The TRPC Family of TRP Channels: Roles Inferred (Mostly) from Knockout Mice and Relationship to ORAI Proteins

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Abstract

Aside from entering into cells through voltage gated Ca channels and Na/Ca exchangers in those cells that express these proteins, for all cells be they excitable or non-excitable, Ca^{2+} enters through channels that are activated downstream of phosphoinositide mobilization (activation of phospholipase C, PLC) and through channels that are activated secondary to depletion of internal stores. Depletion of internal stores activates plasma membrane channels known as ORAIs. Activation of PLCs activates the canonical class of transient receptor potential channels (TRPCs), and, because this activation also causes depletion of Ca^{2+} stores, also ORAI based channels. Whereas the activation of ORAI is a

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well-accepted phenomenon, it appears that TRPC channels also participate in Ca^{2+} entry triggered by store depletion with or without participation of ORAI molecules. Regardless of molecular makeup of TRPC containing channels, a plethora of studies have shown TRPCs to be important both in physiologic systems as well as in pathophysiologic phenomena. Particularly important in defining roles of TRPCs, have been studies with mice with targeted disruption of their genes, i.e., with TRPC KO mice. In this chapter we first focus on TRPCs as regulators of body functions in health and disease, and then focus on the possible make-up of the channels of which they participate. A hypothesis is set forth, whereby ORAI dimers are proposed to be regulatory subunits of tetrameric TRPC channels and serve as structural units that form ORAI channels either as dimers of dimers or trimers of dimers.

Keywords
Ca^{2+} Influx • TRPC • ORAI • Icrac • SOCE • ROCE • TRPC physiology • TRPC pathophysiology • Knockout mice • Phenotype

Abbreviations

CaCaM	Ca^{2+}-calmodulin complex
DAG	Diacylglycerol
ER	Endoplasmic reticulum
GPCR	G protein coupled receptor
IP3	Inositol 1,4,5-trisphosphate
OAG	Oleyl-acetyl-glycerol
PLC	Phospholipase C
PM	Plasma membrane
PMCA	Plasma membrane Ca^{2+}-activated ATPase
ROC	Receptor operated channel
ROCE	Receptor operated Ca^{2+} entry
SERCA	Sarcoplasmic endoplasmic reticulum Ca^{2+}-activated ATPase
SOC	Store operated channel
SOCE	Store operated Ca^{2+} entry
TRPC	Transient receptor potential canonical
VSMC	Vascular smooth muscle cells

1 Introduction

Changes in intracellular Ca^{2+} concentrations constitute a fundamental mechanism by which a host of extracellular stimuli are transduced into cellular responses that can either be helpful in adapting to the extracellular milieu or be detrimental to the cell's survival—Ca^{2+} toxicity. One large class of stimuli acts by activating phospholipase C enzymes which, owing to the formation of inositol trisphosphate (IP3),

promote release of Ca^{2+} from internal stores. The rises in cytosolic Ca^{2+} ($[Ca^{2+}]i$) are transient, owing to the activity of Ca^{2+} pumps that rapidly restore "resting" levels by returning Ca^{2+} to the stores (SERCA pumps) and by extrusion from the cell (PMCA pumps). Persistent stimuli elicit cyclical elevations—oscillations. But, because of each oscillation causing a net loss of cellular Ca^{2+}, signaling—good or bad—would stop if it were not that secondary to PLC activation, a Ca^{2+} influx pathway is activated. This Ca^{2+} entry pathway is commonly referred to as receptor-operated Ca^{2+} entry or ROCE. An entry pathway similar to ROCE can be activated also by mere inhibition of SERCA pumps, which causes gradual emptying of the stores and is referred to as store-operated Ca^{2+} entry or SOCE. This emptying is associated with the appearance of a highly Ca^{+2} selective, Ca^{2+} release activated current, ICRAC, the electrophysiological correlate to SOCE. This chapter deals with TRPC channels—transient receptor potential canonical channels—as structural components of ROCE channels, their functional relationship and interaction with SOCE channels formed by ORAI proteins, and the multiple roles in physiology and pathophysiology (health and disease) that TRPC channels have been found to have.

2 SOCE and ICRAC Are Mediated by Both ORAI-Based Channels and TRPC-Based Channels

With the discovery of ORAI (Vig et al. 2006; Feske et al. 2006; Zhang et al. 2006) and the finding that when expressed together with the endoplasmic reticulum (ER) Ca^{2+} sensor STIM, this gives rise to very large store depletion-activated currents with the bonafide characteristics of CRAC currents (Peinelt et al. 2006), Soboloff et al. 2006; Mercer et al. 2006), the previous interest in the roles and functioning of TRPC channels as molecular participants of SOCE and CRAC channels waned in the minds of many. The reasons for this were twofold: (1) when expressed in model cells, TRPCs had given rise to mostly nonselective cation channels with variable Ca^{2+} selectivity over Na^{+}, none more than 10:1, most less 3:1, whereas CRAC channels are highly Ca^{2+} selective, and (2) store depletion was not a good trigger, if at all, for TRPC activation.

However, one TRPC stood out, TRPC1, which, even if nonselective, is activated by store depletion. This was shown in one series of reports, in which thapsigargin was found to activate a store-operated current or channel (SOC) recorded in the whole-cell configuration of the patch clamp technique from cells transfected with the TRPC1 cDNA (Ong et al. 2007). Interestingly, TRPC1 SOC activity was augmented by expression of STIM1, and this required ORAI1 (Cheng et al. 2008), indicating that the TRPC1 machinery interacted with the ORAI machinery. In support of store depletion being a regulator of TRPC1, single-channel SOC currents with slope conductances of approximately 2pS were recorded from cell-attached and inside-out membrane patches of cells exposed to BAPTA-AM to deplete their Ca^{2+} stores, and these SOCs were absent in cells from *Trpc*$^{-/-}$ mice (Shi et al. 2012). At the biochemical level, STIM1 was discovered to gate not only ORAI channels but also TRPC1 channels, providing a link between

