The TRPC Class of Ion Channels: A Critical Review of Their Roles in Slow, Sustained Increases in Intracellular Ca^{2+} Concentrations*

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Key Words

store operated calcium entry (SOCE), capacitative calcium entry (CCE), calcium release activated calcium current (Icrac), G protein–coupled receptor (GPCR), phospholipase C (PLC)

Abstract

The realization that there exists a multimembered family of cation channels with structural similarity to *Drosophila*'s Trp channel emerged during the second half of the 1990s. In mammals, depending on the species, the TRP family counts 29 or 30 members which has been subdivided into 6 subfamilies on the basis of sequence similarity. TRP channels are nonselective monovalent cation channels, most of which also allow passage of Ca^{2+}. Many members of each of these families, but not all, are involved in sensory signal transduction. The C-type (for canonical or classical) subfamily, differs from the other TRP subfamilies in that it fulfills two different types of function: membrane depolarization, resembling sensory transduction TRPs, and mediation of sustained increases in intracellular Ca^{2+}. The mechanism(s) by which the C-class of TRP channels—the TRPCs—are activated is poorly understood and their role in mediating intracellular Ca^{2+} increases is being questioned. Both of these questions—mechanism of activation and participation in Ca^{2+} entry—are the topics of this review.

INTRODUCTION

The mammalian TRP family of ion channels consists of structurally related ion channels, the majority of which are nonselective Ca^{2+} permeable cation channels. When activated in response to external ligands, G protein–coupled receptors, metabolites, or physical stimuli, the TRP family of ion channels (TRPs) cause the collapse of the membrane potential. Depending on the cellular and physiological context, the collapsed membrane potential activates voltage-gated Na^{+} channels, engendering an action potential, or voltage-gated Ca^{2+} channels, promoting Ca^{2+} channel dependent responses.

TRPs have been subclassified into the C, V, M, A, P, and ML subfamilies, of which C, V, M, and A are structurally much closer to each other than to the P (polycystin) and ML (mucolipin) families, which stand alone as separate subfamilies (1). Many of the TRPs are expressed at the dendritic ends of sensory neurons, giving them a central role in sensory signal transduction, such as for taste, vision, thermosensation, and pain. Whereas direct activation of TRPs by chemical and physical changes in their membrane environment, e.g., temperature, are easily understood if they are assumed to operate akin to ionotropic receptors such as the nicotinic acetylcholine receptors or the NMDA/AMPA glutamate receptors, the mechanism of activation in response to other changes, including activation of phospholipase C (PLC), is still largely unknown.

THE TRP SUPERFAMILY OF ION CHANNELS

The superfamily of TRP channels (**Figure 1**) was discovered independently in several laboratories interested in a diverse array of cell and physiological functions. The first and founding member was *Drosophila* Trp, a gene that affects visual signal transduction. Its closest mammalian homologues are the TRPC channels (more below). Expression cloning led to the discovery of the first of the TRP-related channels, the vanilloid receptor, VR1, now TRPV1. TRPV1 is a nonselective cation channel activated by vanilloids such as capsaicin and is responsible for the hot taste of chili peppers (2). It is also a sensor for heat and pH. A closely related gene, VRL1 or TRPV2, is also a heat sensor but with a different activation threshold and insensitive to vanilloids. Sequence comparisons showed VR1, as well as its homologues, to be distant relatives of TRPC channels. Thus, TRPV1 became the founding member of the TRPV subfamily of TRP channels. Whereas TRPV2, 3, and 4 were discovered by their structural relationship to TRPV1, two others, ECaC1 and CaT1 (also ECaC2 and CaT2, now TRPV5 and V6) were discovered as a consequence of independent studies on cellular Ca^{2+} entry, this time into epithelial cells (3, 4). TRPV2 (VRL1) was also discovered as GRC1, growth receptor channel 1, a gene product whose translocation to the cell surface is promoted by insulin-like growth factor (5).

Melastatin is a gene product that is underexpressed in melanoma cells (6). Analysis of its sequence revealed it to be somewhat similar to TRPCs and to be related to the *Caenorhabditis elegans* osm9 gene product. Melastatin became the founding member of the TRPM type of TRP channels. LTRPC2 and LTRPC7, now TRPM2 and TRPM7 have long C-termini that include unrelated domains such as an ADP-ribose phosphatase-related NUDIX domain (TRPM2) and an atypical α-kinase domain (TRPM7). These domains confer special properties to these channels, including responsiveness to reactive oxygen species and ADP-ribose (TRPM2) and protein:protein interactions with attendant assembly of signaling complexes that still need to be fully explored [TRPM7 also TRP-PLIK (PLIK: phospholipase C interacting kinase)]. Perhaps counterintuitively, the TRPM8 channel is a cold and menthol receptor (CMR1; 7). This molecule extends the range of temperatures over which TRP-related channels confer temperature sensitivity to cells. TRPV2 (VRL1), TRPV1 (VR1), and TRPV3 are activated by a positive temperature change (warming) at

Annu. Rev. Pharmacol. Toxicol. 2009.49:395-426.

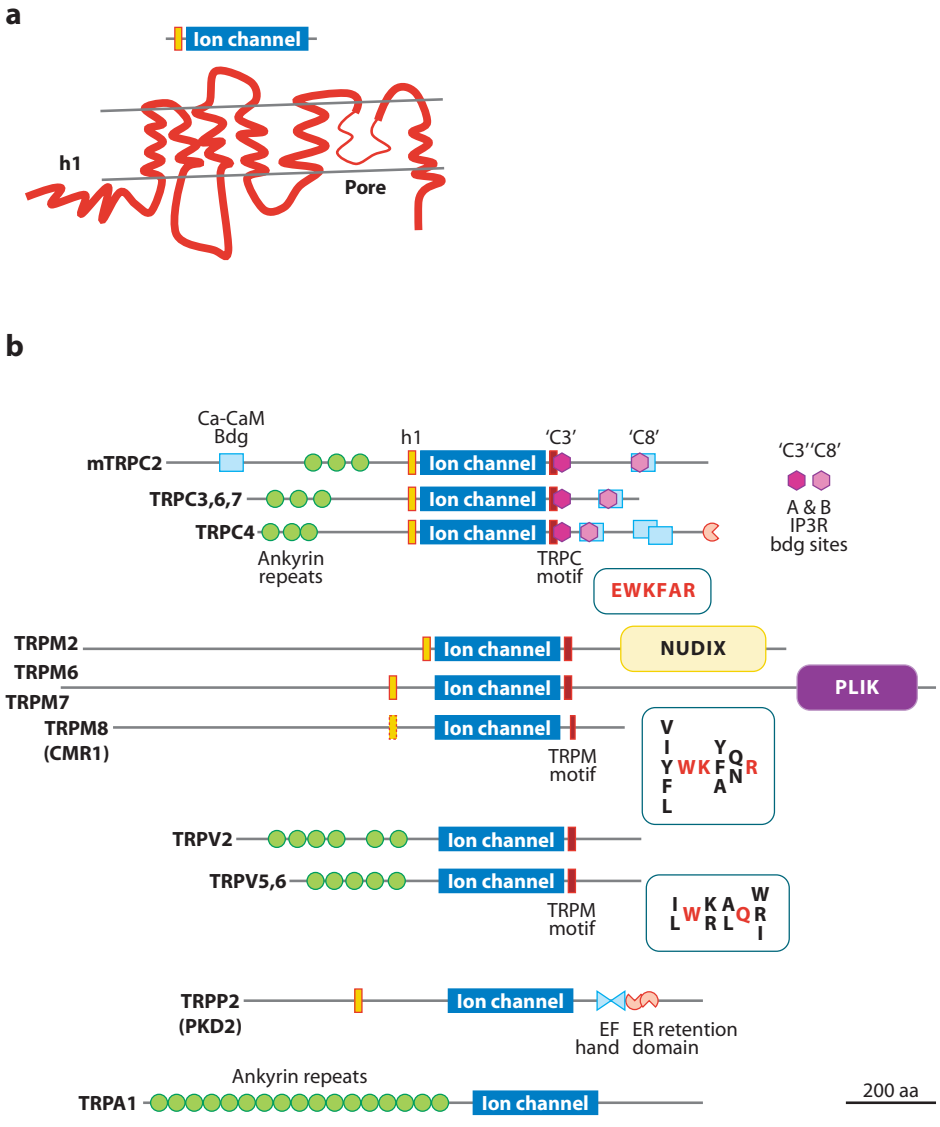

Figure 1

Structural domains of TRP channels of the C, V, M, P, and A types, as deduced from primary amino acid sequence alignment and from biochemical interaction studies. (*a*) Cartoon of the six-TM ion channel domain with preceding hydrophobic segment, as found in the C- and V-type TRPCs. Based on glycosylation scans of TRPC3 (17). (*b*) TRP channels are organized in three general domains: A central 6-transmembrane ion channel forming domain, a cytosolic N-terminus with variable number of protein:protein interaction motifs similar to those found in ankyrin (ankyrin motifs) and a cytosolic C-terminus with protein:protein interaction sites that differ from channel to channel and may include one or more Ca:CaM binding sites, one or more IP3 receptor interacting sites, a PDZ domain, and, in the case of TRPP1 (PKD1, also polycystin 1) an ER retention signal that is occluded by its interaction with TRPP2 (PDK2 or polycystin 2), thus favoring expression at the plasma membrane of the TRPP1-TRPP2 dimer. TRPs of the C, V, and M classes have the so-called TRP domain immediately after the ion channel domain, which includes three variations of the Glu-Trp-Lys-Phe-Ala-Arg (EWKFAR) TRP box, a PKC phosphorylation site, and motifs that in cellular overexpression and/or in vitro studies can be shown to have the ability to mediate or promote interactions of the TRP carboxy-termini with PIP2 and/or STIM.

Annu. Rev. Pharmacol. Toxicol. 2009.49:395-426.

decreasing thresholds of 55, 43, and 32°C, respectively. CMR1 is activated by a negative temperature change (cooling) to below 28°C. Yet it does not belong to the V-type TRPs, raising questions as to the evolutionary relationships among the TRP channel genes. A functional relationship of a similar kind also exists between TRPV4 (OTRPC4) and TRPM3. Both are channels that respond to changes in extracellular osmotic strength, yet they belong to two separate subfamilies of TRP channels (8, 9). A second cold-sensitive TRP, TRPA1, activated by lowering the temperature to <17°C, was discovered in 2003 (10).

The PKD1 and PKD2 genes, for polycystic kidney disease 1 and 2, were discovered through positional cloning efforts that culminated in their identification as the genes responsible for familial autosomal dominant forms of this disease (11). Sequence analysis of PKD2 revealed it to be a distant relative of TRP channels. Likewise, PKD1, a large multidomain protein that interacts with PKD2, also shows sequence characteristics that would predict it to be an ion channel. PKD1 became the founding member of a fourth TRP subfamily, the TRPPs. There are now five members of this family.

TRP channels of the different subfamilies are encoded in 28 nonallelic mammalian genes. As such, these genes are subject to mutations that can give rise to familial diseases referred to as channelopathies. As defined by Ashcroft (12), channelopathies comprise "a class of diseases or syndromes defined as disorders resulting from mutations in ion channel genes." Mutations in over 60 ion channel genes have shown associations with human disease and TRP genes are no exception. At present, mutations in TRP genes are responsible for five channelopathies: *Familial segemental glomerulosclerosis* (FSGS, OMIM 603965), which causes renal proteinuria and has its root in what appears to be an activating mutation in the gene encoding TRPC6 (13). As reviewed in Reference 14, the other recognized TRP channelopathies are *autosomal-recessive hypomagnesemia with secondary hypocalcemia* (HSH/HOMG 1; OMIM 602014) caused by mutations in TRPM6, *amyotrophic lateral sclerosis-Parkinsonism/dementia complex* (ALS-G/PD-G Guam disease; OMIM 105500) caused by mutations in TRPM7, *autosomal dominant polycystic kidney disease* (ADPKD; OMIM 173910) caused by mutations in TRPP1 or TRPP2, and *mucolipidosis IV* (MLIV, OMIM 252650) caused by mutations in TRPML1.

Table 1 summarizes environmental cues that affect TRP activities. All TRPs have a predicted membrane topology of 6 transmembrane (TM) segments with a selectivity filter between the last two, presumably pore-forming TM segments, but lacking the S4 segment with multiple basic amino acids spaced three apart that are typical of voltage-gated ion channels. Members of the C, V, and A subfamilies, but neither M nor P subfamily TRPs, have a variable number of ankyrin repeats of unproven function that are thought to serve as protein:protein interaction modules.

Owing to their nature as nonselective cation channels, many of the TRP channels are transduction channels, i.e., channels that when activated in excitable cells lead to collapse of the membrane potential and activation of voltage-dependent ion channels. This, depending on the cellular context, leads to the generation of action potentials, or, as is the case in myocytes, in contractions triggered by activation of voltage-dependent Ca^{2+} channels.

