The TRPCs–STIM1–Orai Interaction

Seok Choi, Jozsef Maleth, Archana Jha, Kyu Pil Lee, Min Seuk Kim, Insuk So, Malini Ahuja, and Shmuel Muallem

Contents

S. Choi • J. Maleth • A. Jha • M. Ahuja
Epithelial Signaling and Transport Section, Molecular Physiology and Therapeutics Branch, National Institute of Dental and Craniofacial Research, National Institute of Health, Bethesda, MD 20892, USA

K.P. Lee
Department of Physiology, College of Veterinary Medicine, Chungnam National University, Daejeon 305-764, Republic of Korea

M.S. Kim
Department of Oral Physiology, School of Dentistry, Wonkwang University, Iksan City, Jeonbuk, Republic of Korea

I. So
Department of Physiology and Biophysics, Seoul National University College of Medicine, Seoul 110-799, Republic of Korea

S. Muallem (✉)
Epithelial Signaling and Transport Section, Molecular Physiology and Therapeutics Branch, NIDCR, NIH, Building 10, Room 1N-112, Bethesda, MD 20892, USA
e-mail: shmuel.muallem@nih.gov

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Abstract

Ca^{2+} signaling entails receptor-stimulated Ca^{2+} release from the ER stores that serves as a signal to activate Ca^{2+} influx channels present at the plasma membrane, the store-operated Ca^{2+} channels (SOCs). The two known SOCs are the Orai and TRPC channels. The SOC-dependent Ca^{2+} influx mediates and sustains virtually all Ca^{2+}-dependent regulatory functions. The signal that transmits the Ca^{2+} content of the ER stores to the plasma membrane is the ER resident, Ca^{2+}-binding protein STIM1. STIM1 is a multidomain protein that clusters and dimerizes in response to Ca^{2+} store depletion leading to activation of Orai and TRPC channels. Activation of the Orais by STIM1 is obligatory for their function as SOCs, while TRPC channels can function as both STIM1-dependent and STIM1-independent channels. Here we discuss the different mechanisms by which STIM1 activates the Orai and TRPC channels, the emerging specific and non-overlapping physiological functions of Ca^{2+} influx mediated by the two channel types, and argue that the TRPC channels should be the preferred therapeutic target to control the toxic effect of excess Ca^{2+} influx.

Keywords

TRPC channels • STIM1 • Gating • Physiology • Pathology

1 Introduction

The receptor-evoked Ca^{2+} signal is initiated by the hydrolysis of PI(4,5)P2 either by phospholipase Cβ (G protein-coupled receptors) or phospholipase Cγ (tyrosine kinase receptors) to generate IP_3 and diacylglycerol. IP_3 releases Ca^{2+} primarily from the endoplasmic reticulum (ER), which provides a signal to activate Ca^{2+} influx channels at the plasma membrane. The ensuing increase in free cytoplasmic Ca^{2+} ($[Ca^{2+}]_i$) activates the plasma membrane Ca^{2+} ATPase (PMCA) and the sarcoplasmic reticulum Ca^{2+} ATPase (SERCA) pumps that clear Ca^{2+} from the cytosol (Kiselyov et al. 2003). At weak receptor stimulation, this cycle is periodically repeated to generate Ca^{2+} oscillations, while at intense receptor stimulation, the IP_3 receptors (IP_3Rs) remain active to keep the ER depleted of Ca^{2+}, and $[Ca^{2+}]_i$ is determined by a "pump-leak turnover" with the Ca^{2+} influx channels providing the leak and the PMCA the pump (Kiselyov et al. 2006).

The Ca^{2+} influx channels participate in all aspects of the receptor-evoked Ca^{2+} signal. They contribute to the initial increase in $[Ca^{2+}]_i$ upon receptor stimulation, they reload the stores with Ca^{2+} between the Ca^{2+} spikes during Ca^{2+} oscillations, and they remain active for many minutes at the end of the stimulation period until the stores are fully loaded with Ca^{2+} (Muallem et al. 1988; Pandol et al. 1987; Parekh and Putney 2005). In polarized cells the Ca^{2+} signal initiates at the apical pole and propagates to the basal pole in the form of Ca^{2+} waves (Kasai and Augustine 1990; Thorn et al. 1993). Ca^{2+} influx accelerates the rate of Ca^{2+} wave

propagation (Lee et al. 1997). The Ca^{2+} influx channels determine practically all physiological and pathological effects of $[Ca^{2+}]_i$ by determining the size and shape of the Ca^{2+} signal. In fact, all Ca^{2+}-dependent physiological functions occurring after the first 1 min of cell stimulation stops in the absence of Ca^{2+} influx (Parekh and Putney 2005), while excessive activation of the Ca^{2+} influx channels and Ca^{2+} influx is largely responsible for the pathological effects of Ca^{2+} (Lee et al. 2010a; Petersen et al. 2006).