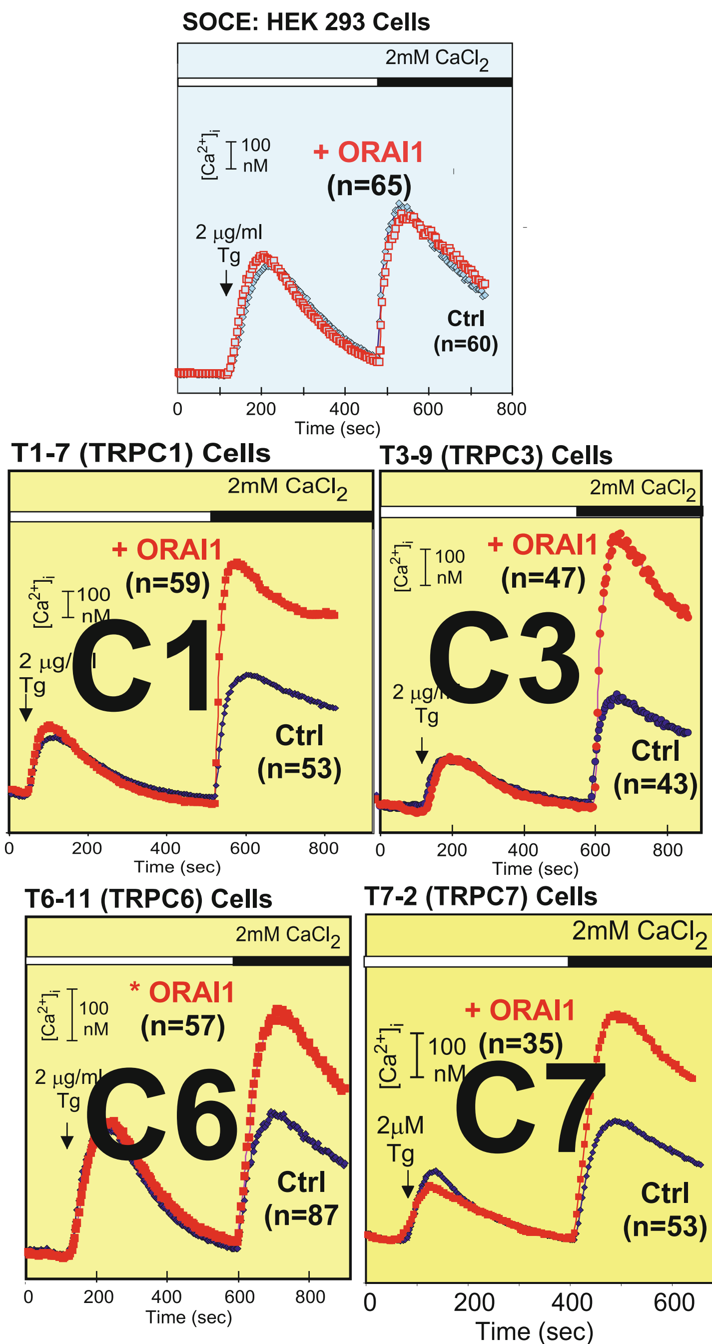

Fig. 1 Functional interaction of ORAI with TRPCs as seen in tests for SOCE. Store-operated Ca^{2+} entry is defined as the Ca^{2+} entry seen upon addition of $CaCl_2$ to the extracellular milieu of cells in which internal stores have been depleted in the absence of external Ca^{2+} by inhibition of SERCA pumps without activation of a PLC enzyme. SOCE was assessed in control HEK-293 cells and in

store depletion and TRPC1 (Zeng et al. 2008). Gating of TRPC1 and ORAI1 by STIM1 differed in that that of TRPC1 occurs by an electrostatic mechanism involving the polybasic C-terminus of STIM and the AspAsp, AspGlu or Glu/Glu (DD, DE or EE) sites next to the TRPC's EWFKAR motif (TRP box) (Zeng et al. 2008), whereas gating of ORAI channels by STIM1 occurs at ORAI sites interacting with STIM's SOAR (Stim-Orai-Activation Region, Yuan et al. 2009) or CAD [for CRAC Activation Domain, Park et al. (2009)].

Independent studies confirmed the existence of functional interactions between TRPCs and ORAI. Thus, when ORAI was expressed in cells with unpaired TRPC channels, but not in control cells not expressing an excess of TRPCs, it promoted a robust increase in store-operated Ca^{2+} entry (SOCE) (Liao et al. 2007; Fig. 1); ORAI1 expression also facilitated recording of CRAC currents (Liao et al. 2008), and under resting conditions, it silenced spontaneous activity of the unpaired TRPCs (Liao et al. 2007). These findings suggested that TRPCs and ORAIs interact at a functional level and possibly also at the physical level. This concept was then supported further by the fact that expression of Orai1[R91W] behaved as a dominant negative element (dnORAI) inhibiting not only SOCE elicited by inhibition of SERCA pumps but also Gd^{3+}-resistant ROCE and OAG-stimulated Ca^{2+} entry in cells overexpressing TRPC3, one of the OAG-activated TRPCs. SOCE channels and ICRAC, activated without participation of PLC-activating stimuli, are inhibited by 0.5–1.0 μM Gd^{3+}. As in the above-mentioned ROCE experiment, the ORAI channels were inhibited by Gd^{3+}; the ROCE channel(s) inhibited by the dnORAI1 can safely be assumed to be TRPC channels (Fig. 2). In the OAG experiment, the Ca^{2+} entry inhibited by the dnORAI1 occurred without store depletion, depended on overexpression of one of the OAG-responsive TRPCs, and was therefore also mediated by a TRPC channel (Liao et al. 2009) and not by an ORAI channel which requires, for its assembly, the molecular orchestration of STIM clusters engendered by store depletion (Luik et al. 2006; Wu et al. 2006).