Structural aspects and subclassification of TRP channels into subfamilies have been reviewed (1), as have been the biophysical characteristics of their ion-permeating pores (15). The fundamental properties and responses to physiologic and pharmacologic agents of the members of the different TRP subfamilies, together with their proposed roles as transduction channels, have also been recently reviewed by Nilius et al. (14) and Venkatachalam & Montell (16) and will for the most part not be recapitulated in the present article. The aim of this article is to highlight the cellular roles that TRPCs play as environmental sensors and transduction channels, and the role they play as regulators of intracellular Ca^{2+} levels in response to the depletion of Ca^{2+} from internal Ca^{2+} stores, without or with simultaneous activation of the C-type phosphoinositidases (PLCs).

Annu. Rev. Pharmacol. Toxicol. 2009.49:395-426.

Table 1 TRP channels are chemical and environmental sensors and as well as sensory transduction channels

Channel	Activating cue(s)	Evidence for translocation
TRPC2	DAG	No
TRPC3	DAG	Yes[b]
TRPC6	DAG	n.r.
TRPC7	DAG	n.r.
TRPC4	DAG[a]	n.r.
TRPC5	DAG[a]	Yes
	Lysophosphatidylcholine	
	Thioredoxin[c]	
	Nitrosylation[c]	
TRPV1	H^+	Yes
	Warming (>43°C)	
	Capsaicin, Anandamide	
TRPV2	Warming (>52°C)	Yes
TRPV3	Warming (33–39°C)	n.r.
	2ABP, camphor	
TRPV4	Warming (27–34°C)	Yes
	5′6′EET, Phorbol esters (4αPDD)	
TRPV5	Klotho	Yes
	Tissue kallikrein	
	Inhibited by $[Ca^{2+}]_i$	
TRPV6	Klotho	Yes
	Inhibited by $[Ca^{2+}]_i$	
TRPM1	?	Yes
TRPM2	ADP-ribose, cADP-ribose, H_2O_2, ROS	
TRPM3	Osmotic cell swelling	n.r.
	Phorbol esters, Sphingosine	
TRPM4	Ca^{2+}	n.r.
	5′6′EET	
TRPM5	Ca^{2+}	n.r.
TRPM6	Mg^{2+}-inhibited	Yes
TRPM7	Mg^{2+}-inhibited	n.r.
TRPM8	Cooling (<28°C)	n.r.
	Menthol>iciin	
TRPA1	Cooling (<17°C)	n.r.
	Iciin>>menthol	
	Isothiocyanates, cannabinoids	

[a]Requires inhibition of PKC to be evident (64).

[b]Dependent on interaction with PLCγ (116).

n.r., not reported; 2ABP, 2-aminoethoxydiphenylborate; 2-aminoethoxydiphenylborate; 4αPDD, 4α-phorbol didecanoate; 5′6′EET, 5′6′epoxyeicosatrienoic acid; Anandamide, arachinoylethanolamide; DAG, diacylglycerol; klotho, β-glucuronidase; ROS, reactive oxygen species.

[c]The report on activation of TRPC5 by nitrosylation of Cys553 and Cys 558 (117) conflicts with the report that TRPC5 is activated by reduced thyrotoxin acting to reduce an extracellular disulfide bond between Cys 553 and Cys 558 (118).

Annu. Rev. Pharmacol. Toxicol. 2009.49:395-426.

Voltage-Gated K^+ Channels as Structural Models for TRP Channels

The membrane topology of the TRP family of ion channels resembles that of voltage-gated potassium, sodium, and calcium channels, having six TM segments (17) and a pore between the last two TMs. C- and M-type TRPs have a characteristic hydrophobic domain of unknown function that precedes TM1 (17) (**Figure 1**). TRPs differ in that they do not have a classic voltage sensor. This is not to say that they may not have some voltage-sensing capability, because, for example, TRPM8 can be gated by voltage and has positively charged amino acids towards the end of the fourth TM and in the putative loop connecting TM4 to TM5-pore-TM6 (18).

It is tempting to speculate that TRP channels resemble voltage-gated cation channels not only in TM topology but also in their structural organization of four subunits each with two domains, a voltage sensor, and a cation channel proper. The structural organization of voltage independent and voltage gated K^+ channels, as deduced from the pioneering work of MacKinnon and his collaborators (19–21), has shown voltage-gated K^+ channels to be formed of four subunits, each being a two-domain protein that spans the membrane six times, as TRP channels do. One of the two domains contributes the last two-TM helices with intervening pore helix and selectivity filter (direct structural and functional homologue of the procaryotic voltage insensitive KscA channels) forming the cation channel proper. The second domain forms a separate voltage-sensing domain of four TMs of which the fourth (S4 in **Figure 2**) is the voltage-sensing helix with basic amino acids in each third position. If this structure-based functional arrangement is conserved in TRP channels, it could be that in TRPs TMs 1-4 are sensors regulating the gating of the TM5-pore-TM6 domain. Under this hypothesis TRP's TM1-4 domains would be chemical sensors and the site of interaction of capsaicin and protons in TRPV1, of menthol in TRPM8, of diacylglycerol (DAG) in TRPCs, of phorbol esters in TRPV4, and of icilin in TRPA1 (**Table 1**). The question as to whether temperature sensing by the various TRPs is also conferred by the sensing domain, or whether a different principle applies, requires extensive experimentation, as does the idea that TMs 1–4 of TRPs are chemical sensors.

THE TRPC SUBFAMILY OF TRP CHANNELS

Among the subfamilies of TRP channels, the TRPC subfamily, discovered in the author's laboratory in 1996 (22), is unique in that its members are not only responsible for agonist-activated nonselective cation currents, but in that they also participate in the so-called slow sustained mode of Ca^{2+} signaling, which requires sustained elevations of intracellular Ca^{2+} ($[Ca^{2+}]_i$). Depending on cellular and physiological context, activation of C-type TRPs leading to changes in $[Ca^{2+}]_i$ appears to come about through a functional association with Orai (also CRAM or Ca^{2+} release activated modulator), the appearance of the Ca^{2+}-release activated Ca^{2+} current, Icrac, and the concomitant development of a form of Ca^{2+} influx referred to as store operated Ca^{2+} entry or SOCE (formerly termed capacitative Ca^{2+} entry or CCE). In contrast to the TRPC-mediated nonselective cation current, which requires cells to be stimulated by a receptor-PLC activating cascade, typically by engaging a G protein–coupled receptor (GPCR)-Gq-PLCβ signaling pathway, SOCE and Icrac can be triggered without activation of a PLC by passive depletion of the internal Ca^{2+} stores. The participation of TRPCs as structural members of the SOCE or Icrac channels is, at this time, a matter of dispute.

Ca^{2+} as a Second Messenger with Two Distinct Spatio-temporal Properties

The levels of cytosolic free Ca^{2+} oscillate between 50 and 150 nM in resting, unstimulated cells. The concentration gradients of free Ca^{2+} between cytosol and the extracellular milieu

Annu. Rev. Pharmacol. Toxicol. 2009.49:395-426.

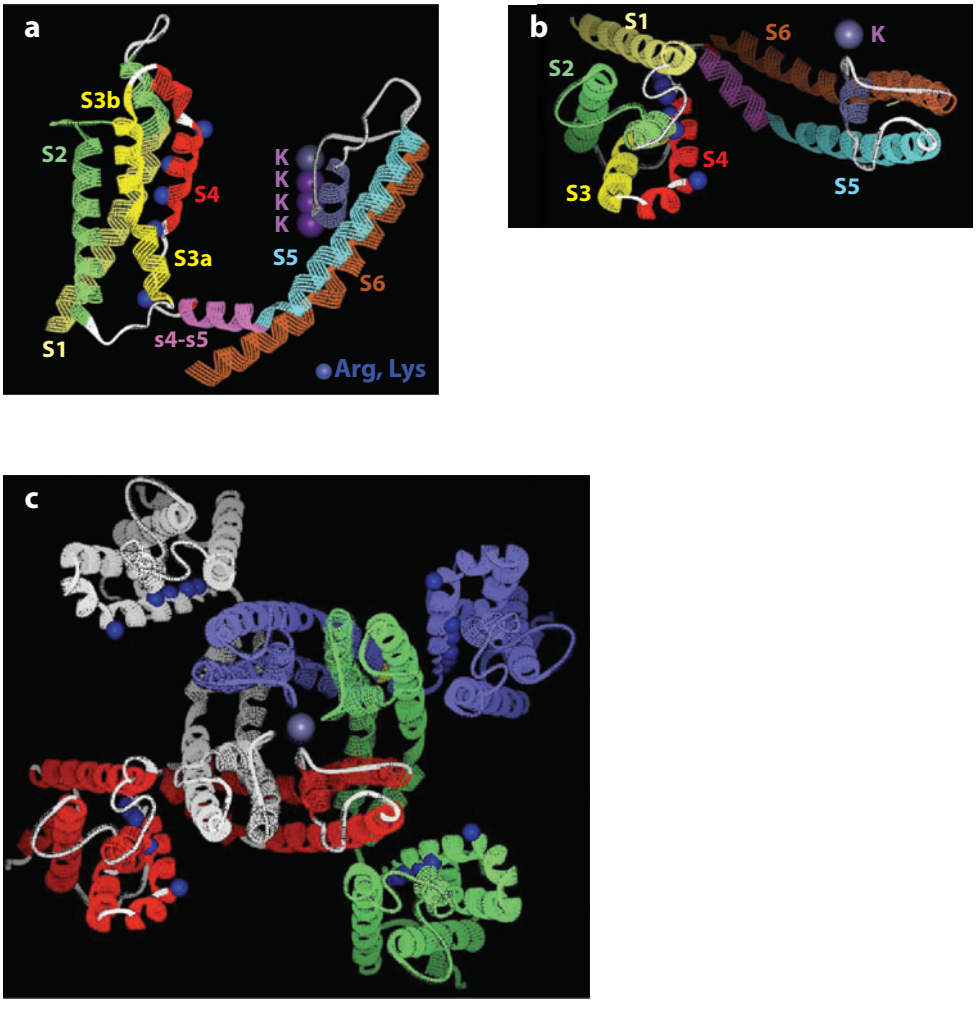

Figure 2

The voltage-gated K^+ channel may be a structural model for TRP channels. (*a*) Side view of the monomeric subunit of a voltage-gated potassium channel (*PDB accession number 2R9R.pdb*). The figure shows the structures of the N-terminal TM1-TM4 voltage-sensing domain in relation to the TM5-pore-TM6 pore/gate forming domain of the channel. Note the clear separation of the voltage-sensing domain from the selectivity filter- and gate-forming domain. This structure, a 4TM regulatory environment–sensing domain plus a 2TM ion channel domain may be a general feature of 6TM ion channels. (*b*) Top view of the monomer. (*c*) Four 4TMplus2TM 6TM ion channel monomers form the fully assembled channel. Shown is a view of the tetramer from the (extracellular) top, emphasizing the preservation of the relative structural independence of the 4TM regulatory sensor domain from the 2TM ion channel forming domain. Changes in the sensing domain are transmitted to the ion channel domain through the s4-s5 connecting α helix.

(at ca. 2 mM), and between cytosol and the intracellular stores (at 0.1–1.0 mM), are maintained by Ca^{2+} -dependent ATPases: the plasma membrane Ca^{2+} ATPases (PMCAs) that pump Ca^{2+} out of cells and the the sarco-endoplasmic reticulum Ca^{2+} ATPases (SERCAs) that return Ca^{2+} into the stores. Signaling processes mediated by Ca^{2+} use the energy embedded in the existing concentration gradients and operate by controlling delicate gating mechanisms intrinsic to various

Annu. Rev. Pharmacol. Toxicol. 2009.49:395-426.

types of Ca^{2+}-permeable ion channels. These gates are activated (opened) at either very fast rates or, comparatively, at slow rates, giving rise to fast and slow cellular responses to extracellular signals mediated by Ca^{2+}. Fast responses include muscle contraction and neurosecretion and are triggered by activation of voltage-dependent Ca^{2+} channels. Slow responses include activation of the NFAT (nuclear factor of activation of T cells) family of transcription factors and of nitric oxide (NO) synthases leading, respectively, to the activation of gene transcription and to paracrine effects such as vascular smooth muscle relaxation. Fast responses are associated with fast changes in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) and with $[Ca^{2+}]_i$ spikes. Fast responses are often not only temporally but, due to their short duration, also spatially restricted, whereas slow responses are often dependent on temporally more extended and sustained increases in $[Ca^{2+}]_i$.