Two types of channels mediate the bulk of the receptor-stimulated Ca^{2+} influx, the TRPC and Orai channels (Lee et al. 2010a). The contribution and role of the TRPC channels to the receptor-stimulated Ca^{2+} influx has been extensively studied (Pandol et al. 1987; Lee et al. 1997) before the discovery of the Orai channels (Feske et al. 2006; Zhang et al. 2006; Vig et al. 2006) and of STIM1 (Roos et al. 2005; Liou et al. 2005). In this chapter we will argue that both the TRPC and Orai channels are gated by STIM1, that the contribution of both channel types is vital for cell function, that the TRPC and Orai channels sense and respond to the activity of each other, that increasing evidence suggests that each channel type may regulate separate Ca^{2+}-dependent cell functions, and that the TRPC channels may provide an important and perhaps preferred therapeutic target to guard against Ca^{2+}-dependent toxicity and pathology.

2 The STIM1 Functional Domains

2.1 The STIM1 Domains

The relationship between the ER Ca^{2+} load and the function of the Ca^{2+} influx channels was resolved with the discovery of the ER Ca^{2+} content sensor STIM1 (Roos et al. 2005; Liou et al. 2005). STIM1 is a multidomain protein, the function of which is continually being updated as more information becomes available. The currently known STIM1 domains are depicted in Fig. 1a. These include the ER lumen resident Ca^{2+} binding EF hand and SAM domains. The EF hand is the Ca^{2+}-binding site, while the SAM domain participates in STIM1 clustering (Stathopulos et al. 2008; Zheng et al. 2008). Binding of Ca^{2+} to the EF hand keeps STIM1 in a non-clustered form and restrict its access to the Ca^{2+} influx channels. The luminal domain also has reactive cysteines that appear to participate in ER redox sensing through S-glutathionylation of cysteine 56 (Hawkins et al. 2010). The SAM domain is followed by a short transmembrane domain and a long cytoplasmic domain. The cytoplasmic domain of STIM1 mediates the opening of the TRPC and Orai channels (Huang et al. 2006). The cytoplasmic domain starts with the first coiled-coil domain (CC1), which includes a short helix at its C terminus that functions as an inhibitory helix (IH) (Yu et al. 2013; Yang et al. 2012). CC1 is followed by the SOAR domain (Yuan et al. 2009), which is also known as CAD (Park et al. 2009) or CCb9 (Feske et al. 2010). SOAR is the minimal STIM1 domain needed to fully activate Orai1 (Yuan et al. 2009; Park et al. 2009; Feske et al. 2010). SOAR may also participate in the regulation of TRPC channels by STIM1 (see below). The

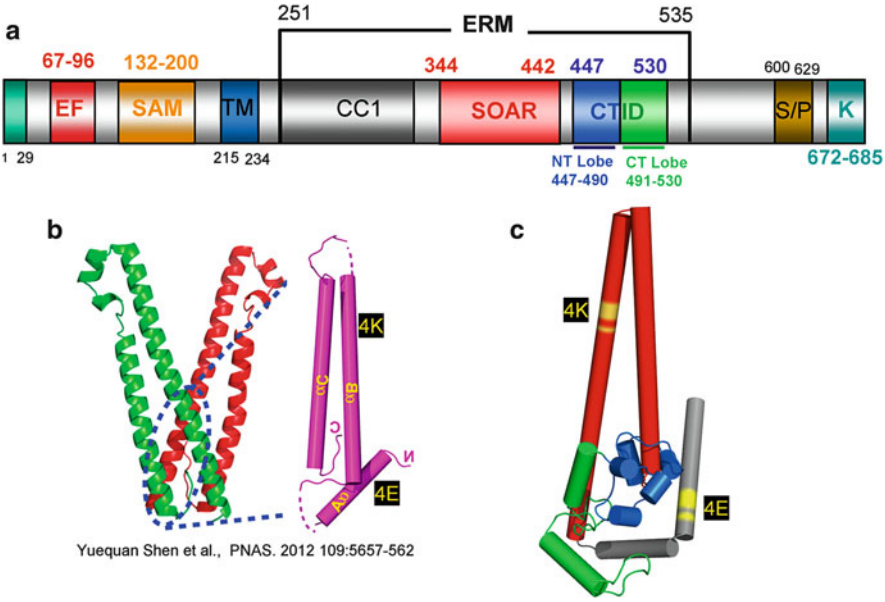

Fig. 1 The STIM1 domains. The currently known domains of STIM1 are shown in (**a**). Panel (**b**) shows the crystal structure of the SOAR dimer and monomer and the position of the four lysines (4K) and four glutamates (4E) in IH taken from Archana Jha Malini Ahuja et al. (2013). Panel (**c**) is a predicted structure of SOAR (*red*) and the N terminal (*blue*) and C terminal (*green*) lobes of CTID

crystal structure of the SOAR domain was resolved recently (Jha et al. 2013; Fig. 1b). Recent analysis identified a new highly conserved domain immediately C terminus to SOAR that we named CTID (for C terminus inhibitory domain). The predicted structure of CTID is shown in Fig. 1c (see also Jha et al. 2013). CTID is followed by a long sequence, the function of which is only beginning to emerge. Recent study reported that the function of this C terminus portion of STIM1 is to keep SOAR in an inactive state (Yu et al. 2013). The C terminal end of STIM1 is polybasic with multiple lysine residues (K-domain). The K-domain interacts with PI(4,5)P2 at the plasma membrane to stabilize the clustered STIM1 at the ER/plasma membrane microdomain (Liou et al. 2007). Most significantly, the K-domain gates open the TRPC channels (see Zeng et al. (2008) and below).