3 Structural Considerations

ORAIs, of which there are three, are membrane proteins with four transmembrane (TM) domains. Biochemical studies on the structure of ORAI1 in resting cells and after activation by STIMs found ORAI1 to exist as dimers and the assembled CRAC channel to be dimers of dimers (Penna et al. 2008). Scoring of the inhibitory effect, another dnORAI1, the pore-dead mutant of ORAI1 (ORAI1[E106Q]), on the CRAC channel activity of wild-type ORAI1 transfected as concatenated dimers, trimmers, and tetramers also led to the conclusion that the ORAI1-based CRAC

Fig. 1 (continued) HEK cells that stably expressed TRPC1 (T1-7 cells), TRPC3 (T3-9 cells), TRPC6 (T6-11 cells), and TRPC7 (T7-2 cells), into which non-inhibitory amounts of myc-tagged ORAI1 expression plasmids had been transfected 48 h before imaging (adapted from Liao et al. 2007)

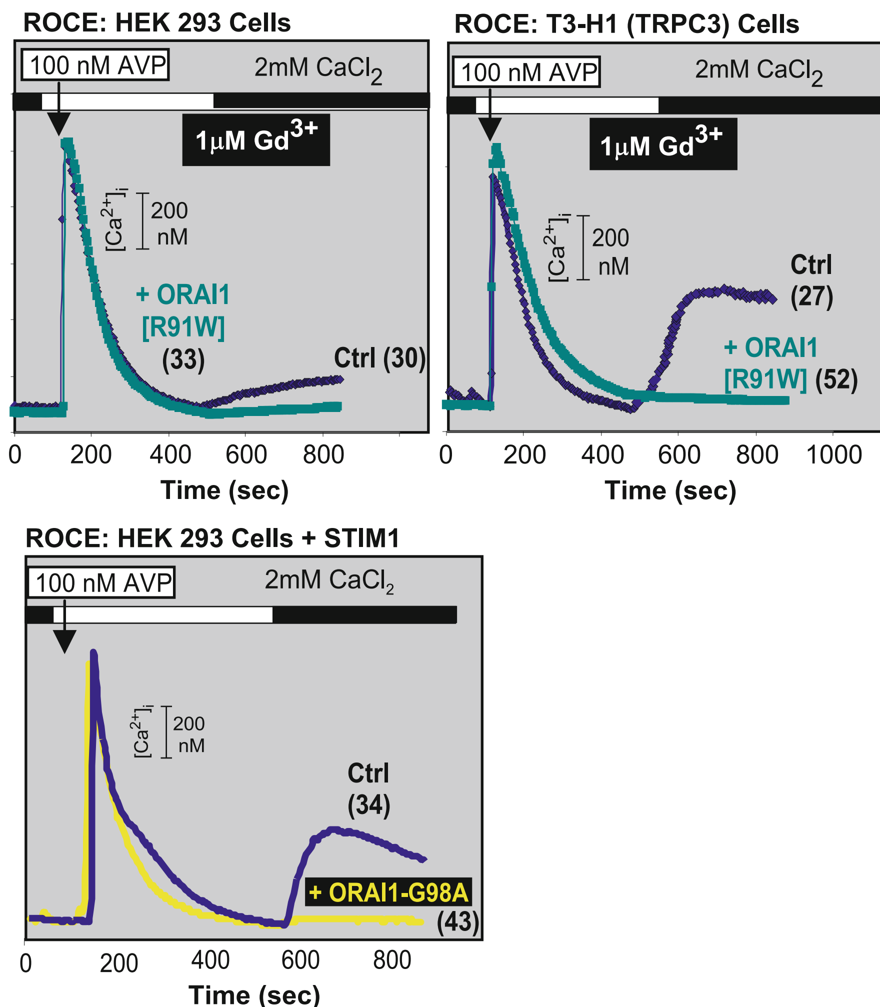

Fig. 2 Functional interaction of ORAI with TRPCs as seen in tests for ROCE. Receptor-operated Ca^{2+} entry is defined as the Ca^{2+} entry seen upon addition of Ca^{2+} to the extracellular milieu of cells in which a Gq-coupled G protein receptor agonist had been used to stimulate PLCβ activity in the absence of extracellular Ca^{2+}. The intracellular Ca^{2+} transient had been allowed to run its course—release of Ca^{2+} from the ER and reduction of cytosolic Ca by the action of PMCA and SERCA pumps. *Top two panels*: ROCE elicited by V1a receptor stimulation is inhibited by Gd^{3+} in HEK 293 cells but not in T3-H1 cells which stably overexpress TRPC3. *Bottom panel*: ROCE was assessed in HEK-293 cells transfected with the V1a receptor expression plasmid, a eYFP-STIM1 expression plasmid, with or without a plasmid driving the expression of ORAI1[G98A], a dominant negative mutant of ORAI1

channel is an ORAI1 tetramer (Mignen et al. 2008). To the surprise of many, if not all, the crystal structure of ORAI—Drosophila ORAI—showed it to be assembled as an hexamer formed of three dimers (Hou et al. 2012; Fig. 3a). ORAI1 can

