boulay G, Birnbaumer L, Stefani E (1998) Ionic currents underlying HTRP3 mediated agonist-dependent Ca^{2+} influx in stably transfected HEK293 cells. FEBS Lett 422:333–338
109. Kamouchi M, Philipp S, Flockerzi V, Wissenbach U, Mamin A, Raeymaekers L, Eggermont J, Droogmans G, Nilius B (1999) Properties of heterogenously expressed hTRP3 channels in bovine pulmonary artery endothelial cells. J Physiol (Lond) 518:345–358
110. Helliwell RM, Large WA (1997) α_1-Adrenoreceptor activation of a non-selective cation current in rabbit portal vein by 1,2-diacyl-*sn*-glycerol J Physiol (Lond) 499:417–428
111. Garcia RL, Schilling WP (1997) Differential expression of mammalian TRP homologues across tissues and cell lines. Biochem Biophys Res Commun 239:279–283
112. Wissenbach U, Schroth G, Philipp S, Flockerzi V (1998) Structure and mRNA expression of a bovine TRP homologue related to mammalian TRP2 transcripts. FEBS Lett 429: 61–66
113. Mori Y, Takada N, Okada T, Wakamori M, Imoto K, Wanifuchi H, Oka H, Oba A, Ikenaka K, Kurosaki T (1998) Differential distribution of TRP Ca^{2+} channel isoforms in mouse brain. Neuroreport 9:507–515