Several recent studies revealed that when the ER stores are filled with Ca^{2+}, STIM1 resumes a compact structure. Upon Ca^{2+} store depletion, STIM1 undergoes massive conformational transition to a more extended conformation (Yu et al. 2013; Korzeniowski et al. 2010; Muik et al. 2011). Sequence analysis identified four conserved lysines in SOAR (marked by 4K in Fig. 1b, c) that potentially interact either with four conserved glutamates in the IH domain in CC1 (marked by 4E in Fig. 1b, c) or with conserved glutamates in Orai1 that may fold similarly (Korzeniowski et al. 2010). Mutations of the glutamates in IH resulted in a constitutively active STIM1, while mutations of the lysines in SOAR inhibited

STIM1 function. Based on these findings, it was suggested that CC1 interacts with SOAR to keep it in inactive state (Yu et al. 2013; Korzeniowski et al. 2010; Muik et al. 2011). Direct evidence for folding and interaction of CC1 and SOAR was provided by convincing experiments using a STIM1 conformational FRET reporter (Muik et al. 2011). These studies suggested that the CC1–SOAR interaction is mediated by hydrophobic interactions. This conclusion is supported by further analysis of the role of the glutamates in the CC1 domain (Yu et al. 2013). In addition, recent crystal structure of SOAR and IH revealed that the lysines in SOAR and the glutamates in CC1 do not interact (Yang et al. 2012). However, they are consistent with the possibility that the lysines in SOAR may mediate SOAR interaction with Orai1 (Korzeniowski et al. 2010). Our studies suggest that mutation of the lysines in SOAR likely disrupts its structure to the extent that STIM1 with the lysines mutated to alanines aggregates in small intracellular inclusions (Jha et al. 2013). Another fold that occludes SOAR is present in STIM1 C terminus to SOAR (Yu et al. 2013; Jha et al. 2013). Initially, it was shown that replacing the STIM1 C terminus with GFP resulted in activation of STIM1 by making SOAR available for interaction with Orai1 (Yu et al. 2011). Subsequently, another STIM1 conformational reporter that includes the entire STIM1 cytoplasmic domain was used to show that the STIM1 C terminus folds to contact CC1, likely in the IH, and participates in the occlusion of SOAR (Yu et al. 2013). Together, the conformational reporters and SOAR structure suggest that in the basal state, STIM1 is folded in a manner that CC1 interacts with SOAR and the C terminus folds back to interact with the IH in CC1, resulting in occlusion of SOAR.

2.2 STIM1 Interactors

Additional regulation of STIM1 function is due to its interaction with other proteins. Comprehensive analysis of the STIM1 interactome is not available as yet. The two established interactors are the microtubule-plus-end-tracking protein end binding (EB1) (Grigoriev et al. 2008) and the SOCE-associated regulatory factor (SARAF) (Palty et al. 2012). Interaction of STIM1 with EB1 forms comet-like accumulations at the sites of microtubule end-ER contacts. The interaction is regulated by phosphorylation of STIM1 in serine/threonine residues in the C terminus region of STIM1 (Smyth et al. 2012). Interestingly, this interaction appears to participate in ER remodeling (Grigoriev et al. 2008; Smyth et al. 2012). In particular, STIM1 interaction with EB1 is inhibited during mitosis, resulting in exclusion of STIM1 from the mitotic spindle, and this is reversed by phosphorylation-compromised STIM1 mutant (Smyth et al. 2012). Importantly, STIM1 phosphorylation at the sites that affect EB1 interaction and spindle exclusion has no role in the regulation of Ca^{2+} influx by STIM1 (Smyth et al. 2012), suggesting that STIM1 has roles outside the regulation of Ca^{2+} influx channels. This has also been shown for the regulation of endothelial barrier function that is regulated by STIM1-mediated coupling of the thrombin receptor to activation of

RhoA, formation of actin stress fibers, and the eventual loss of cell–cell adhesion (Shinde et al. 2013).

High-throughput screen has identified SARAF as a negative regulator of STIM1 (Palty et al. 2012). SARAF is a single transmembrane span, ER resident protein with the N terminus in the ER lumen and the C terminus in the cytoplasm. SARAF interacts with STIM1 upon Ca^{2+} store depletion to facilitate inactivation of Orai1 by Ca^{2+}. It appears that the C terminus of SARAF mediates the interaction with STIM1 (Palty et al. 2012). Once activated, Orai1 undergoes two types of Ca^{2+}-mediated inactivation, fast (FCDI) and slow (SCDI) inactivation with time courses of ms and min (Parekh and Putney 2005). Prior work identified a negatively charged STIM1 sequence (^{475}DDVDDMDEE^{483}) that mediates the FCDI (Lee et al. 2009; Mullins et al. 2009; Derler et al. 2009). Before the discovery of SARAF, no information was available on the mechanism of the SCDI. In a systematic domain analysis, we identified SOAR as the STIM1 domain that interacts with SARAF (Jha et al. 2013). Moreover, we found that CTID, the highly conserved STIM1 domain C terminus to SOAR, controls the access of SARAF to SOAR to mediate the SCDI. As shown in Fig. 1c, CTID appears to have two lobes, with the N terminal lobe [STIM1(447–490)] restricting the access of SARAF to SOAR and the C terminal lobe [STIM1(491–530)] facilitating interaction of SARAF with SOAR (Jha et al. 2013). Finally, CTID appears to also have a role in the FCDI of Orai1, as modification of the interaction of SARAF with STIM1 alters the kinetics of or prevents FCDI (Jha et al. 2013).