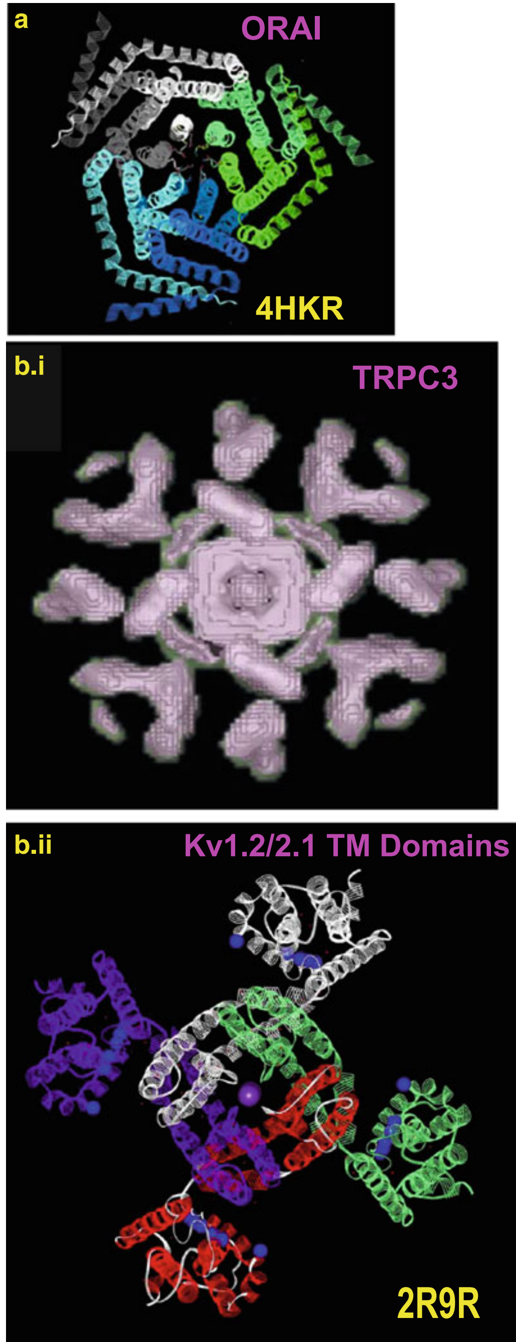

Fig. 3 Models of ORAI (**a**) and TRPCs (**b**). (**a**) Hexameric ORAI (PDB: 4HKR, Hou et al. 2012). (**b**-i), Tetrameric TRPC3 reconstructed from cryo-electron micrographs (adapted from Mio et al. 2007). (**b**-ii), Tetrameric voltage-gated K+ channel (PDB: 2R9R, Long et al. 2007) as surrogate for that of a TRPC channel

therefore be expected to coexist in three formats—dimer, tetramer, and hexamer—of which the relative abundance depends on the interaction with activated STIMs and the level of expression, with hexamers being favored in cells overexpressing ORA1, a situation that may mimic the artificially high concentrations at which proteins are set up to drive their crystallization.

Glycosylation scanning of the transmembrane topology of TRPC3 showed it to pass the membrane six times with cytosolic N- and C-termini akin to voltage-gated K^+ channels (Vannier et al. 1998) but lacking the voltage sensing Arg- and Lys-rich TM4 of voltage-gated K^+ channels. Voltage-gated K^+ channels are tetramers (e.g., Long et al. 2007; PDB: 2R9R; Fig. 3b-ii). Analysis of cryo-electron micrographs of TRPC3 expressed in HEK293 cells by Mio et al. (2007) (Fig. 3b-i) confirmed the generally held assumption that assembled TRPCs channels are tetramers. The most plausible model of a tetrameric TRPC channel is thus one resembling that of voltage-gated K^+ channel, shown in Fig. 3b-ii.

4 Complexity in the Subunit Composition of TRPCs

There are seven *TRPC* genes in the nonhuman mammalian genomes and six in Old World monkeys and human genomes in which *TRPC2* is a pseudogene. Of the seven (six) TRPCs, most cells and tissues express between three and four forming heteromeric complexes that vary with cell type and approach used to determine the subunit composition. The degree of TRPC subunit complexity was well documented by the subunit analysis of SOC channels in vascular smooth muscle cells (VSMCs) in which selective peptide-directed antibodies were added to medium bathing inside-out membrane patches in which SOCs had been activated by store depletion. As illustrated in Fig. 4, single-channel SOCs in mesenteric artery SMVCs are immunoneutralized by either anti-TRPC1 or anti-TRPC5 when derived from wild-type mice but only by anti-TRPC5 in *Trpc1*$^{-/-}$ mice, in which the unpaired TRPC5 channel appeared with properties that differed from those seen in the WT SOC and were those expected from TRPC5 expression in model cells (Shi et al. 2012). The likely TRPC subunit makeup of SOCs in WT mesenteric artery VSMCs is that of a heteromeric tetramer formed to TRPC1 and TRPC5. When the same analysis was applied to VSMCs from coronary artery and portal vein using four antibodies, anti-C1, anti-C5, abti-C6, and anti-C7, all SOCs were immunoneutralized by ant-C1 and anti-C5 (Fig. 4b–d), but anti-C6 immunoneutralized also coronary artery VSMC SOCs (Fig. 4b), whereas portal vein SOCs were immunoneutralized by anti-C7, but not anti-C6 (Fig. 4d). Thus, what appears to be similar cell types, vascular smooth muscle cells, show differences at the level of TRPC channel makeup. Although anti-C6 did not affect mesenteric artery VSMCs, this does not mean that the difference is due to expression or not of the *Trpc6* gene, because similar studies on single-channel currents activated by angiotensin II, instead of store depletion, showed presence of TRPC6-based cation currents in these cells (Shi et al. 2010).