Receptor-triggered activation of C-type phospholipases (PLCs), with attendant formation of DAG plus inositol 1,4,5-trisphoshate (IP3) (**Figure 3*a***), generates the prototypical slow types of Ca^{2+} signals (**Figure 4*a***). A similar response is also seen upon activation of the receptor-tyrosine kinase-PLCγ signaling pathway (e.g., 23–25) The sustained aspect of PLC signaling is dependent

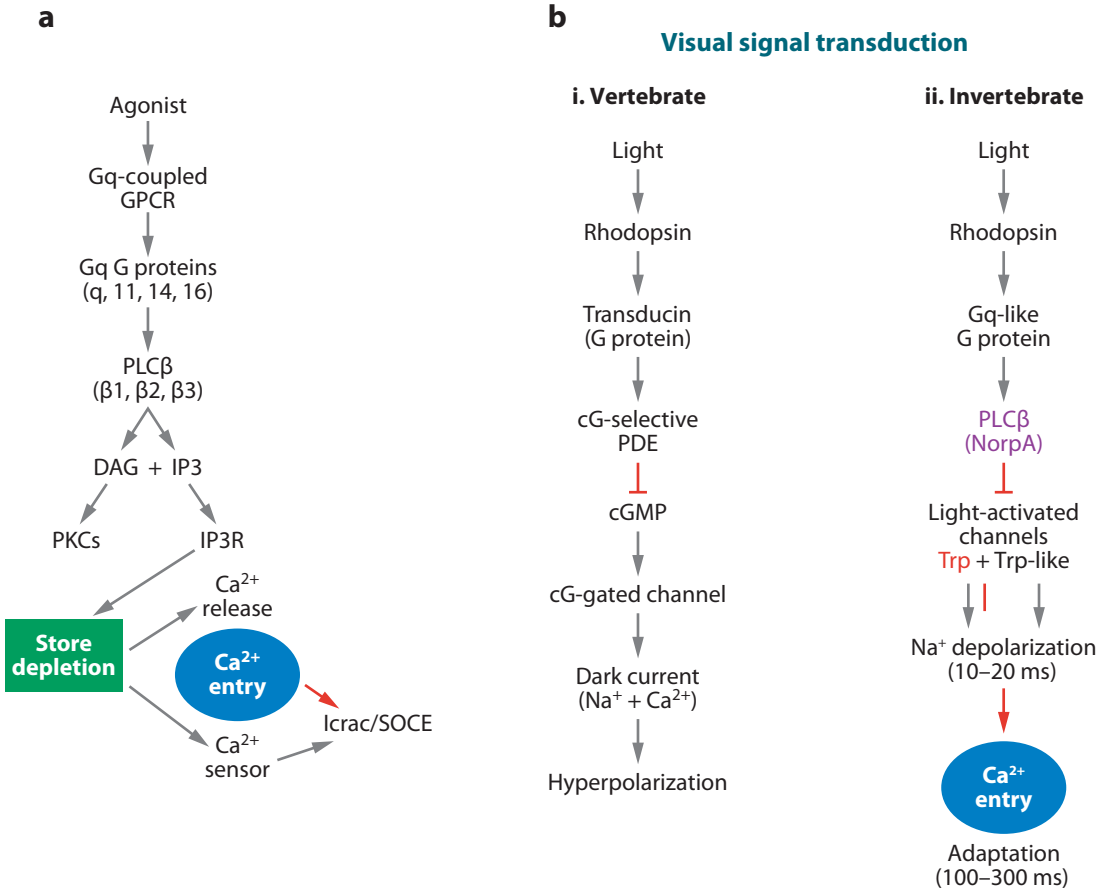

Figure 3

G protein–coupled signal transduction pathways. (*a*) Pathways leading to changes in intracellular Ca^{2+} concentrations. (*b*) Similarity of vertebrate activation of phospholipase C to signal transduction in invertebrate photoreceptor cells, but not vertebrate photoreceptor cells.

Annu. Rev. Pharmacol. Toxicol. 2009.49:395-426.

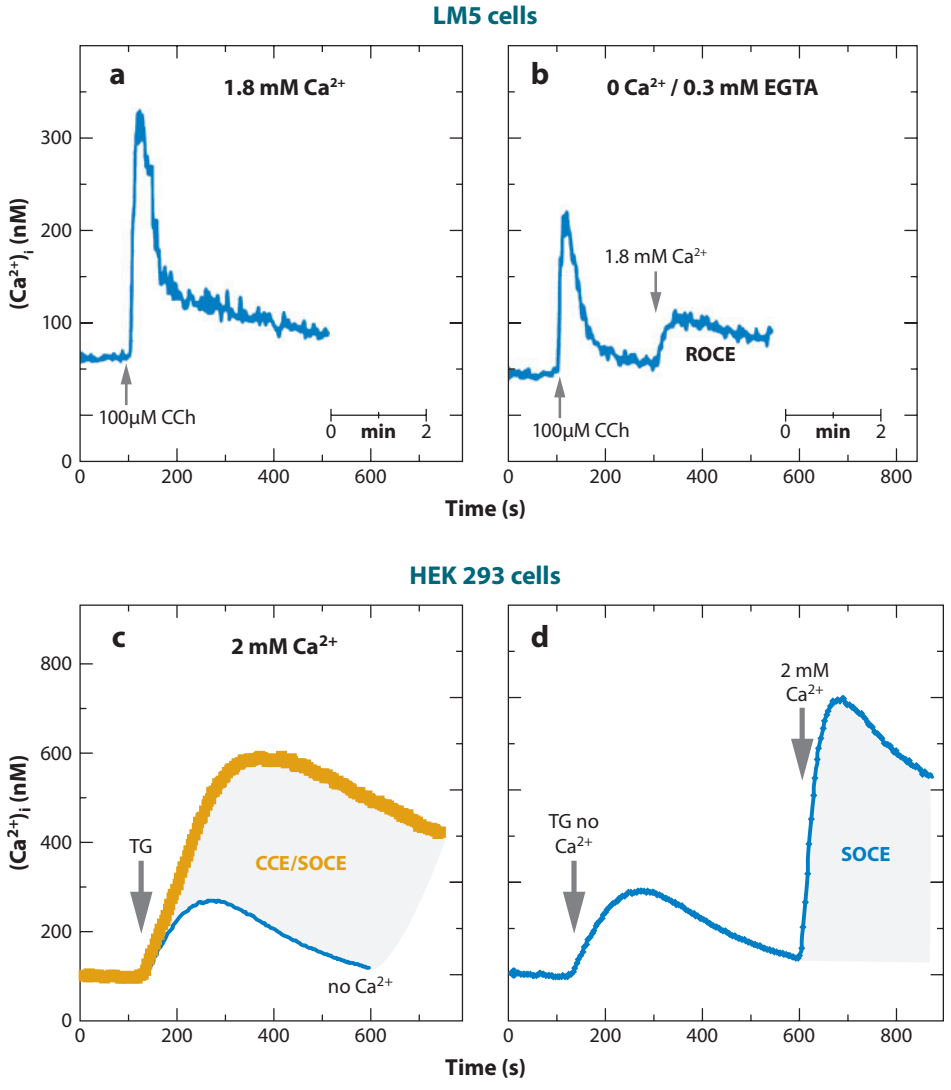

Figure 4

Receptor and store-depletion induced changes in intracellular Ca^{2+}. (*a*) and (*b*) Changes in cytosolic Ca^{2+} concentrations ($[Ca^{2+}]_i$) as a function of time in response to activation of the receptor-Gq-PLCβ signaling pathway as seen in mouse L cells expressing the M5 muscarinic acetylcholine receptor. (*c*) and (*d*) Changes in $[Ca^{2+}]_i$ in response to depletion of internal stores due to irreversible inactivation of the sarco-endoplasmic reticulum Ca^{2+} ATPases (SERCAs) by thapsigargin in HEK 293 cells. $[Ca^{2+}]_i$ was monitored by ratiometric fluorescence video microscopy in cells preloaded with the fluorescent Ca^{2+} indicator dye Fura2. In (*a*) and (*c*), $[Ca^{2+}]_i$ was monitored in the continued presence of extracellular Ca^{2+} (one step protocol). In (*b*) and (*d*), a two-step protocol was used in which cells are stimulated in the absence of extracellular Ca^{2+}, clearly revealing an increase in $[Ca^{2+}]_i$ due to release from intracellular stores. The increase in $[Ca^{2+}]_i$ is transient, returning to basal levels due to extrusion of Ca^{2+} into the extracellular milieu by the action of plasma membrane Ca^{2+} ATPases. Readmission of Ca^{2+} to the extracellular milieu reveals the presence of an activated influx pathway, as reflected by the influx of Ca^{2+} into the cytosol that does not occur in cells not exposed to a Gq-coupled receptor agonist or thapsigargin. The Ca^{2+} influx seen in the two step protocol in receptor- or thapsigargin-stimulated cells is referred to as receptor-operated Ca^{2+} entry (ROCE) or store-operated Ca^{2+} entry (SOCE). Panels (*a*) and (*b*) are adapted from (26).

Annu. Rev. Pharmacol. Toxicol. 2009.49:395-426.

on continued presence of agonists, as is the sustained aspect of elevated cytosolic Ca^{2+} (c.f. figure 5 in 26).

Slow Ca^{2+} signaling depends on the sequential activation of two types of gates. One is the IP3-stimulated IP3 receptor, a Ca^{2+} release channel embedded in the endoplasmic reticulum (ER) membrane. The second is a Ca^{2+} influx channel embedded in the plasma membrane. Existence of these two types of gates was deduced in the mid 1970s from among others, the Ca^{2+} fluxes displayed by guinea pig pancreatic acinar cells in response to secretagogues (27). Because exocrine secretion was known to depend on extracellular Ca^{2+}, the first change in Ca^{2+} flux upon addition of the secretagogue cholecystokinin (CCK) had been assumed to be Ca^{2+} entry. Yet, by comparing $^{45}Ca^{2+}$ influx from the extracellular milieu to $^{45}Ca^{2+}$ efflux from prelabelled cells, efflux was found to precede influx. The molecular basis of the efflux phase was uncovered in the early 1980s with the discovery of IP3 as the second messenger responsible for releasing Ca^{2+} from internal stores (28), followed by the characterization, purification, and cloning of its receptor (29, 30).

SOCE

The molecular basis for the Ca^{2+} influx phase triggered by activation of the Gq-PLCβ signaling pathway is still under investigation and is intimately related to the molecular basis of the Ca^{2+} influx activated by store depletion without activation of the Gq-PLCβ or tyrosine kinase-PLCγ pathways. The existence of store depletion-activated Ca^{2+} entry was uncovered as a sequel to inactivation of the SERCA pumps with the inhibitor thapsigargin (31, 32) (**Figure 4*c***). As illustrated in **Figure 4*d***, Ca^{2+} influx that follows the emptying of internal stores is referred to either as store depletion-activated Ca^{2+} entry or store-operated Ca^{2+} entry channels (SOCE).

ROCE

Ca^{2+} influx triggered as a consequence of PLC activation—presumably enhanced by IP3-induced store depletion—is referred to as receptor-operated Ca^{2+} entry or ROCE (**Figure 4*b***). Although the distinction between ROCE and SOCE did not exist in the initial definition of CCE, prior to discovery of the effects of thapsigargin on Ca^{2+} influx and prior to knowing that Icrac existed, it is important to consider ROCE and SOCE as separate but overlapping/related phenomena when the mechanisms responsible for one or the other are discussed. In contrast to SOCE and Icrac, which are molecularly defined phenomena mediated by the SOCE channels activated by store depletion and generating Icrac without involvement of plasma membrane receptors or their signaling pathways, ROCE is an operational definition that describes Ca^{2+} entry occurring through both nonselective cation channels, as well as the more slowly activated SOCE/Icrac channels. The nonselective cation channels are thought to be TRPC channels. The identity and/or subunit composition of SOCE/Icrac channels is unclear and a matter of controversy.

Icrac

Intimately associated with SOCE is Icrac (**Figure 5**). Icrac, or Ca^{2+} release-activated Ca^{2+} current, was discovered as a slowly developing inward current with high selectivity for Ca^{2+} when mast cells were patch-clamped with pipettes containing as part of their internal solution high concentrations of the Ca^{2+} chelator BAPTA (33). Serving as a sink for Ca^{2+} leaking from the internal stores, BAPTA promotes store depletion and the appearance of the highly Ca^{2+}-selective, strongly

Annu. Rev. Pharmacol. Toxicol. 2009.49:395-426.