That STIM1 has both Ca^{2+} influx-dependent and Ca^{2+} influx-independent functions strongly suggest that STIM1 interacts with many more proteins in addition to EB1 and SARAF within signaling complexes. It is likely that the STIM1 interactome will become available in the near future.

3 Gating of Ca^{2+} Influx Channels by STIM1

3.1 Gating of Orai Channels by STIM1

This topic is discussed only briefly. Further details can be found in recent comprehensive reviews (Engh et al. 2012; Shaw and Feske 2012; Muik et al. 2012). STIM1 activates the Orai channels to mediate the Ca^{2+}-release-activated Ca^{2+} (CRAC) current (Zhang et al. 2006). The Orai channels are four transmembrane span proteins with cytoplasmic N and C termini. Early biochemical and single molecule photobleaching studies suggested that the Orai channels are tetramers (Demuro et al. 2011; Penna et al. 2008; Ji et al. 2008). However, the recent crystal structure of Orai1 revealed that the channel is a hexamer with the selectivity filter composed of a ring of glutamates (Hou et al. 2012). Cytoplasmic extensions of the fourth transmembrane domain of four of the six subunits fold into coiled-coil domains. Functional (Muik et al. 2008; Frischauf et al. 2009) and the structural (Hou et al. 2012) information showed that the C terminus coiled-coil domains are essential for activation of the Orai channels by STIM1. SOAR opens all three

Orai channels by interaction with the C-terminus coiled-coil domains of the Orais (Lee et al. 2009). A second interaction of SOAR/CAD was found with the N-terminus of Orai1 downstream of residue 73 (Park et al. 2009). Recently, it was suggested that STIM1 interaction with the Orai1 C terminus mediates the binding between the proteins, while interaction of STIM1 with the N terminus of Orai1 gates the opening of Orai1 (Zheng et al. 2013). However, farther recent examination of this topic shows that interaction of STIM1 with both the C and N termini of the Orai1 participates in channel gating (McNally et al. 2013). Moreover, both interactions are required for the effect of STIM1 on Orai1 channel selectivity (McNally et al. 2012, 2013). The later findings are also compatible with the role of the Orai channels C termini in the extent and mode of channel regulation. The three Orai channels have a typical FCDI, with Orai1 showing the least prominent and Orai3 the most prominent inactivation (Lis et al. 2007). The information for the type of inactivation is coded in their C termini, as revealed by translocation of the inactivation kinetics with the C-termini when they are swapped among the Orais (Lee et al. 2009). Since removal of CTID eliminates both the FCDI and SCDI (Jha et al. 2013) and SARAF accelerates the SCDI of Orai1 (Jha et al. 2013; Palty et al. 2012), it should be of interest to determine whether and how SARAF affects the FCDI and SCDI of Orai2 and Orai3.

4 TRPC Channels and STIM1

The regulation of TRPC channels by STIM1 is poorly understood and sparsely studied and thus remains controversial to some extent. Several aspects of TRPC channels function and regulation are generally accepted. TRPC channels mediate significant portion of the receptor-stimulated Ca^{2+} influx, TRPC3, TRPC6, and TRPC7, but not TRPC1, TRPC4, and TRPC5 are activated by diacylglycerol, and Ca^{2+} influx by TRPC channels has multiple roles in virtually every cell type (Parekh and Putney 2005; Pedersen et al. 2005; Freichel et al. 2005). Biochemical interaction between TRPC channels and STIM1 is a highly consistent finding. It is also a consistent finding that knockdown or deletion of Orai1 or STIM1 eliminates the store-dependent and receptor-activated Ca^{2+} influx (Lee et al. 2010a). At the same time, numerous studies in multiple cell types consistently find that knockout or knockdown of TRPC channels significantly or markedly reduce the store-mediated and receptor-activated Ca^{2+} influx. Examples of the effect of TRPC1 and TRPC3 knockout of receptor- and store-dependent Ca^{2+} influx in pancreatic acini are illustrated in Fig. 2. Deletion of TRPC1 (green traces and columns) or TRPC3 (red traces and columns) reduces the receptor-stimulated Ca^{2+} influx (reduced plateau) and Ca^{2+} influx evoked by store depletion (Fig. 2a, c, d) and thus the frequency of Ca^{2+} oscillations (Fig. 2b). Additional examples of the effect of TRPC channels knockdown on Ca^{2+} influx are in Lee et al. (2010a), Ng et al. (2012), Antigny et al. (2013), Zhang et al. (2010), Rao et al. (2012), and Sundivakkam et al. (2012). Given the complete inhibition of Ca^{2+} influx by deletion of STIM1 and Orai1 and the substantial inhibition of Ca^{2+} influx by deletion of TRPC channels, it