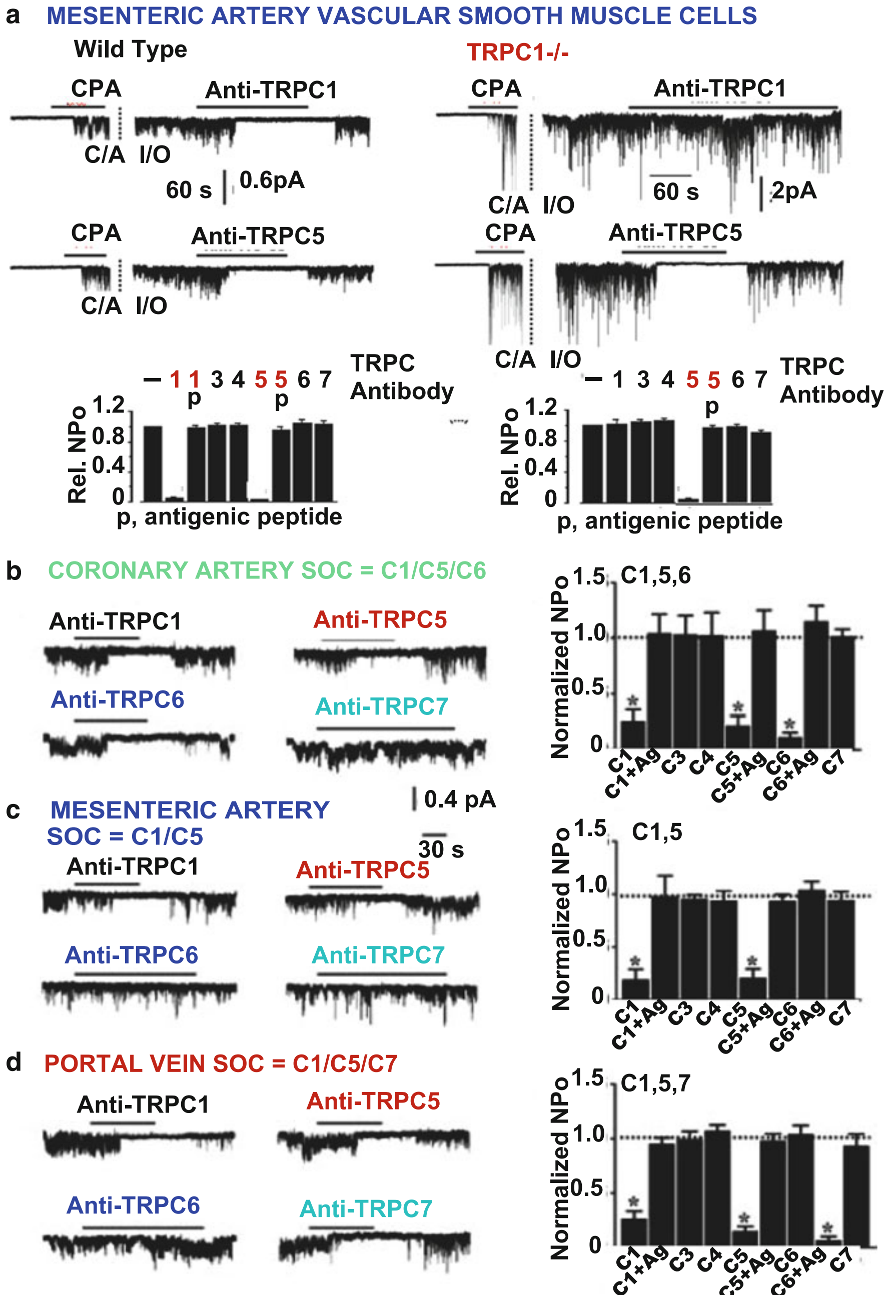

Fig. 4 Heterogeneous TRPC subunit makeup of vascular smooth muscle store-operated channels (SOCs). Vascular smooth muscle cells were patched in the cell-attached configuration of the patch clamp technique (C/A patches), their SOCs activated by treatment with the SERCA pump inhibitor cyclopiazonic acid (CPA) in the cell, and patches excised to expose the cytosolic aspect of the membrane patches [inside-out patches (I/O patches)]. The indicated antibodies were then added to test for their inhibitory activity. Washout restored activity and immunogenic peptides neutralized

Given that similar cell types assemble TRPC channels with a different subunit makeup, it stands to reason that the complexity in any given cell type cannot be predicted and has to be assumed to be unknown unless explicitly tested for. It also stands to reason that phenotypes that may develop upon single- or double-gene disruptions may differ with the cell type under study.

5 Dependence of TRPC's Channel Function on ORAI

The fact that ORAI1[R91W] inhibits OAG-activated TRPC (TRPC3 or TRPC6) raised the interesting possibility that whereas "pure" CRAC channels—activated by store depletion in the absence of PLC activation—are ORAI tetramers or hexamers, the ORAI dimers may play dual roles: structural CRAC channel subunits assembled into an active ion channel and regulatory subunits of TRPC-based channels. In this view a TRPC-based channel is formed of four TRPC molecules and four ORAI dimers. One corollary of this view is that the functioning of a TRPC channel depends on ORAI: "No ORAI, no channel-competent TRPC".

ORAI1 has been knocked out in two laboratories and it is possible to prepare *Orai1*$^{-/-}$ embryonic fibroblasts (ORAI1 KO MEFs). We used 35-cycle RT-PCR analysis of ORAI1 KO MEF RNA and found the MEFs to express both *STIM* genes (*Stim1* and *Stim2)* and the remaining *Oraii2* and *Orai3* genes. Whereas wt MEFs express *Trpc1*, *Trpc2*, and *Trpc6*, the ORAI1 KO MEFs express *Trpc1*, *Trpc2*, and *Trpc4* and therefore have an altered gene expression profile. In tests for ROCE and SOCE, we found that ORAI1 KO MEFs have essentially no SOCE or ROCE responses when challenged with thapsigargin to activate their SOCE or with a Gq-coupled GPCR agonist to activate their ROCE, respectively (Fig. 5). These experiments are in agreement with the view that TRPCs depend on an ORAI, because ROCE should not have been interfered with by the loss of ORAI1, as ORAI and TRPC channels should have been able to operate independently of each other. Consistent with this view, TRPC1 and ORAI1 co-immunoprecipitate (Ong et al. 2007).

The results with ORAI1 KO MEFs are interesting in that they indicate that endogenous levels of ORAI2 and ORAI3 cannot compensate for loss of ORAI1. Yet overexpression of ORAI2 or ORAI3 in ORAI1 KO MEFs reconstitutes SOCE, albeit to a lower extent (Fig. 5, top panel). ORAI1 is thus the dominant protein in setting SOCE and ROCE activities of a cell, and ORAI2 and ORAI3 can replace ORAI1 to varying degrees that may differ from cell to cell. In vivo studies with *Orai1* KO mice reached similar conclusions (Vig et al. 2008; Gwack et al. 2008; Kim et al. 2011).