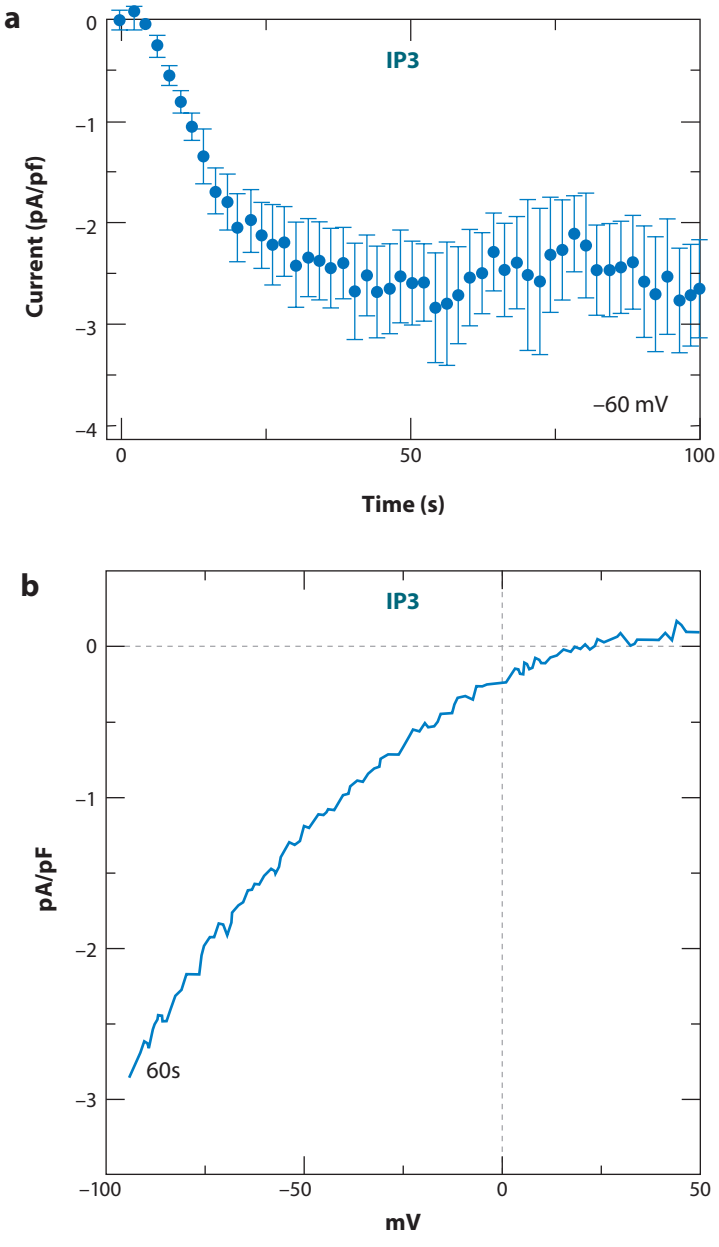

Figure 5

Development of Icrac in Jurkat cells. Cells were patched with pipettes containing 10 mM of the Ca^{2+} chelator BAPTA and 10 μM IP3. The strongly rectifying inward current that develops as Ca^{2+} stores lose Ca^{2+} is referred to as Ca^{2+} release activated Ca^{2+} current or Icrac (33). It is highly selective for Ca^{2+} and inhibited by 0.5 to 1 μM of the lanthanides La^{3+} and Ga^{3+}. (*a*) Time course of Icrac appearance; (*b*) inward rectification. Adapted from (88).

Annu. Rev. Pharmacol. Toxicol. 2009.49:395-426.

inwardly rectifying Icrac current. As expected for a phenomenon elicited by store depletion, the development of Icrac is facilitated (accelerated) by inclusion of IP3 in the patch-clamp pipette.

ROLE OF TRPCS IN SOCE

Visual signal transduction in vertebrates involves the sequential activation of the photoreceptor rhodopsin by light, the activation of the specialized trimeric G protein transducin by rhodopsin, the activation of a cGMP-specific phosphodiesterase by activated transducin, reduction of cytosolic cGMP levels, closure of a cGMP-activated nonselective cation channel, hyperpolarization of the photoreceptor cell in which this is occurring, and cessation of glutamate release at the glutamatergic photoreceptor synapse (**Figure 3*b,i***). Visual signal transduction in invertebrates (**Figure 3*b,ii***) differs in that, while it is also initiated by light-activated rhodopsin and subsequent activation of a trimeric G protein, the G protein is not of the transducin type, but of the PLC-activating Gq type. Flies devoid of a visual phospholipase C—called *norpA* in *Drosophila*, for No receptor potential A—are blind. A typical electroretinogram recorded from a compound eye has a fast-rising phase and a sustained phase which, at low levels of illumination, persists until the light stimulus is interrupted. The light-evoked receptor potential is absent in electroretinograms recorded from the compound eyes of mutant *norpA* flies. Transient receptor potential (*trp*) is a *Drosophila* mutant that exhibits the fast depolarizing response to light but lacks the sustained phase of the electroretinogram (34, 35). *trp* flies are not blind, but fail to adapt to constant light that requires a Ca^{2+}-dependent progressive reduction of the gain of the light-activated pathway. The trp gene was cloned and characterized as a putative membrane protein (36). Trp-like is a gene that codes for a calmodulin-binding protein (34). As its name indicates, it is a structural homologue of trp. Flies that have double null *trp* and *trp-like* alleles are blind and lack a significant receptor potential in their visual transduction system (38, 39).

A detailed characterization of the light-activated ionic conductances of wild-type and mutant flies has shown that the cellular correlates to the electroretinogram are initially mediated by both trp and trp-like, which are activated within 10–20 ms of the initiation of the light pulse, causing the cell to depolarize with concomitant exit of K^+ and entry of both Ca^{2+} and Na^+. Trp has a Ca:Na selectivity of 10:1, whereas trp-like is non-selective and feedback-inhibited by the Ca^{2+} entering as a consequence of light stimulation. If the light source persists, the cell remains depolarized under the control of trp, leading to a sustained phase during which the bulk of the current is carried by Ca^{2+}, as the cell repolarizes rapidly in the absence of external Ca^{2+} (**Figure 6**). Interestingly, the electrical response to light of a wild-type photoreceptor cell in the absence of Ca^{2+} resembles that obtained in the presence of Ca^{2+} with cells from flies lacking trp (**Figure 6**; 40).

Strikingly, trp-like and trp were found to have amino acid sequence similarity to voltage-gated ion channels with limited but significant sequence similarity to voltage-gated sodium and calcium channels in the presumptive pore domains (37).

In 1993, Minke and collaborators formally raised the possibility that the Trp and Trp-like proteins may be functional homologues of mammalian capacitative Ca^{2+} entry channels and, by extension, the pore forming molecules of SOCE/Icrac channels (41, 42). The finding that *Drosophila* Trp-like could be activated by store depletion in SF9 cells (43) lent strong support to the hypothesis proposed by Hardie, Selinger, Minke, and their collaborators.

The mammalian homologues of *Drosophila* trp (TRPs) were cloned to test Hardie and Minke's hypothesis. Six such homologues were identified in three laboratories between 1995 and 1998 (22, 44–47). A seventh was discovered shortly thereafter (48). Initially called TRPs they are now referred to as TRPCs (49).

Annu. Rev. Pharmacol. Toxicol. 2009.49:395-426.

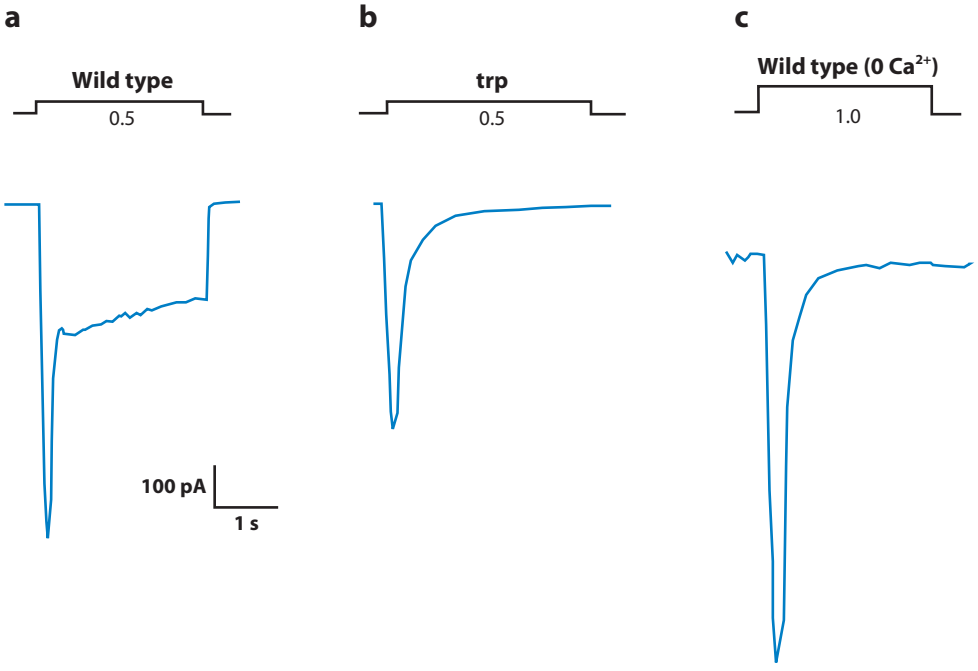

Figure 6

Drosophila trp mutant lacks light activated Ca^{2+} influx. (*a*), (*b*), and (*c*), Whole-cell voltage-clamped inward currents in response to prolonged (3 s) light stimuli at the indicated relative log intensities. Adapted from Hardie and Minke (40).

Annu. Rev. Pharmacol. Toxicol. 2009.49:395-426.

TRPCs Are Not, or Only Minimally, Ca^{2+} Selective

Although *Drosophila* Trp was found to exhibit an approximately 10:1 selectivity for Ca^{2+} over monovalent cations, Trp-like and the mammalian TRPCs do not exhibit the Ca^{2+} selectivity and inward rectification expected for the SOCE/Icrac channel (48, 50–52). Moreover, even though *Drosophila* phototransduction served to develop the hypothesis that mammalian TRP proteins may form SOCE/Icrac channels, the store-operated Ca^{2+} entry did not seem to participate in invertebrate phototransduction as the membranes that delimit the stores are physically removed from the rhabdomere membranes in which phototransduction and membrane depolarization occurs.

Expression and assembly studies showed that TRPCs have the potential of forming heteromultimers with certain specificity rules, such as 1:2, 1:3, 1:5, 4:5, 3:6:7, and 1:4:5 but not 3, 6, or 7 with 4 or 5 [53–55; but see Poteser et al. (56)]. The proposal that the lack of Ca^{2+} selectivity of the cloned mammalian TRPCs is due to inability of the model expression systems to assemble correct heteromultimers, with the Ca^{2+} selectivity of normal SOCE/Icrac channels (50), is probably incorrect, depending instead on insufficient expression of Orai (*vide infra*).

MULTIPLE MECHANISMS OF ACTIVATION OF TRPCS

Activation by Phospholipase C

Maneuvers that activate phospholipase C via the GPCR/Gq pathway resulting in activation of the IP3 receptor invariably also activate TRPC channels as seen by monitoring changes in $[Ca^{2+}]_i$ with cytosolic fluorescent Ca^{2+} indicator dyes. Inhibitors of PLCβ such as U73122 invariably

inhibit agonist-stimulated Ca^{2+} entry, i.e., inhibit ROCE (c.f. 57). Thus, activation of vertebrate TRPCs resembles activation of *Drosophila* Trp and Trp-like in that both depend on PLC activation. However, the mechanism by which PLC activation leads to activation of either TRPCs or invertebrate Trp channels is not known.

Conformational Coupling between IP3 Receptor and TRPC

Early studies suggested that IP3 receptors may participate in the activation of TRPCs, as pull-down experiments showed these proteins to interact both in vitro and in intact cells (58, 59), and expression of IP3 receptor peptides comprising the TRPC interacting sequences, but not of peptides from noninteracting regions, affected SOCE development and SOCE duration (58). In agreement with these findings and the hypothesis that activation of IP3Rs may lead to a conformational coupling between IP3R and TRPCs with attendant activation of the TRPC channel, the type II IP3R and TRPC1 were shown to coprecipitate from lysates of human platelets that had been prestimulated with either a receptor agonist, thrombin, that activates the GPCR-Gq-PLC pathway or by a maneuver that promotes store depletion, such as exposure to low concentrations of ionomycin (60).

Diacylglycerol (DAG) and Lysophosphatidylcholine (LysoPC)

DAG is the additional product along with IP3 that results from activation of PLCs. Hofmann et al. (61) discovered that TRPC3 and TRPC6—later shown also for TRPC7 (48)—are activated by DAGs such as OAG (oleyl-acetyl-glycerol) and SAG (stearoyl-arachidonoyl-glycerol). The physiologic importance of this mechanism is in question, however, as maneuvers leading to DAG formation in response to agonist stimulation of the GPCR-Gq-PLC pathway, in cells expressing cloned TRPC3, under ionic conditions that allow for differentiation of transfected TRPC3 from endogenous TRPCs, fail to activate TRPC3 under conditions that allow for activation of the same TRPC3 by exogenous OAG (62). The converse is also true: in spite of expressing TRPC3 and TRPC6, HEK 293 cells do not respond to exogenous OAG with Ca^{2+} influx, whereas transfected TRPC3, C6 and C7 are activated by addition of OAG (63). In contrast, TRPC5 and TRPC4 are activated by GPCR-Gq-PLCβ-generated diacylglycerol provided PKC is inhibited. In the absence of PKC inhibitor, the GPCR-Gq-PLCβ-DAG pathway does not activate these TRPCs (61). This type of maneuver has not been reported for other TRPCs, leaving open the possibility that all TRPCs are activated by DAGs.

The second lipid known to activate a TRPC is lysophosphatidylcholine (LysoPC), which activates TRPC5 (65). Whether it acts on other TRPCs has not as yet been reported.