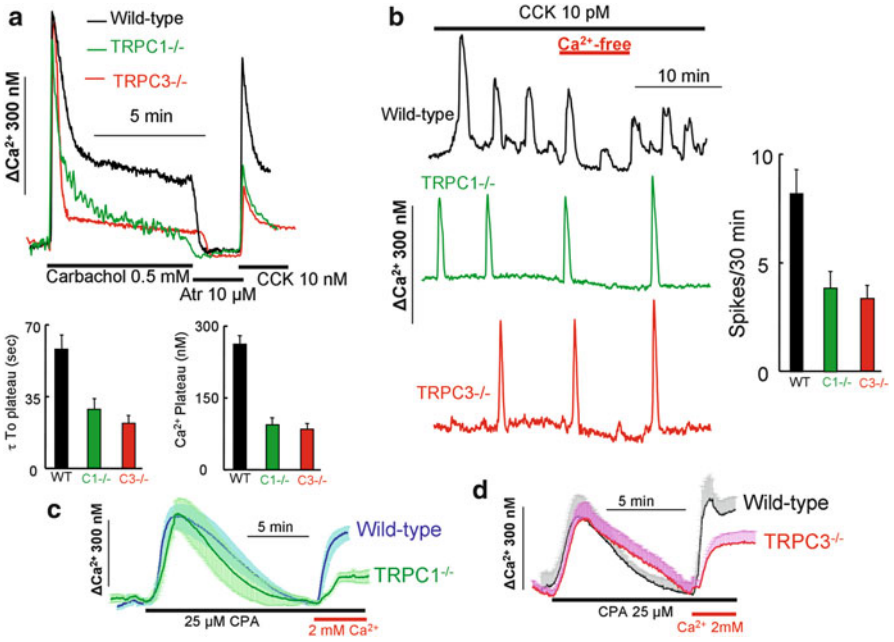

Fig. 2 TRPC1 and TRPC3 as store-operated Ca^{2+} influx channels in pancreatic acini. $[Ca^{2+}]_i$ was measured in wild-type (*black traces and columns*), *Trpc3*$^{-/-}$ (*red traces and columns*), and *Trpc1*$^{-/-}$ (*green traces and columns*) pancreatic acini stimulated with the indicated concentration of agonists to generate a sustain response (**a**) or Ca^{2+} oscillations (**b**) or treated with 25 μM CPA to deplete the stores and measure Ca^{2+} influx by Ca^{2+} re-addition (**c**, **d**). Results were reproduced from Kim et al. (2009b)

follows that TRPC channels required Orai1 function and/or Orai1 requires TRPC channels function for their activity and, moreover, that TRPC channels are regulated by STIM1.

4.1 STIM1-Dependent and STIM1-Independent Function of TRPC Channels

In spite of the findings mentioned above, regulation of TRPC channels by STIM1 remains poorly understood and is not fully accepted or appreciated. This is mostly because regulation of TRPC channels by STIM1 is not always observed when the two are co-expressed in model systems. How can this be reconciled with the knockout/knockdown results? A major reason is that TRPC channels can function as both STIM1-dependent and STIM1-independent channels while the Orai channels function is strictly dependent on STIM1. Thus, the early studies already showed that TRPC1, TRPC2, TRPC4, and TRPC5 can directly interact with STIM1, while TRPC3, TRPC6, and TRPC7 do not (Huang et al. 2006). However,

it is well established that TRPC channels can heteromultimerize, including TRPC1–TRPC3 (Yuan et al. 2007; Liu et al. 2005) and TRPC4–TRPC6 (Yuan et al. 2007). It turned out that TRPC3 can function as STIM1-dependent channel only in the presence of TRPC1 and TRPC6 functions as STIM1-dependent channel only in the presence of TRPC4 (Yuan et al. 2007). Notably, in the absence of TRPC1 and TRPC4, TRPC3 and TRPC6, respectively, are fully active, but their activity is STIM1 independent. This indicates that (1) the cellular composition of TRPC channel isoforms will determine whether their function is STIM1 dependent or not and (2) a precise, physiological expression level and ratio of TRPC channels isoforms is required to observe and study their STIM1 dependence. Hence, a common problem in the field is that often marked overexpression of the channels is used to study their regulation by STIM1, conditions that ensure their STIM1-independent function.