Fig. 4 (continued) the inhibitory effects of the corresponding antibodies. The heteromeric composition of the TRPCs activated by store depletion was concluded to be C1/C5 for cells from mesenteric artery, C1/C5/C6 for cells from the coronary artery, and C1/C5/C7 for cells from the portal vein. Panel (**a**) was adapted from Shi et al. (2012), and panels (**b**), (**c**), and (**d**) were adapted from Saleh et al. (2008)

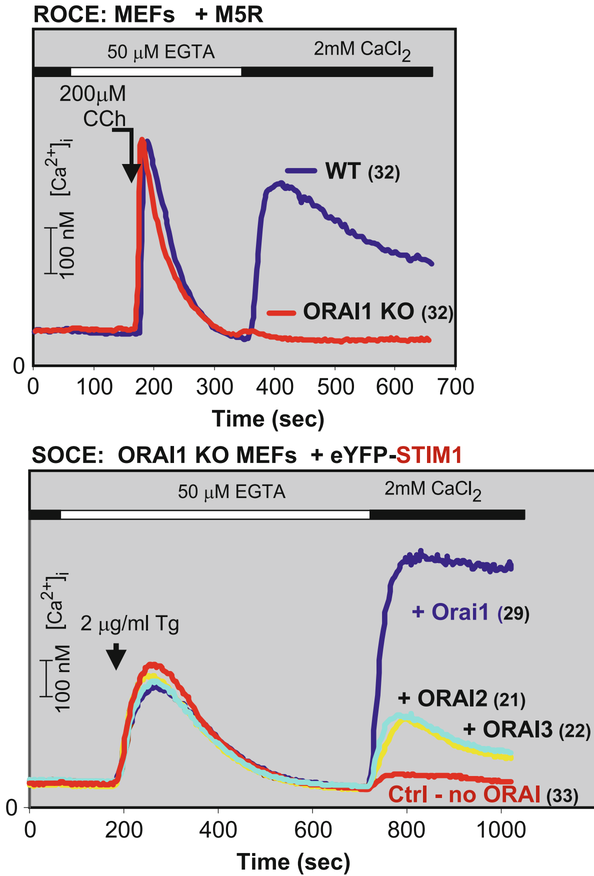

Fig. 5 ROCE and SOCE are critically dependent on ORAI. *Left Panel*: ROCE in WT and ORAI1 KO mouse embryonic fibroblasts (MEFs) activated by a transfected M5 mACh receptor. *Right panel*: SOCE in ORAI1 KO MEFs transfected with an empty pCMV expression vector (no ORAI) and with pCMV directing the expression of myc-tagged human ORAI1, ORAI2, or ORAI3

6 To What Extent Is ORAI Function Dependent on a TRPC?

Whereas TRPCs appear to depend on ORAI, the answer to the converse question is unknown. Another unknown, related to the previous, is in how many formats ORAIs may adopt. Taken together the evidence discussed so far may suggest that ORAI molecules exist in one of four configurations (Fig. 6): (1) as a non-active dimeric precursor of (2) tetrameric and (3) hexameric ORAI-only channels and (4) in association with TRPCs, where they act as regulatory subunits of TRPC channels. In turn, TRPCs may exist in one of two configurations: (a) inactive tetramers, often located within the cells and trafficking to the cell surface in response to an external stimulus (e.g., Chaudhuri and Colles 2008), and (b) active TRPC-ORAI hetero-multimers in the plasma membrane. ORAI dimers may or may not have changed conformation upon binding to TRPCs.

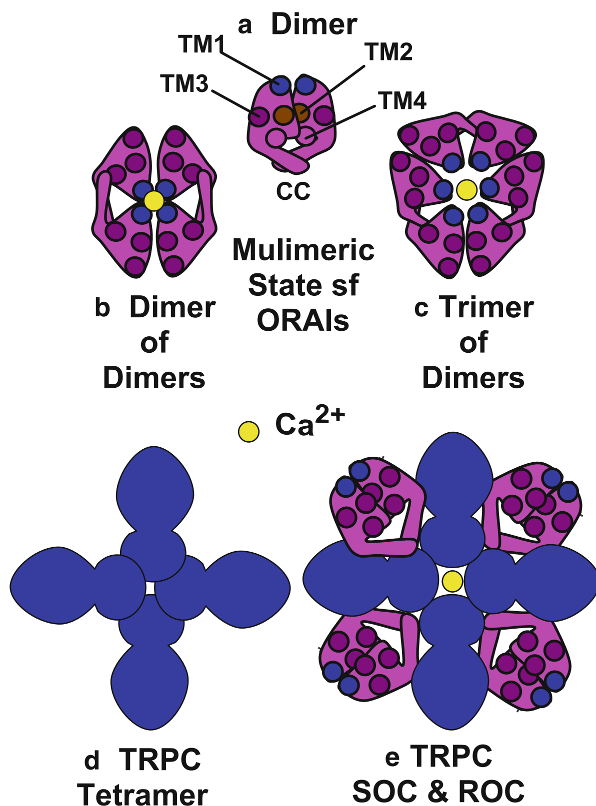

Fig. 6 Models of molecular makeup of CRAC and TRPC channels. (**a**) Cartoon of an ORAI dimer based on the crystal structure of the hexameric trimer of dimers ORAI (PDB 5HKR, Fig. 3). *Blue circle*, pore lining TM1, CC, coiled coil formed of the C-termini of the two monomers. The SOAR/CAD interacting domains located on the ORAI CC region. Mutations that disrupt the CC structure impede activation by STIM. (**b**) Hypothetical channel-competent ORAI tetramer with Ca^{2+} atom in its pore. (**c**) Cartoon of hexameric ORAI channel with Ca^{2+} in its pore. (**d**) Inactive tetrameric TRPC complex. (**e**) Channel-competent TRPC-tetramer with four ORAI dimers bound to it. It is hypothesized that ORAI tetramers and hexamers are CRAC channels and that the [TRPC]4-[ORAI2]4 complex is a CRAC-like SOC, whereas nonselective ROCs are TRPC tetramers with less than four ORAI dimers

The central roles of ORAIs in Ca^{2+} signaling downstream of PLC activation became evident early on, as a missense mutation of *Orai1*, *ORAI1[R91W],* was found to be the molecular cause of a familial severe combined immune deficiency (Feske et al. 2006). Thus, loss of ORAI leads to strong phenotypes, which was how ORAI was discovered: RNAi-mediated suppression of *Orai1* caused loss of SOCE, and homozygous *Orai1* KO mice tend to die perinatally; do not procreate and have impaired mast cell, T cell, and B-cell function, suffer from hair loss; and have stunted growth (Vig et al. 2008; Gwack et al. 2008; Kim et al. 2011). Since ORAIs have not been expressed and/or studied in cells proven null for all seven TRPCs, the

question whether perhaps the channel-competent ORAI1 tetramer may coexist and depend with a TRPC-tetramer cannot be answered at this time. In contrast, loss of any one or two of the TRPCs does not compromise normal growth nor does it interfere with procreation. The difference presumably lies in that there are only three ORAIs with one, ORAI1, being dominant, whereas there are seven TRPCs and loss of one or two can be compensated by the remaining TRPCs. This is not to say that loss of TRPCs do not bring about distinct phenotypes.