Rapid, PLC-Dependent Activation of the Visual Trp:Trp-like Transduction Channel without Involvement of Either IP3R or, Very Likely, DAG

As mentioned earlier, the activation of the visual transduction channel in photoreceptor cells of the invertebrate compound eye is very fast, having a signal response rise time of 10–20 msec (40) (**Figure 6**). This type of coupling implies that protein:protein interaction is the coupling mechanism. Yet such conformational coupling has to be discarded as the mechanism between TRP and IP3R in the photoreceptor cell of the fly This is because the rhabdomere's structure in which light-activated rhodopsin and the NorpA protein reside are physically distant from the location of the IP3 receptor-containing membrane compartment that delimits the Ca^{2+} store. Likewise, formation of DAG with attendant diffusion from the point of catalysis by PLC to the Trp and Trp-like channels is inconsistent with the rapid depolarizing response.

Annu. Rev. Pharmacol. Toxicol. 2009.49:395-426.

Based on current knowledge, the most likely transduction mechanism between NorpA and Trp channels, and by extension between PLCβ and TRPCs is protein:protein interaction. Whereas it has been possible to coimmunoprecipitate PLCβ and TRPC1 in cells stably overexpressing HA-tagged TRPC3 (66), stable, agonist-induced interactions between PLCβ and TRPCs have not been reported in the absence of overexpression of the interacting partners. It remains to be proven whether physiologic levels of PLCβ and TRPCs operate as a transduction unit to activate TRPC channels.

PHARMACOLOGY OF TRPC CHANNELS

Interpretation of the mechanism by which pharmacologic agents affect TRPC channels is intimately connected to and dependent on their structural makeup. It is widely accepted, but not yet proven, that TRPCs form tetramers in analogy to voltage-gated K^+, Na^+, and Ca^{2+} channels with whom they share sequence similarity and transmembrane topology. TRPCs have been shown to form heteromeric complexes by a variety of approaches, including rescue from intracellular compartments (53), co-immunoprecipitation as shown for TRPC1 and TRPC5 (67), and the appearance of novel currents generated by co-expression, as shown for TRPC1 and TRPC5 in embryonic brain (55), for endothelial cell TRPC3 and TRPC4 (56), and for heterologously expressed TRPC1 and TRPC3 (57). Not only may channels formed by co-expression display different permeation characteristics from those of the channels formed upon expression of single TRPCs, but also pharmacologic properties change. For example, channels formed by co-expression of TRPM6 and TRPM7 (M6/7 channels) are activated at high, millimolar concentrations of 2-APB, whereas homomeric TRPM7 channels are inhibited by low micromolar concentrations of 2-APB, which inhibit TRPCs and store operated Ca^{2+} entry, but activate homomeric TRPM6 channels (68). The description as to which TRPCs (as well as which TRPs, in general) operate as homomeric complexes and which operate as heteromeric complexes, and how this changes from tissue to tissue, is still evolving. Development of drugs that address these differences by showing specificities that differentiate between homomeric and different heteromeric forms of TRPs (including TRPCs) will be both difficult and surely also rewarding.

So far, TRPC channels are targets for only a few compounds, all thought to act directly by interacting with the TRPC molecules. None of these compounds is specific. In spite of this, it is possible to describe a limited number of profiles that allow for discrimination between some of the TRPCs when expressed in heterologous systems [e.g., TRPC3, C6, and C7 generate ROCE resistant to 1-5 μM Gd^{3+}; TRPC5 (and TRPC4), but none of the other TRPCs, is activated by 100 μM Ga^{3+}; all TRPCs are inhibited by 2-APB and SKF96365]. However, none of these chemicals acts at low, nanomolar concentrations and some act on related channels with opposite effects. Moreover, the direct effects of both diacylglycerols and 2-APB have been questioned (69, 70).

Table 2 lists agents that are currently known to affect TRPCs. Effects on other TRPs and related functions (e.g., channels formed upon co-expression of Orai and STIM in TRPC expressing cells) are listed only when an effect on a TRPC is known. There are instances in which the reports conflict. The apparently disparate findings may relate to cellular context (which other members of the same subfamily are co-expressed and/or to expression levels). Not having information other than what has been published, both claims are listed.

TRPCS AND SOCE/ICRAC

While the absence of the all-important Ca^{2+} selectivity should have prompted the hypothesis that TRPCs are structural candidates for pore-forming members of the SOCE/Icrac channels to be

Annu. Rev. Pharmacol. Toxicol. 2009.49:395-426.

Table 2 Drugs that affect TRPC channels[a]

Compound	Affected TRPC or TRPC related function	Nature of effect	Reference (No)
SKF96365			
5–25 μM	ROCE	Inhibition	Merritt et al. 1990 (119)
	TRPC		
	-C3	Inhibition	Zhu et al. 1996 (22)
	-C4	Inhibition	Kinoshita et al. 2000 (120)
	-C5	Inhibition	Okada et al. 1998 (121)
	-C6	Inhibition	Boulay et al. 1998 (51)
	-C7	Inhibition	Okada et al. 1999 (48)
	TRPM8	Inhibition	Mälkiä et al. 2007 (122)
	VGCC	Inhibition	Merritt et al. 1990 (119)
100 μM	Ca^{2+}-permeable cation channel	Activation	Schwarz et al. 1994 (123)
40–80 μM	K_{ir} channel	Inhibition	Schwarz et al. 1994 (123)
2-APB			
5–20 μM	TRPC		
	-C1, -C6	Inhibition	Delmas et al. 2002 (124)
	-C5, -C6	Inhibition	Xu et al. 2005 (125)
	-C3, -C6, -C7	Inhibition	Livremont et al. 2005 (69)
	TRPV		
	-V1, -V2, -V3	Activation	Hu et al. 2004 (126) Chung et al. 2004 (127)
	-V5, -V6	Inhibition	Hu et al. 2004 (126)
	TRPA1	Inhibition	Kim & Cavanaugh 2007 (128)
	TRPM		
	-M3	Inhibition	Xu et al. 2005 (125)
	-M2	No effect	Xu et al. 2005 (125)
	-M2	Inhibition	Togashi et al. 2008 (129)
	-M6	Activation	Li et al. 2006 (68)
	-M7	Inhibition	Li et al. 2006 (68)
	-M8	Inhibition	Hu et al. 2004 (126)
	Icrac: Orai1/STIM1	Inhibition	Lis et al. 2007 (130) DeHaven et al. 2008 (131) Zhang et al. 2008 (132)
	Icrac: Orai2/STIM1	Partial inhibition	DeHaven et al. 2008 (131)
	Icrac: Orai3/STIM1	Stimulation	Lis et al. 2007 (130) DeHaven et al. 2008 (131) Zhang et al. 2008 (132)
1–10 μM	HEK293 SOCE (Tg)	Stimulation	DeHaven et al. 2008 (131)
10–100 μM	HEK293 SOCE (Tg)	Inhibition	DeHaven et al. 2008 (131)
1–2 mM	TRPM7, -M6/7	Activation	Li et al. 2006 (68)
BTP2			
<0.5 μM	SOCE (HEK293, DT40, T cells)	Inhibition	He et al. 2004 (133)
	TRPC3 ROCE	Inhibition	He et al. 2004 (133)
	TRPC3 OAG	Inhibition	He et al. 2004 (133)
	TRPC5 ROCE	Inhibition	He et al. 2004 (133)
	TRPV6 ROCE	No effect	He et al. 2004 (133)

(*Continued*)

Annu. Rev. Pharmacol. Toxicol. 2009.49:395-426.

Table 2 (*Continued*)

Compound	Affected TRPC or TRPC related function	Nature of effect	Reference (No)
DAGs, 10–100 μM			
OAG	Hetrologously expressed TRPC		
	-C1	No effect	Delmas et al. 2002 (124)
		Activation	Lintschinger et al. (54)
	-C5	No effect	Flemming et al. 2006 (65)
	-C3, -C6	Activation	Hofmann et al. 1999 (61)
	-C7	Activation	Okada et al. 1998 (121)
	Endogenous TRPC		
	-C3, -C6	No effect	Liao et al. 2007 (63)
SAG	-C3, -C6,	Activation	Liao et al. 2007 (63)
	-C2	Activation	Lucas et al. 2003 (134)
LysoPC, 2–20 μM	TRPC5	Activation	Flemming et al. 2006 (65)
Gd^{3+}	TRPC		
1–5 μM	-C1, -C4, -C5	Inhibition[b]	
	-C3, -C6, -C7	No effect[b] (Gd-resistant ROCE)	
100 μM	-C5, -C4	Stimulation	Schaefer et al. 2000 (135) Jung et al. 2003 (136) Zheng et al. 2004 (137)
Reduced TRX	TRPC1, TRPC5		
DTT	TRPC1, TRPC5	Activation	Xu et al. 2008 (118)
TRX	TRPC1, TRPC5	Inhibition	Xu et al. 2008 (118)
DTT	TRPC5	Inhibition	Yoshida et al. 2006 (117)
Nitric oxide (NO)	TRPC5	Activation	Yoshida et al. 2008 (117)

[a], The table addresses the pharmacology of TRPCs and only lists other TRPs and related channel activities in those cases in which the drug affects a TRPC.

[b]M. X. Zhou, personal communication; 2ABP, 2-aminoethoxydiphenylborate; BTP2, (4-methy-4-[3,5-bis(trifluoromethyl)-1H-pyrazol-1-yl]-1,2,3-thiadiazole-5-carboxanilide); DTT, dithioerytritol; K_{ir}, inwardly rectifying potassium channel; LysoPC, lysophosphatidylcholine; OAG, oleyl-acetyl-glycerol; SAG, stearoyl-arachidonoyl-glycerol; SKF96365, 1[2-(4-methoxyphenyl)-2-[3-(4-methoxyphenyl)propoxy]ethyl]-1H-imidazole; Tg, thapsigargin; TRX, thioredoxin; VGCC, volatge-gated calcium channel.

Annu. Rev. Pharmacol. Toxicol. 2009.49:395-426.

discarded, a wealth of data has been accumulated to indicate that the mechanisms responsible for activation of SOCE/Icrac channels also activate cloned TRPC channels. This was shown for TRPC1 expressed in CHO cells by Zitt et al. (71) and in human salivary gland cells (HSG cells) by Liu et al. (72); for TRPC2 expressed in HEK cells by Vannier et al. (73); for TRPC3 by Vazquez et al. (74) and for a splice variant of TRPC3 (TRPC3a) by Yildirim et al. (75); for TRPC4 and TRPC5 by Philipp et al. (46, 47) [illustrated for TRPC5 (CCE2) in **Figure 7**], and for TRPC7 by Riccio et al. (76) and Lievremont et al. (77). The only TRPC missing in this list is TRPC6 (but see below the TRPC6-dependent enhancement of SOCE by Orai1).

Complementing these studies on activation of cloned TRPCs by store depletion are studies in which interference with naturally expressed TRPCs led to loss of SOCE or Icrac. Thus, Jungnickel et al. (78) reported that a peptide-directed anti-TRPC2 antibody inhibited not only Ca^{2+} entry into mouse sperm cells promoted by zona pellucida proteins, but also Ca^{2+} entry activated by thapsigargin. Freichel et al. (79) found that Icrac is absent in vascular endothelial cells of TRPC4 (CCE1) knockout mice (**Figure 8**). Liu et al. (80) reported that in submaxillary gland acinar cells of mice lacking TRPC1 the thapsigargin-activated Ca^{2+} entry is reduced by about 80% when

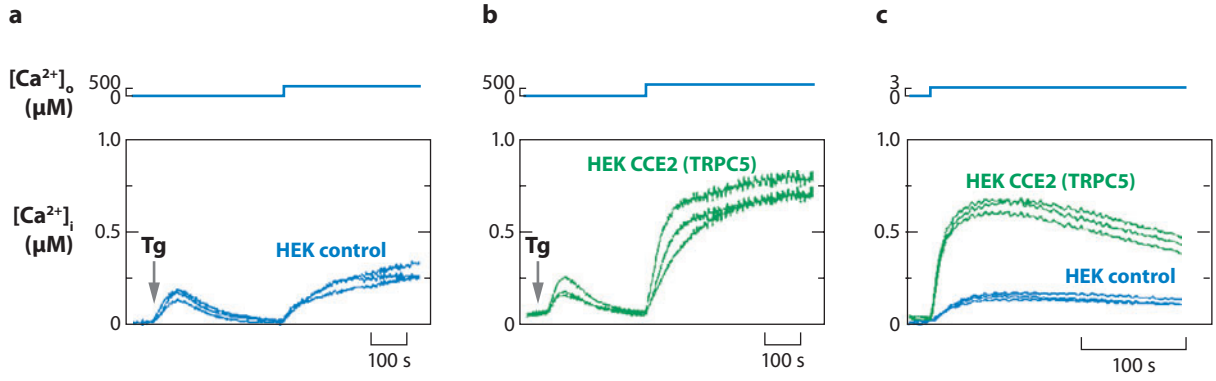

Figure 7

Activation of the TRPC5 channel by thapsigargin-induced store depletion in HEK cells stably expressing TRPC5 (CCE2). Control and TRPC5 expressing cells were loaded with Fura2 and tested for thapsigargin-induced Ca^{2+} entry using either a two-step protocol [(*a*) and (*b*)] or a one-step protocol (*c*). Note the markedly enhanced Ca^{2+} entry in the TRPC5 (CCE2) expressing cells when compared with control cells. Adapted from Philipp et al. (47).