The study of the regulation of TRPC channels by STIM1 is further complicated by the requirement of Orai1 for TRPC channels function. Undoubtedly, the Orai and TRPC channels can function independent of each other to mediate the CRAC current (Zeng et al. 2008) and nonselective, Ca^{2+}-permeable current (Pedersen et al. 2005; Freichel et al. 2005; Nilius et al. 2007) and dominate Ca^{2+} influx in particular cell types. For example, Orai1-mediated current is prominent in all blood-born cell types (Engh et al. 2012; Shaw and Feske 2012). However, in most cell types, it is difficult to record Icrac current, where TRPC channels likely mediate most of the Ca^{2+} influx. In such cells, both the Orai and TRPC channels appear to be required for SOC by affecting the activity of each other. Initial indication for this was obtained by demonstrating that deletion of Orai1 inhibits all forms of Ca^{2+} influx. Subsequently it was shown that all Orai channels interact with TRPC channels to complex with STIM1 and enhance TRPC channels store dependence (Liao et al. 2007, 2008, 2009). Additional relationship between the channels was shown by demonstrating that functional Orai1 and TRPC channels are required to restore the physiological store-mediated Ca^{2+} influx, with channel-dead Orai1 or TRPC channel mutants unable to do so (Kim et al. 2009a). The interdependence of the channels is attributed to their insertion in the plasma membrane and stabilization of the Orai1–STIM1–TRPC complexes in plasma membrane microdomain (Kim et al. 2009a; Ong et al. 2007; Cheng et al. 2011). Importantly, modulation of TRPC channels function by the Orais and reconstitution of SOCs were observed only when the Orai and TRPC channels are expressed at very low, close to physiological levels (Liao et al. 2007, 2008, 2009; Kim et al. 2009a). The latter suggest that the ratio of Orai1/TRPC channels is important for the STIM1-dependent function of the TRPC channels.

Another factor contributing to the problem in studying TRPC channels regulation by STIM1 is the activation/regulation of TRPC channels by other ligands/regulators. For example, TRPC3, TRPC6, and TRPC7 are activated by diacylglycerol (Pedersen et al. 2005; Freichel et al. 2005), TRPC4 and TRPC5 are activated by Gα subunits (Jeon et al. 2012), TRPC5 is activated by Ca^{2+} (Blair et al. 2009), and TRPC4 and TRPC5 are activated by redox compounds (Xu et al. 2008; Takahashi and Mori 2011). These forms of activation are

STIM1-independent and, when functioning, may overwhelm the STIM1-dependent activation of the channels.

The discussion above points to the caution needed when selecting the system and conditions to study regulation of TRPC channels by STIM1. However, the important point is that this topic is neglected and grossly understudied by investigators interested in TRP channels and in the role of TRPC channels in receptor-mediated Ca^{2+} influx and its functions. Biochemical interaction between STIM1 and the TRPC channels is a highly consistent observation. Therefore, understanding how STIM1 interacts with and affects the function of TRPC channels is paramount for the understanding receptor-mediated Ca^{2+} signaling.

4.2 Gating of TRPC Channels by STIM1

Probably the strongest evidence that TRPC channels are gated by STIM1 is the finding that STIM1 gates TRPC channels by electrostatic interaction (Zeng et al. 2008). Initial structure-function studies revealed that deletion of the STIM1 K-domain prevents activation of TRPC1 by STIM1, Ca^{2+} influx, and translocation of NFAT to the nucleus (Huang et al. 2006). The K-domain is predicted to fold as a helix with the positive charge of the last two lysines (K) on the surface at the end of the helix. This prompted us to search for a helix with negatively charged residues that might complement the positive charges in the STIM1 K-domain. Such two conserved residues (DD/DE/EE) were identified in all TRPC channels (Park et al. 2009). Mutation analysis revealed that the negatively charged residues (D^{639} and D^{640} in the case of TRPC1) interact with the last two lysines (K^{684} and K^{685}) in STIM1, that interaction of both lysines with the two negative charges is required, that insertion or deletion of a single glycine within 100 residues upstream of the STIM1 C terminus disrupted the gating, and, most notably, that gating of TRPC channels by STIM1 occurs whether the positive or negative charges are on STIM1 or TRPC channels, as long as the negative charges on one protein are matched with positive charges on the other protein (Zeng et al. 2008).

Subsequent studies examined gating by electrostatic interaction with other TRPC channels to show that the same mechanism operates with TRPC3, TRPC4, TRPC5, and TRPC6 (Lee et al. 2010b). These studies also resulted in an excellent tool to selectively inhibit TRPC channels independent of the inhibition of Orai1. Since the STIM1 K-domain is not required for activation of Orai1 (Yuan et al. 2009), STIM1($K^{684}E/K^{685}E$) has no effect on Orai1, but inhibits the native TRPC channels activity. This tool was used together with mutations of the conserved TRPC3($D^{697}K/D^{698}K$) to demonstrate gating of TRPC3 by electrostatic interaction and the STIM1-dependent and STIM1-independent function of TRPC3. As illustrated in Fig. 3 (taken from Lee et al. 2010b), while wild-type STIM1 increased, the mutant STIM1($K^{684}E/K^{685}E$) strongly inhibited the current of wild-type TRPC3 (Fig. 3a–c). The TRPC3($D^{697}K/D^{698}K$) mutant that is not gated by STIM1 retained full activity. Notably, the current of TRPC3($D^{697}K/D^{698}K$) was not