7 Phenotypic Changes in TRPC KO Mice Reveal a Broad Spectrum of Physiological and Pathophysiological Roles for TRPCs

All mouse *Trpc* genes have been disrupted as reported in Dietrich et al. (2007) for *Trpc1*, Stowers et al. (2002) for *Trpc2*, Hartmann et al. (2008) for *Trpc*3, Freichel et al. (2001) for *Trpc4*, Phelan et al. (2013) for *Trpc5*, Dietrich et al. (2005) for *Trpc6*, and Perez-Leighton et al. (2011) for *Trpc7*, and the literature is replete with functional roles for TRPCs in health and disease stemming from studies on the TRPC KO mice. As detailed in Table 1, these roles span from neuronal to smooth and skeletal muscle, the immune system, inflammation, cardiac hypertrophy, pathologic remodeling of endothelial cells in response to static stretch, wound healing, and myogenic tone and blood pressure regulation. They also include specialized systems such as vomeronasal pheromone sensing (TRPC2), light responses initiated by melanopsin in intrinsically photosensitive retinal ganglion cells (ipRGCs) that project to the suprachiasmatic nucleus to entrain circadian rhythms (TRPC6 plus TRPC7), normal touch (TRPC3 plus TRPC6), and auditory neurotransmission (TRPC3). Independent studies of human mutations and syndromes have pointed to a role of TRPC6 in normal glomerular filtration in renal kidney (Reiser et al. 2005; Winn et al. 2005) and raised the involvement of a TRPC in a host of other syndromes that are outside of the scope of this review. The phenotypic changes ascribed to loss of TRPC function listed in Table 1 are primarily those seen in knockout mice, though there are also some phenotypic changes listed as seen in cultured cardiac myocytes [TRPC3 and TRPC6; Onohara et al. (2006)] and organotypic cultures of cerebral resistance artery segments denuded of their endothelial cell layer (Welsh et al. 2003). In skeletal muscle, TRPC1 has been shown to counteract muscle fatigue (Zanou et al. 2010), to aid in muscle regeneration (Zanou et al. 2012). In a model of dopaminergic neurons, TRPC1 was shown to aid in maintenance of ER Ca^{2+} homeostasis and in so doing to counteract the effects of neurotoxin-induced ER stress and development of the unfolded protein response (Selvaraj et al. 2012), relevant in the context of Parkinson's disease development.

Gain-of-function mutations of *TRPC6* are responsible for familial forms of focal segmental glomerulosclerosis in man (Winn et al. 2005; Reiser et al. 2005), and a gain-of-function mutation of *Trpc3* in mice is responsible for the "Moonwalker" ataxia caused by loss of Purkinje cells (Becker et al. 2009). The "Moonwalker" phenotype develops in mk/+ mice; the mwk/mwk genotype is embryonic lethal.

Table 1 Examples of roles of TRPC channels (nonselective calcium-permeable cation channels) as seen from different points of view

Biochemical roles	
NFAT activation—Calcineurin (CaN) activation by Ca–calmodulin (CaCaM) (Seth et al. 2009; Poteser et al. 2011)	TRPC1; TRPC3
TLR4-CD14 signaling—MLCK (CaCaM) (Tauseef et al. 2012)	TRPC6
NFkB activation in endothelial cells (ECs)	
by GPCR—CaMKKβ (CaCaM) in ECs (Bair et al. 2009)	TRPC4
by LPS—MLCK (CaCaM) MyD88-IRAK4-MLCK complex in ECs (Tauseef et al. 2012)	TRPC6
TNFα signaling—CaMKII (CaCaM) in monocytes (Smedlund et al. 2010)	TRPC3
CaN activation by CaCaM—NFAT (Poteser et al. 2011)	TRPC3
CaMKKIIβ activation by CaCaM in activation of NFkB (Bair et al. 2009)	TRPC4
CaMKII activation by CaCaM—TNFα signaling—CaCaM-CaMKII (Tano and Vazquez 2011)	TRPC3
MLCK activation by CaCaM—activation of NFkB by LPS (Tauseef et al. 2012)	TRPC6
cGMP-independent signaling of the ANP receptor GC-A (membrane guanylyl cyclase A) (Klaiber et al. 2011)	TRPC3–C6
Physiological roles	
Agonist-induced Ca-mediated neurotransmitter release from dendrites (Munsch et al. 2003)	TRPC4
Synaptic transmission and motor control; slow EPSCs (Hartmann et al. 2008)	TRPC3
Neuronal after depolarization (Stroh et al. 2012)	TRPC1–C4[a]
Plateau potentials in hippocampal CA1 pyramidal neurons (Tai et al. 2011)	TRPC5
Control of vascular tone (Welsh et al. 2003; Dietrich et al. 2005)	TRPC6
Endothelial cell NO-EDRF (endothelium-derived relaxing factor) generation—vascular smooth muscle relaxation (Freichel et al. 2001)	TRPC4
Endothelial cell NO-independent EDH (endothelium-dependent hyperpolarization—vascular smooth muscle relaxation (Senadheera et al. 2012)	TRPC3
Endothelial cell migration—wound healing, fibroblast transdifferentiation (Davis et al. 2012)	TRPC6
Static stretch response of endothelial cells—stretch-ATR1-Gq-TRPC-Ca-ET1-ANP-GC-A-cGMP-PKG-zyxin-gene transcription (Suresh Babu et al. 2012)	TRPC3
Intestinal motility regulation by vagus (Tsvilovskyy et al. 2009)	TRPC4 + TRPC6
Cold transduction in the peripheral nervous system (Zimmermann et al. 2011)	TRPC5
Exocrine secretion (saliva) (Liu et al. 2007)	TRPC1
Efferocytosis and survival signaling in macrophages (Tano et al. 2011)	TRPC3
Normal touch (Quick et al. 2012)	TRPC3-C6[a]
Light entrainment by ipRGCs (melanopsin signaling) (Xue et al. 2011)	TRPC6-C7[a]
Innate immunity (LPS-induced NFkB activation) (Tauseef et al. 2012)	TRPC6
Platelet activation by thrombin plus collagen-related peptide—PS exposure (Harper et al. 2013)	TRPC3–C6[a]
Short-term postsynaptic memory—burst firing-induced after depolarization (Phelan et al. 2012)	TRPC1–C4[a]