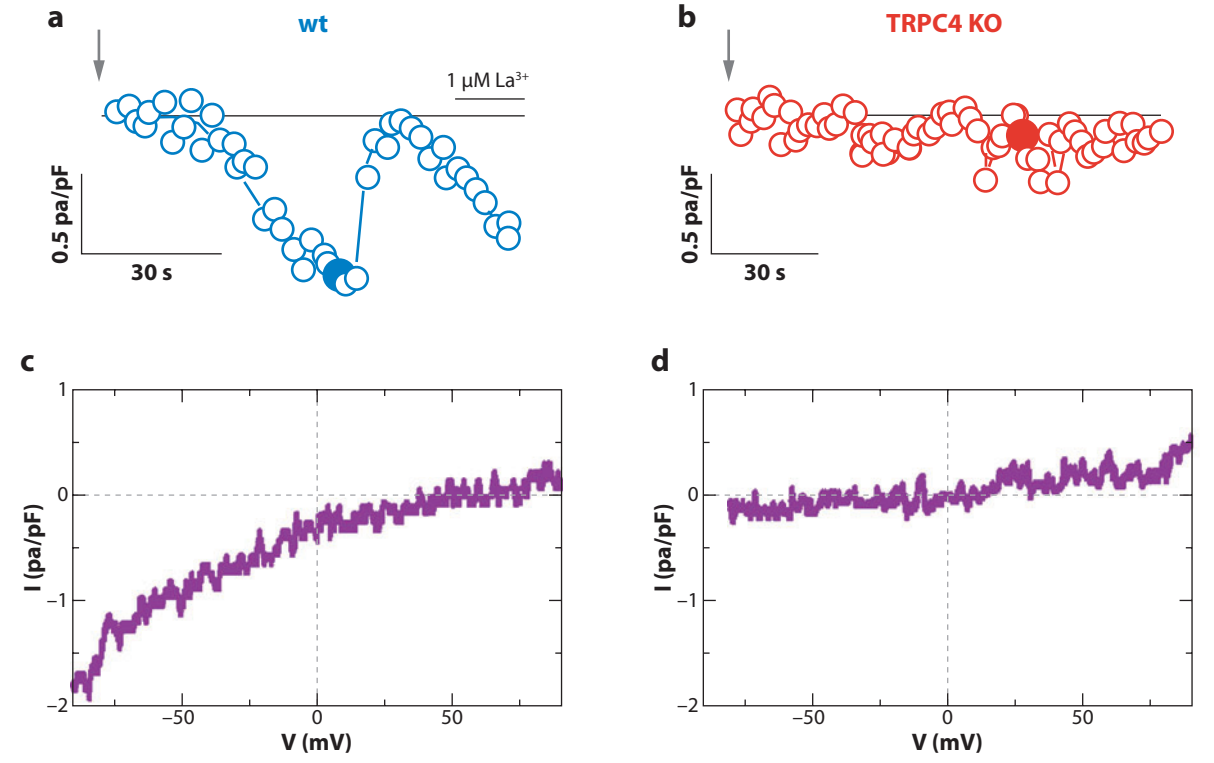

Figure 8

Total loss of Icrac in vascular endothelial cells from mice lacking TRPC4. The left panels show development of Icrac recorded at –80 mV of a vascular endothelial cell. (*a*) Time course of the appearance of Icrac recorded at –80 mV. (*b*) Current-voltage relationship of the active channels at the time indicated by the filled circle in (*a*). Note that the fully developed Icrac is fully inhibited by 1 μM La^{3+}, a typical property of Icrac. (*c*) and (*d*) The same as (*a*) and (*b*), but recorded from a vascular endothelial cell obtained from a mouse lacking TRPC4. Adapted from Freichel et al. (79).

Annu. Rev. Pharmacol. Toxicol. 2009.49:395-426.

compared to controls. Although Wedel et al. (81), upon testing inhibitory RNAs designed to target 34 members of the TRP family, found no effect on SOCE, similar experiments by Zagranichnaya et al. (82), with accompanying assessment of the effectiveness of the siRNA treatment, found that reduction in mRNA levels coding for TRPC1, TRPC3, and TRPC7, but not TRPC4, reduced thapsigargin-stimulated SOCE in HEK-293 cells (shown for TRPC1 and TRPC3 in **Figure 9**). Taken together, these findings constitute an impressive array of data in support of the hypothesis that TRPCs are involved not only in ROCE, but also in SOCE and Icrac. The most likely role for TRPCs is that they structurally form part of the Icrac/SOCE channel. Further, the data suggest the participation of one or more additional components involved in conferring Ca^{2+} selectivity to store-operated Ca^{2+} entry channels.

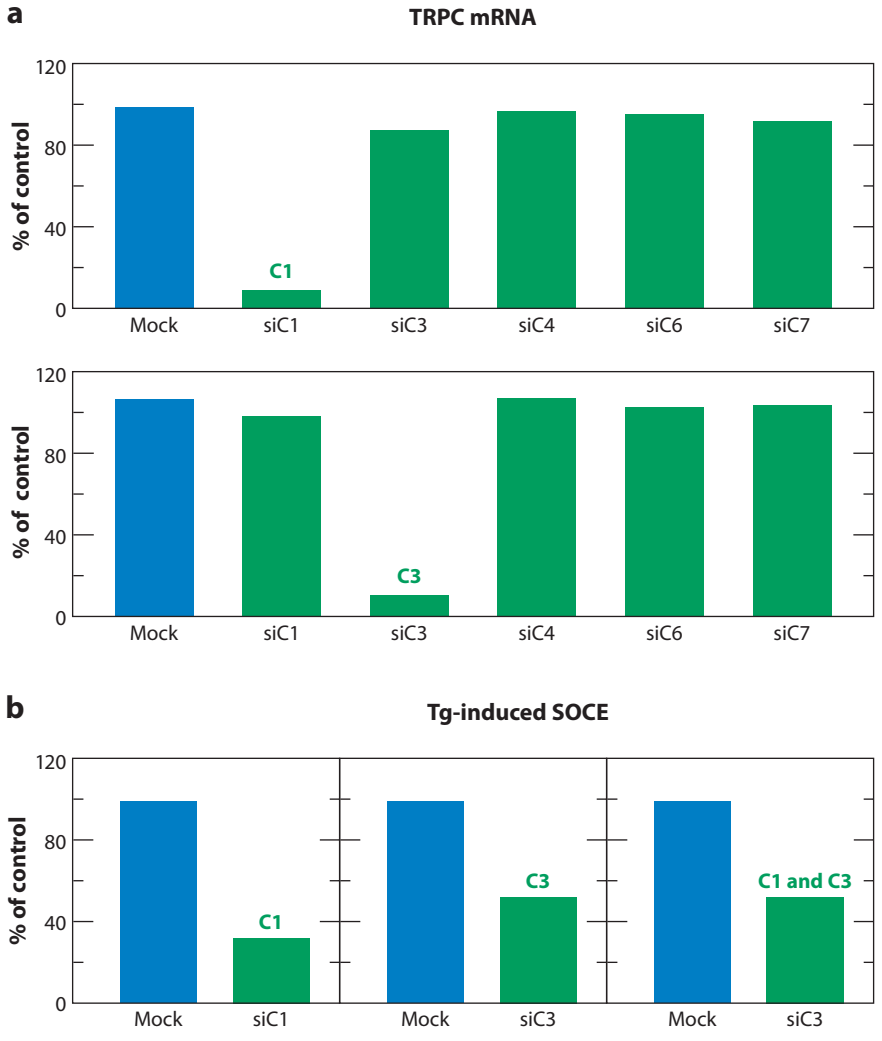

Figure 9

Severe blunting of SOCE in HEK-293 cells upon down-regulation of mRNA coding for TRPC1 and TRPC3. (*a*) Effectiveness of siRNA treatment. (*b*) Effect on SOCE. Adapted from Zagranichnaya et al. (82).

Annu. Rev. Pharmacol. Toxicol. 2009.49:395-426.

ORAI AS ESSENTIAL COMPONENT OF SOCE AND ICRAC CHANNELS

Although TRPCs are sensitive to store depletion, to be detected, this property requires special conditions and is not always easily observable. Observation of activation of TRPCs by store depletion often depends on the expression system studied. For TRPCs of the 3-6-7 subfamily, and possibly also for the other TRPCs, the overriding condition appears to be that they be expressed at low densities (75, 83, 84). The reason for this has not been clarified. One possibility is that at high expression levels the transfected TRPCs titrate one or more regulatory subunit/proteins that confer SOCE/Icrac characteristics to TRPCs in terms of both sensitivity to store depletion and Ca^{2+} selectivity. The discovery in 2005 of STIM (85, 86) and in 2006 of Orai (87–89) as essential components of SOCE and Icrac appear to have uncovered two such components.

STIM (85, 86) is a single pass transmembrane protein that resides primarily in the endoplasmic reticulum membrane and has an unpaired Ca^{2+}-sensing EF hand near its lumenal N-terminus. There is one stim gene in *Drosophila* but two STIM genes in the mammalian genome, which encode proteins of 685 and 833 amino acids (aa). In contrast to human STIM1, which when expressed by itself in normal cells has either no or only a slight enhancing effect on thapsigargin-stimulated Ca^{2+} influx, overexpression of STIM2 inhibits STIM1-dependent SOCE (90). STIM1's function is accepted as being the ER Ca^{2+}-sensor that conveys the depletion signal to the SOCE/Icrac channel in the plasma membrane, causing the activation of the influx channel by an as-yet not well defined mechanism. The finding that the cytosolic C-terminus, as well as a mutant that has lost its capacity to bind Ca^{2+}, can activate SOCE and Icrac (91) lends strong support to this conclusion.

Like STIM (85, 86), Orai (also CRACM, for CRAC modulator) was identified in RNA suppression screens carried out independently in three laboratories testing for suppression of thapsigargin-activated Ca^{2+} entry into cells loaded with a fluorescent Ca^{2+} indicator dye (87–89). RNA that suppressed SOCE also suppressed Icrac. An independent approach pointing to Orai as essential for SOCE and Icrac tracked the molecular basis of the loss of T cell receptor function in a familial case of severe combined immunodeficiency (SCID) described one year earlier (92). Positional cloning of the mutant locus identified an interval containing a mutant form of Orai1 (89).

Orai proteins, of which there is one in *Drosophila* and three in the mammalian genome, are predicted to span the plasma membrane four times and range in size from 250 to 301 aa. One of the extracellular loops of Orai1 has a consensus glycosylation site. Accordingly, expression of epitope-tagged Orai1 shows both a glycosylated and a smaller, unglycosylated form. Expression of wild-type Orai1 in cells from SCID patients reconstituted not only SOCE but also Icrac. The SCID-causing mutation was identified as an Arg to Trp mis-sense mutation in the human Orai1 gene (89).

Co-expression of STIM1 and Orai1 results in thapsigargin-induced Ca^{2+} entries as well as Icrac currents that are increased between 10 to 100 times compared to the effect of either alone (93–95). The fact that Icrac currents generated by co-expression of Orai and STIM (Orai1 and STIM1) were much larger than ever seen before and developed without requiring cotransfection of a TRPC de-emphasized the idea that TRPCs are part of SOCE/Icrac channels and has been taken to mean that Orai is a pore-forming Ca^{2+} channel activated by its interaction with STIM. In apparent support, mutations in Orai were found to change the ion permeation characteristics of Icrac channels induced by the coexpression of STIM1 and the mutated Orai (96–98) (**Figure 10**). This has led to the proposal that Orai is the only channel responsible for Icrac and SOCE. Indeed, two recent reviews of molecular aspects of SOCE and Icrac (99, 100) and one recent review on the role of Ca^{2+} in immune cells including neutrophils (101) do not discuss TRPCs as proteins related to Ca^{2+} entry into cells, while prominently displaying the STIM-Orai/CRAC pathway.

Leaving the question as to whether Orai is a channel or a regulatory subunit of TRPCs aside, several lines of evidence indicate the participation of both Orai1 and TRPCs in store

Annu. Rev. Pharmacol. Toxicol. 2009.49:395-426.