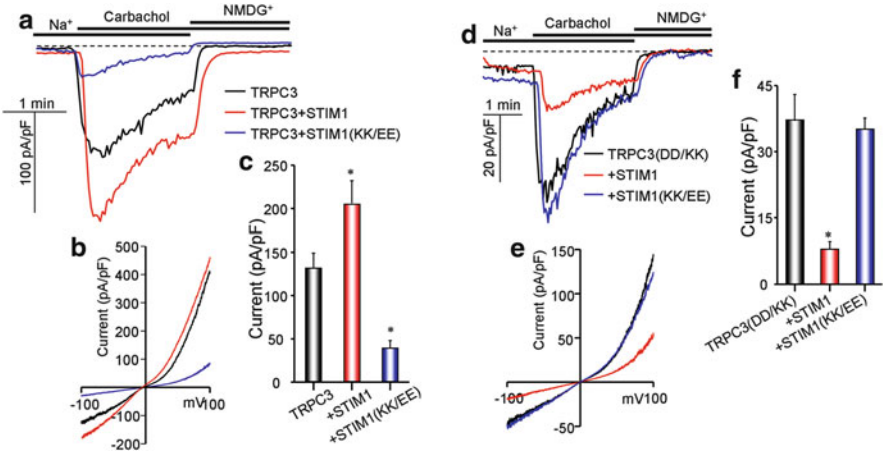

Fig. 3 Function of TRPC3 in STIM1-dependent and STIM1-independent modes. HEK cells were transfected with wild-type TRPC3 (**a**–**c**) or the mutant TRPC3(DD/KK) (**d**–**f**) and either empty vector (*black traces and columns*), STIM1 (*red traces and columns*), or STIM1(KK/EE) (*blue traces and columns*), and the carbachol-activated current was measured. Note that STIM1 activated wild-type TRPC3 but strongly inhibited the mutant TRPC3(DD/KK) while the reverse charge STIM1(KK/EE) had the opposite effects. Results were reproduced from Lee et al. (2010b)
* In c,f denotes $p<0.05$

affected by the reverse charge STIM1($K^{684}E/K^{685}E$), but was fully inhibited by wild-type STIM1 (Fig. 3d–f).

An important remaining question in the gating of TRPC channels by STIM1 is their interacting modules. The STIM1 K-domain that gates the channel is not required for STIM1–TRPC channels interaction (Huang et al. 2006). The most likely STIM1 domain that interacts with the TRPC channels is SOAR. SOAR folds into a coiled-coil domain structure and crystallizes as a V-shaped dimer with lysines lining both sides of the V interior (Yang et al. 2012; Fig. 1b) that may interact with the Orai1 C terminus (Korzeniowski et al. 2010). The Orai1 C terminus folds into a coiled-coil domain structure (Hou et al. 2012), and the coiled-coil domain fold is required for activation of Orai1 by STIM1 (Muik et al. 2008; Frischauf et al. 2009). The same mechanism may hold for TRPC channels, which are predicted to have N- and C terminal coiled-coil domains. The role of the TRPC channels coiled-coil domains in interaction with STIM1 and in regulation of TRPC channels function by STIM1 was reported recently in Lee et al. 2014.

4.3 TRPC Channels in Physiology and as Therapeutic Target

The presence of two STIM1-gated Ca^{2+} influx channels in the same cells that affect the activity of each other raises the question of their expression pattern and whether Ca^{2+} influx through each of the channels mediate specific cell function. Expression

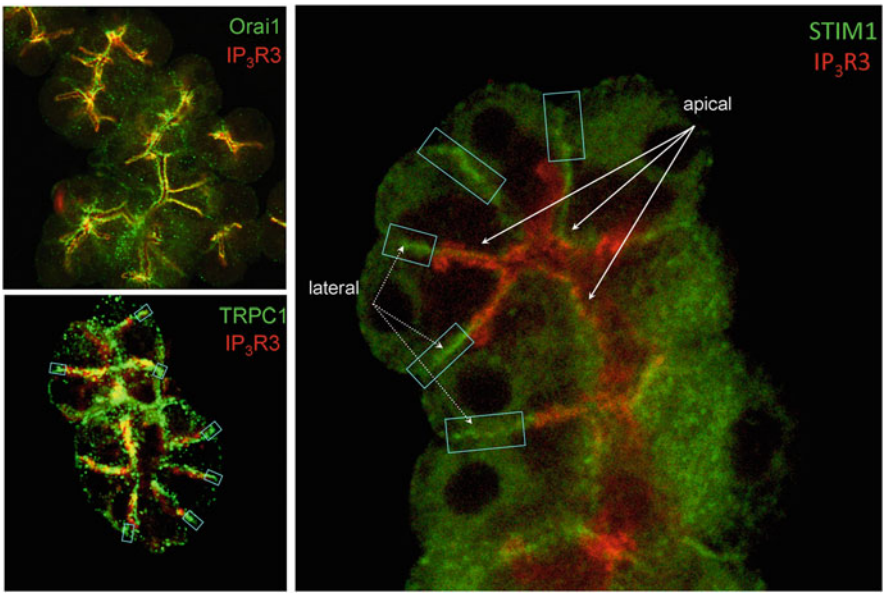

Fig. 4 Localization of the native STIM1, Orai1, and TRPC1 in polarized cells. Localization of Orai1, TRPC1, and STIM1 was determined relative to localization of IP_3Rs in the apical pole. Note that Orai1 is confined largely to the apical pole whereas TRPC1 and STIM1 are found at the apical and lateral membrane with minimal expression of the proteins in the basolateral membrane. The *turquoise squares* mark regions expressing STIM1 and TRPC1 but devoid of Orai1. Results were reproduced from Archana Jha Malini Ahuja et al. (2013)