(continued)

Table 1 (continued)

Pheromone signal transduction in vomeronasal sensory neurons (Stowers et al. 2002); lost in evolution between New and Old World monkeys and man (Liman and Innan 2003)	TRPC2
Sound transduction and auditory neurotransmission (Wong et al. 2013)	TRPC3
In disease (pathophysiological roles)	
Cardiac hypertrophy induced by Ang II (Onohara et al. 2006)	TRPC3 + TRPC6
Cardiac hypertrophy induced by transverse aorta constriction (TAC) (Seth et al. 2009)	TRPC1
Albuminuria associated with Ang II-induced cardiac hypertrophy (Eckel et al. 2011)	TRPC6
Epileptogenic postsynaptic regenerative plateau potentials (Phelan et al. 2012)	TRPC1–C4[a]
Calcium toxicity in secretory epithelia (Kim et al. 2011)	TRPC3
Neuronal excitotoxicity (Phelan et al. 2012)	TRPC1–C4[a]
Neurotoxin-induced ER stress response and ER calcium homeostasis (Selvaraj et al. 2012)	TRPC1 loss
Proinflammatory in murine allergic asthma (Yildirim et al. 2012)	TRPC1

Most of the roles listed here were deduced from studying phenotypes developed in the corresponding knockout mice or cells
[a]Likely operating as obligatory heteromeric channels

Gain-of-function phenotypes are rare however. An explanation of this may come from studies with gain-of-function mutations in TRPC4 and TRPC5, which when expressed in HEK cells caused their death due to Ca^{2+} toxicity. Cell death was reduced by buffering extracellular Ca^{2+} to below 1 μM (Beck et al. 2013).

An analysis of the molecular basis for the phenotypes described to loss of TRPCs points to two mechanisms (Fig. 7): (1) TRPCs cause the collapse of the membrane potential owing to their nonselective cation channel property, and (2) TRPCs directly mediate Ca^{2+} entry. In many cell types the membrane depolarization triggers additional Ca^{2+} entry either by activation of voltage-gated Ca^{2+} channels or as a consequence of Na^{+} influx driving reverse mode Na^{+}/Ca^{2+} exchange, by the Na^{+}/Ca^{2+} exchangers (NCX1–3). In neurons, TRPC activation may of course trigger action potentials as is the case in ipRGCs. The opposite may also occur. In cells expressing Ca^{2+}-activated K^{+} channels (SKca, IKca, BKca), the Ca^{2+} entering through the TRPC causes hyperpolarization of the cell, as happens in endothelial cells (endothelium-derived hyperpolarization or EDH), which may thus contribute to vascular smooth muscle relaxation in those vessels in which the endothelium is connected to the surrounding smooth muscle layer by myoendothelial gap junctions (Sandow et al. 2002). The EDH response to stimuli that activate the Gq-PLC pathway in endothelia sums to the production of EDRF (NO) by CaCaM-activated soluble nitric oxide synthase, a response shown to be due to Ca^{+2} entering through TRPC4, as it is impaired in TRPC4 KO mice (Table 1; Freichel et al. 2001).

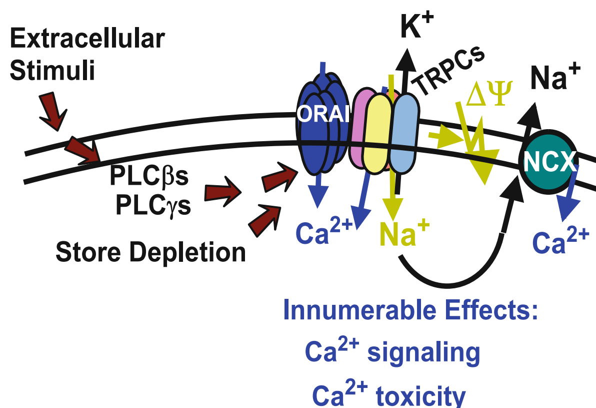

Fig. 7 Signaling initiated by TRPC and ORAI channels in response to stimuli that activate PLCs and deplete intracellular Ca^{+2} stores

Concluding Remarks

The fact that TRPC activation has a component driven by store depletion (via STIM)—an argument that was limited to TRPC1 in this article, but may also apply to other TRPCs whose presence allows for increased SOCE by fortification with ORAI (Fig. 1)—would predict that at some point, consequences of pharmacological manipulation of ORAI would overlap with consequences arising from pharmacological manipulation of TRPCs. In this context, it is interesting that loss of TRPC3 or inhibition of TRPC3 by the TRPC3-selective antagonist Pyr3 ameliorate cerulein-induced acute pancreatitis (Kim et al. 2009) as does inhibition of ORAI1 in a model of store depletion (thapsigargin)-induced pancreatitis (Gerasimenko et al. 2013). TRPC3, as TRPC1, is gated by STIM1 (Zeng et al. 2008), and both models of pancreatitis are based on prolonged increases in intracellular Ca^{2+}.

Signaling in response to ORAI and TRPC activation downstream of PLC activation and store depletion is summarized in Fig. 7. ORAI is placed next to TRPCs and does not distinguish between the regulatory roles of ORAI dimer independent of the CRAC activity of the assembled ORAI channel. The resolution of the relative roles of the different configurations of ORAI and how they regulate TRPCs awaits the results from further investigations. The dependence of a functional TRPC channel on an ORAI is supported by experimental data, several of which were presented in this review. The inverse, to what extent ORAI channels may or may not operate in the absence of a TRPC will require the creation of TRPC-null cell line. We (our laboratory) are well on the way in creating such a cell line. So far we have found that MEFs lacking TRPC1/2/3/5/6 (TRPC4 and TRPC7 remaining) have unaltered SOCE.

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