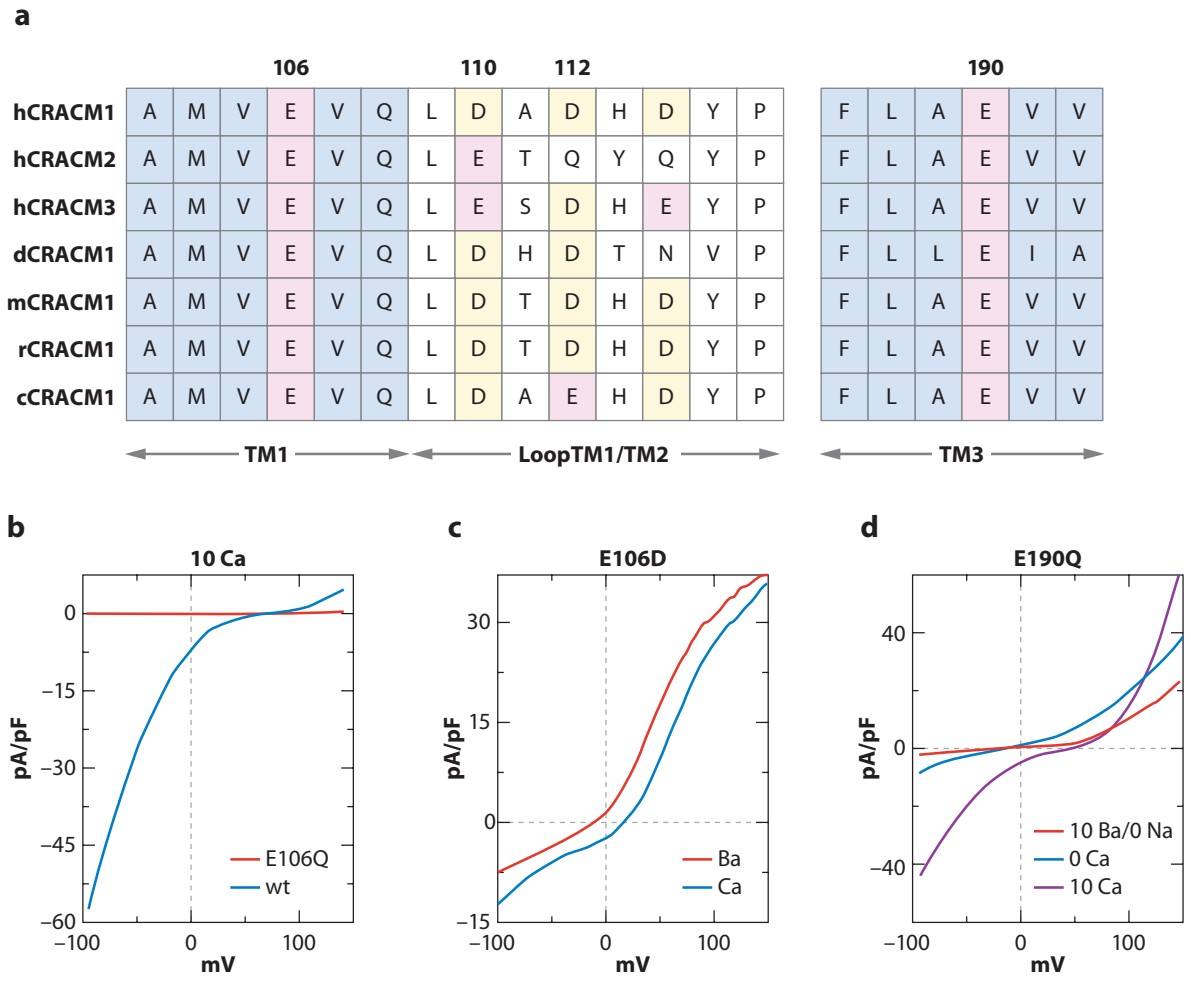

Figure 10

Evidence for ion channel nature of human Orai1. I-V curves of HEK cells co-transfected with STIM1 and wild-type or mutated Orai1. (*a*) Partial amino acid alignment of human (h), *Drosophila* (d), mouse (m), rat (r) and chicken (c) Orai highlighting locations of E106 and E190 in TMs 1 and 3, close to the external surface of the plasma membrane. (*b*) through (*d*) Icrac currents evoked in HEK cells by wild-type and mutant forms of Orai1 in the presence of excess STIM1. The fact that permeation characteristics are altered by the mutations in (*c*) and (*d*) is taken as proof positive that Orai1 is an ion channel [Vig et al. (96); Prakriya et al. (97); Yeromin et al. (98)]. Adapted from Vig et al. (96).

Annu. Rev. Pharmacol. Toxicol. 2009.49:395-426.

depletion-activated Ca^{2+} influx. One showed TRPC-dependent enhancement (augmentation) of thapsigargin-induced SOCE by Orai1 (63) (**Figure 11**). The second showed that [R91W]Orai1—the mutation responsible for a familial form of SCID (89)—exhibits dominant negative properties when expressed in HEK-293 cells or in HEK cells stably expressing TRPC3, reducing not only thapsigargin-stimulated SOCE, but also classical ROCE (102) (**Figure 11**). The third, and perhaps more important, piece of evidence that Orai interacts functionally with TRPCs affecting SOCE was the finding that expression of Orai1 in TRPC-expressing cells reconstituted classical Icrac (102) (**Figure 12**). These studies also revealed the presence in membranes of TRPC3- and TRPC6-expressing cells of spontaneously active nonselective cation channels of 120 and

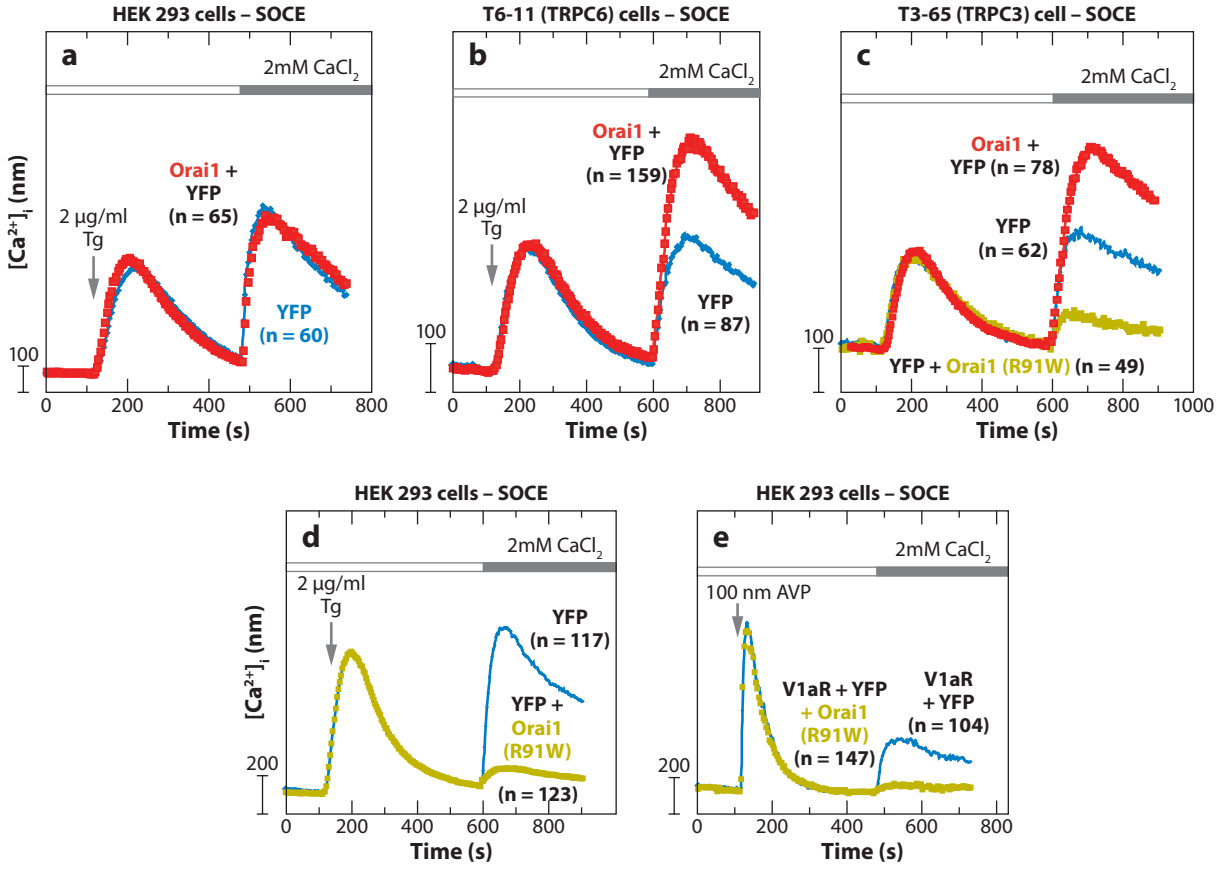

Figure 11

TRPC-dependent enhancement of SOCE by Orai1 and inhibition of SOCE and ROCE by SCID-causing [R91W]Orai. (a), (b), and (c), wild-type Orai1 does not affect SOCE in HEK-293 cells but increases it in cells expressing a TRPC [TRPC6 in (b) and TRPC3 in (c)]. (c), (d), and (e), Mutant human Orai1[R91W] acts as a dominant-negative, reducing SOCE and ROCE in TRPC-expressing cells and in control HEK-293 cells. Inhibition of SOCE is shown in TRPC3-expressing cells and in HEK-293 cells (c) and (d); inhibition of ROCE is shown for HEK-293 cells (e). Adapted from Liao et al. (102).

60 pS, respectively, that had their Po markedly reduced by expression of SOCE-enhancing levels of Orai1. TRPC7-expressing cells have much higher levels of spontaneous activity that is unaffected by expression of Orai1. Membranes from TRPC1-expressing cells showed no spontaneous activity, in spite of the fact that TRPC1-expressing cells respond to expression of Orai with an increase in SOCE and Icrac (102).

LIPID RAFTS AS SITES OF ASSEMBLY OF ORAI, TRPC, AND STIM DEPENDENT SOCE CHANNELS

Transduction of the Ca^{2+} store's signal, generated by Ca^{2+} loss, from the lumenal side to the cytosolic side may involve STIM's dimerization (103), in analogy with the mechanism by which other single pass transmembrane receptors, such as the EGF receptor, signal across membranes. As seen in cells overexpressing fluorescently tagged STIM1 and Orai1, upon store depletion, ER

Annu. Rev. Pharmacol. Toxicol. 2009.49:395-426.

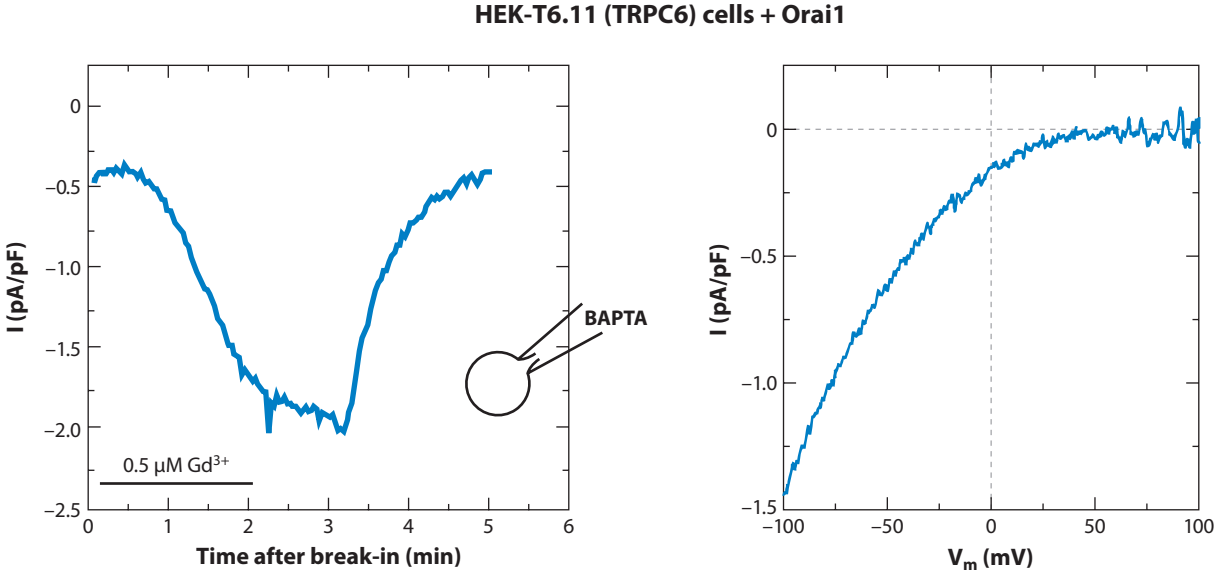

Figure 12

Expression of Orai1 in TRPC6-expressing cells reconstitutes Icrac. HEK-293 cells or HEK cells stably expressing a TRPC, when patch-clamped with BAPTA-containing pipettes, develop only minimal Icrac [<0.2pA/pF; Liao et al. (102)]. This is not changed upon expression of Orai1 in HEK-293 cells (Liao et al. (102), but leads to 10-fold increases in Icrac if Orai is expressed in cells that stably express a TRPC, as shown in the figure for TRPC6. Adapted from Liao et al. (102).

Annu. Rev. Pharmacol. Toxicol. 2009.49:395-426.

STIM1 co-clusters with plasma membrane Orai1, forming so-called junctional complexes through which Ca^{2+} enters into cells (104, 105), also referred to as puncta (106).

In cells overexpressing TRPC1, STIM1 and Orai1, the three molecules form ternary complexes thought to constitute Ca^{2+} influx channels (107). In agreement with these studies, STIM1 has been shown to have the ability to interact with TRPC1 in vitro and to heteromultimerize TRPC channels in intact cells (108). Cahalan and collaborators co-immunoprecipitated transfected *Drosophila* STIM and Orai from S2 cell lysates (98) and TRPC1 and STIM1 were co-immunoprecipitated from submaxillary gland cells by Ong et al. (107). Stable associations in untransfected cells between other TRPCs and STIM or Orai, or between STIM and Orai, have apparently not as yet been reported. Although the molecular make-up of SOCE and Icrac channels and the molecular interactions between STIM and plasma membrane components of SOCE/Icrac channels remain unclear, two groups have reported that their functional assembly involves lipid raft domains of the plasma membrane (109, 110). Pani et al. (109), working with human submandibular gland (HSG) and HEK-293 cells, showed that STIM containing puncta partition into both submembrane domains and into lipid rafts of plasma membranes, where STIM1 colocalizes with TRPC1 allowing for their coimmunoprecipitation. Disruption of lipid rafts reduced partitioning of STIM1 and TRPC1 into lipid raft fractions and reduced thapsigargin-induced SOCE. Interestingly, down-regulation of TRPC1 did not affect either reorganization of STIM1 or its partitioning into lipid raft domains, but reduced thapsigargin-stimulated (store-operated) inward currents (107). Under whole cell patch-clamp conditions with 2 mM EGTA in the pipette and 1 mM Ca^{2+} in the bath, Sampieri et al. (110) recorded a nonrectifying store-operated current (SOC) from untransfected HEK29-3T cells that had a reversal potential of 25 mV, suggesting some Ca^{2+} selectivity. This SOC (*a*) remained essentially unchanged upon down-regulation of Orai1-3; (*b*) was reduced

by greater than 80% at −100 mV and Icrac-like (strongly rectifying) upon down-regulation of TRPC1, and (*c*) was absent in cells in which either STIM1 was down-regulated or Orai1-3 plus TRPC1 were down-regulated with the corresponding siRNAs. In cells transfected with TRPC1, SOC recorded under the same conditions was barely detectable, even though the cells developed a robust carbachol-induced ROCE. Expression of STIM1 together with TRPC1 led to a dose-dependent inhibition of ROCE and development of thapsigargin-induced SOC. Expression of STIM1 also caused a redistribution of the TRPC1 protein to lipid raft domains on the plasma membrane. Similar results, with the exception that the nonrectifying SOC in STIM1 plus TRPC1-transfected cells was suppressed by down regulation of Orai1 with siRNA, were obtained by Cheng et al. (111). It would appear, therefore, that the observations describing coclustering of STIM1 and Orai as junctional complexes at the plasma membrane (104, 105), of STIM1 having the ability to promote heteromultimerization of TRPC channels (108), and of store depletion or STIM1 induced partitioning of TRPC into lipid rafts (109–111) as well as the coimmunoprecipitation of TRPC1 with caveolin (66), all paint a consistent picture wherein SOCE channels are assembled at lipid rafts which are rich in caveolin (66, 104–111).

Thus, although the molecular architecture of STIM-activated TRPC-Orai complex has yet to be elucidated in terms of whether STIM interacts with TRPC, Orai, or with both, the accumulated evidence strongly suggests SOCE and Icrac channels are likely to be formed of both TRPC and Orai proteins and that STIM directs them to assemble SOCE channels in lipid raft domains of the plasma membrane (see Note Added in Proof).

IS ORAI AN ION CHANNEL?

Liao et al. (63) have proposed that Orai may be a regulatory subunit of TRPC channels, similar to minK's and MiRP's roles as regulatory subunits of the KvLQT1 and HERG1 voltage gated K^+ channels. MinK and MiRP are single pass transmembrane proteins that associate with KvLQT and HRG1 with a stoichiometry of two regulatory subunits per tetrameric K channel. The stoichiometric arrangement of Orai1 expressed in cells stably transfected with STIM1 was explored by Shuttleworth's laboratory (112), who took advantage of the fact that the inactive Orai1[E106Q] (**Figure 10**) has dominant-negative properties. These authors concatenated wild-type Orai1 into dimers, trimers, and tetramers and showed that only tetramers were insensitive to the dominant-negative action of Orai1[E106Q], leading to the conclusion that STIM1 activates a tetrameric Orai. It is difficult to visualize Orai as a covalently linked tetrameric regulatory complex of a tetrameric ion channel.

Hv1, the Voltage-Gated H^+ Channel, as a Structural Model for Orai

In 2001, Starace and Bezanilla analyzing the voltage sensor of the *Drosophila* Shaker K^+ channel by His-scanning mutagenesis discovered that the voltage sensor could operate as a pH-dependent proton channel (113). The concept of voltage-sensing domains operating as proton channels was proven true by the discovery of a voltage-gated proton channel-1 (Hv1) (114). More recently, MacKinnon's laboratory established that Hv1, when expressed in HEK cells, assembles as a dimer (115), leading to the prediction of the two-pore model shown in **Figure 13**. Although there is no sequence similarity between Hv1 or voltage sensors of cation channels, Orai proteins may nevertheless be structural homologues of voltage-sensing domains with ion conducting paths optimized for Ca^{2+}. Regardless of the state of oligomerization of active Orai, it stands to reason that if Orai proteins form bonafide Ca^{2+} channels, they are bound to be working in parallel with

Annu. Rev. Pharmacol. Toxicol. 2009.49:395-426.

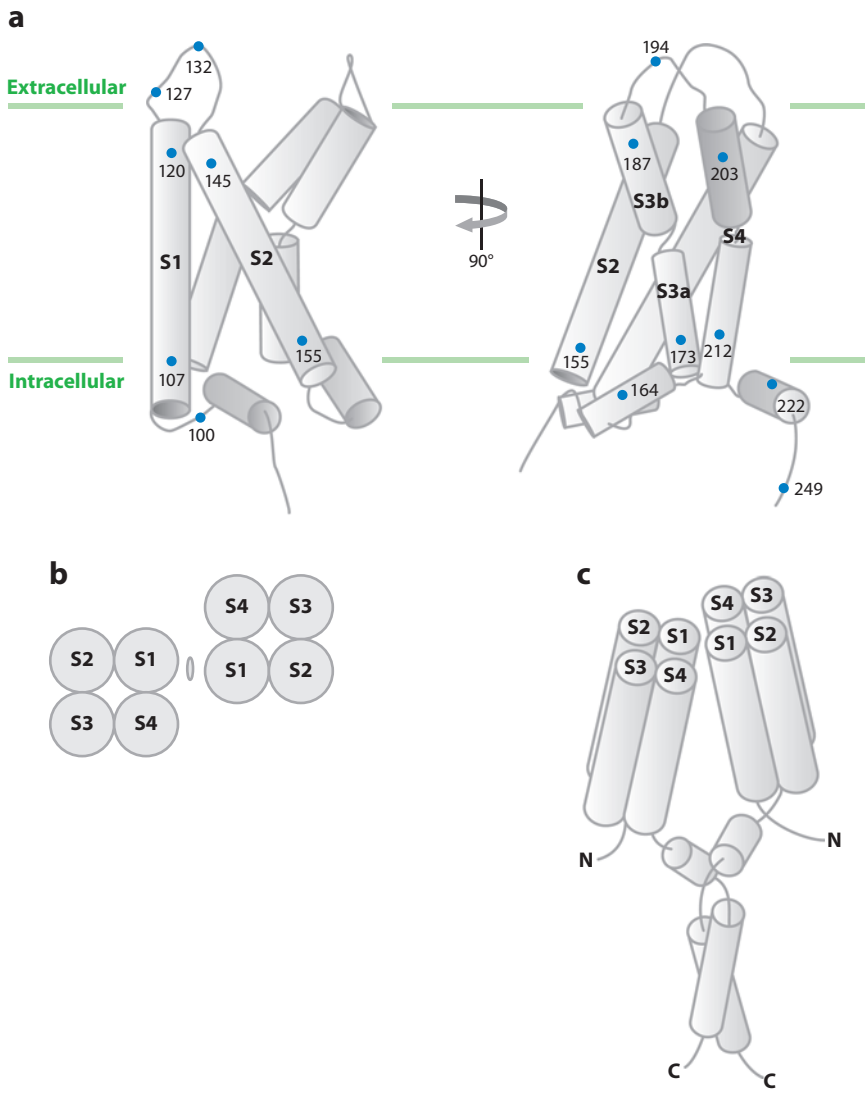

Figure 13

Orai as a 4TM cation channel based on a model of the 4-TM voltage-gated proton channel, Hv1. The Hv1 model is based on the crystal structure of K^+ channel voltage sensors (*a*) and the experimental finding that Hv1 proteins self-organize as dimers in which the respective S1 helices form the interacting interface (*b*). (*c*) Hv1 model as depicted by (115). Adapted from Lee et al. (115).

TRPC channels. It remains to be determined how TRPCs and Orai interact and regulate each other in the context of lipid raft environments.

CONCLUDING REMARKS

It is our contention that receptor-operated and store-operated Ca^{2+} entries both depend on TRPCs as well as on Orai. Store-operated Ca^{2+} entry and Icrac are assembled under the control of STIM and appear to occur in lipid rafts. In contrast, whereas receptor-operated Ca^{2+} entry in response to phospholipase Cβ activation by Gq/11 does not require partitioning of TRPCs or Orai into lipid rafts, it is likely—although not yet proven—to be independent of STIM. In addition to these modes of activation, some TRPCs are also chemical sensors. It is possible that all TRPCs are chemical sensors. Which chemicals activate which TRPCs needs to be determined. Given that the information so far available does not distinguish between Orai being a regulatory

Annu. Rev. Pharmacol. Toxicol. 2009.49:395-426.

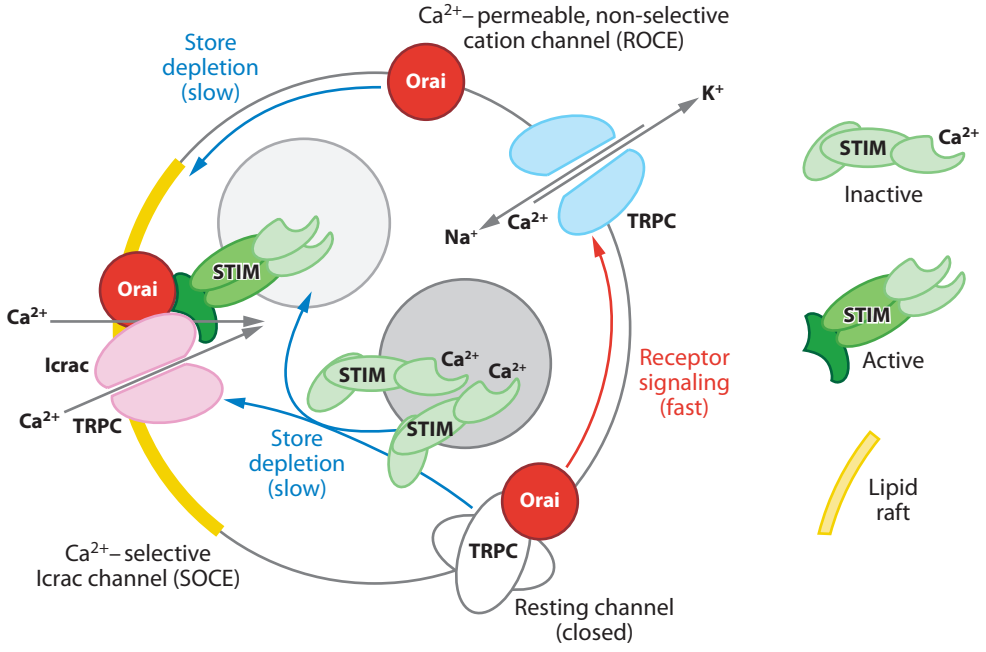

Figure 14

Model of sequential activation of TRPC channels. The receptor-Gq-PLC signaling pathway rapidly activates TRPCs leading to ROCE. Depletion of Ca^{2+} stores then activates STIM, which causes the redistribution of Orai and TRPCs and their partitioning into lipid rafts where the SOCE channel complex is assembled. This leads to Ca^{2+} entry and the development of Icrac. For some (C3 and C6), but not all TRPCs, their intrinsic tendency to spontaneous activity may be repressed by Orai in the resting state, as shown in the figure. Adapted from Liao et al. (102).

subunit, a regulatory subunit with Ca^{2+} channel properties, or a free standing Ca^{2+} channel, we offer the model of **Figure 14** as a working hypothesis for experimentation. This, along with the resolution as to which molecule STIM interacts with so as to organize the SOCE/Icrac channels, is the major issue calling for answers in the field that addresses store-operated Ca^{2+} entry, Icrac and the relative roles of Orai and TRPCs in this(these) process(es) and the mechanism by which STIM activates these channels.

DISCLOSURE STATEMENT

The author is not aware of any biases that might be perceived as affecting the objectivity of this review.

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NOTE ADDED IN PROOF

After completion of this article for publication, Rosado's laboratory reported the pair wise coimmunoprecipitation of Orai, TRPC1, and STIM from human platelet membranes (138).

Annu. Rev. Pharmacol. Toxicol. 2009.49:395-426.

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Contents

Annu. Rev. Pharmacol. Toxicol. 2009.49:395-426.

Indexes

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