pattern of the *native* STIM1, Orai1, and TRPC channels has been examined to a very limited extent, especially in animal tissues (rather than in cell lines). Such studies were performed in the polarized pancreatic acinar cells and an example is shown in Fig. 4 that was taken from Hong et al. (2011). Localization of the proteins was examined relative to IP_3 receptors (IP_3Rs) that are clustered at the apical pole (Lee et al. 1997; Hong et al. 2011; Yule et al. 1997). Orai1 shows perfect colocalization with IP_3Rs, while the localization of TRPC1 and STIM1 only partially overlap with the localization of IP_3Rs. This indicates that STIM1 forms several Ca^{2+} influx complexes in the same cells, Orai1–STIM1, Orai1–STIM1–TRPC1, and STIM1–TRPC1 complexes. Complexes of TRPC1–STIM1 are formed when cells are stimulated and can be readily immunoprecipitated (Hong et al. 2011).

Emerging evidence points to selective roles of the STIM1-regulated Ca^{2+} influx pathways in cellular physiology. Several of these examples are described below. Orai1-mediated Ca^{2+} influx activates the Ca^{2+}-dependent phosphatase calcineurin that dephosphorylates NFAT, resulting in translocation of NFAT to the nucleus (Shaw and Feske 2012; Darbellay et al. 2010). Selective inhibition of Ca^{2+} influx by TRPC channels was reported to have no effect on NFAT translocation to the nucleus (Cheng et al. 2011; Darbellay et al. 2010). Conversely, Ca^{2+} influx

mediated by TRPC1 activates the Ca^{2+}-dependent transcription factor NFκB that is less affected by Orai1-mediated Ca^{2+} influx (Cheng et al. 2011; Darbellay et al. 2010). The TRPC1-mediated Ca^{2+} influx activates the Ca^{2+}-activated K^+ channel in salivary glands acinar cells (Cheng et al. 2011) that are localized at the apical pole (Ong et al. 2012). This is despite the findings that Orai1 channels are present in and mediate Ca^{2+} influx across the luminal membrane (Almassy et al. 2012).

The Orai1–STIM1-mediated Ca^{2+} influx was found to be essential for postnatal human myoblast differentiation through activation of the transcription factors MEF2 and myogenin (Hong et al. 2011; Balghi et al. 2011). On the other hand, TRPC1 and TRPC4 mediate a portion of STIM1-dependent store-operated Ca^{2+} influx necessary for activation of MEF2 that mediates myoblasts fusion. Ca^{2+} influx by Orai1 did not substitute for the TRPC channel-mediated Ca^{2+} influx that facilitates the myogenic fusion (Antigny et al. 2013). Proliferation of adult neuronal progenitor cells is regulated by store-dependent Ca^{2+} influx. Knockdown of Orai1 and TRPC1 was equally effective in inhibition of the proliferation and causing cell cycle arrest (Li et al. 2012). Similarly, Ca^{2+} influx by TRPC1 is essential for the platelet-derived growth factor-BB-mediated restoration of neuronal progenitor cells proliferation that had been impaired by HIV Tat-cocaine via the cognate receptors (Yao et al. 2012). Similar effect was proposed for TRPC5 in the A2B5 neuronal progenitor cells (Shin et al. 2010).

In a Parkinson's disease model induced by neurotoxin, loss of dopaminergic neurons in the substantia nigra is associated with a decrease in TRPC1 expression and interaction of TRPC1 with STIM1. Ca^{2+} influx through the STIM1-regulated TRPC1 was essential and sufficient for activating the AKT pathway to mediate the neuroprotection (Selvaraj et al. 2012).

Vascular permeability is controlled by endothelial cells, a function regulated by TRPC channels, in particular by TRPC1 and TRPC4 when activated by STIM1 to mediate SOC-dependent Ca^{2+} influx (Di and Malik 2010). Knockdown of *Trpc4* or STIM1 inhibited vascular endothelial cells Ca^{2+} influx and caused disruption of the endothelial barrier (Sundivakkam et al. 2012). Notably, knockdown of Orai1 or Orai3 in these cells had minimal effect of Ca^{2+} influx and barrier integrity. Similarly, overexpression of Orai1 or STIM1 in $Trpc4^{-/-}$ cells failed to rescue Ca^{2+} influx (Sundivakkam et al. 2012), further demonstrating the essential role of TRPC channels in endothelial cell SOCs.

Another, potentially important aspect of TRPC channels relative to the Orai1 channel is their potential as therapeutic targets. Several diseases have been associated with mutations in TRPC channels. They are not discussed here, but information on this topic can be found in other chapters of this book and in Nilius and Owsianik (2011). However, the reason TRPC channels should be considered as therapeutic target is that deletion of TRPC1 (Liu et al. 2007), TRPC3 (Kim et al. 2009b, 2011; Hartmann et al. 2008), TRPC4 (Freichel et al. 2005), TRPC5 (Riccio et al. 2009), and TRPC6 (Dietrich et al. 2005) has relatively minor phenotypes, while deletion of Orai1 is embryonically lethal (Vig et al. 2006; McCarl et al. 2009). Moreover, deletion of the ubiquitous Orai1 eliminates all

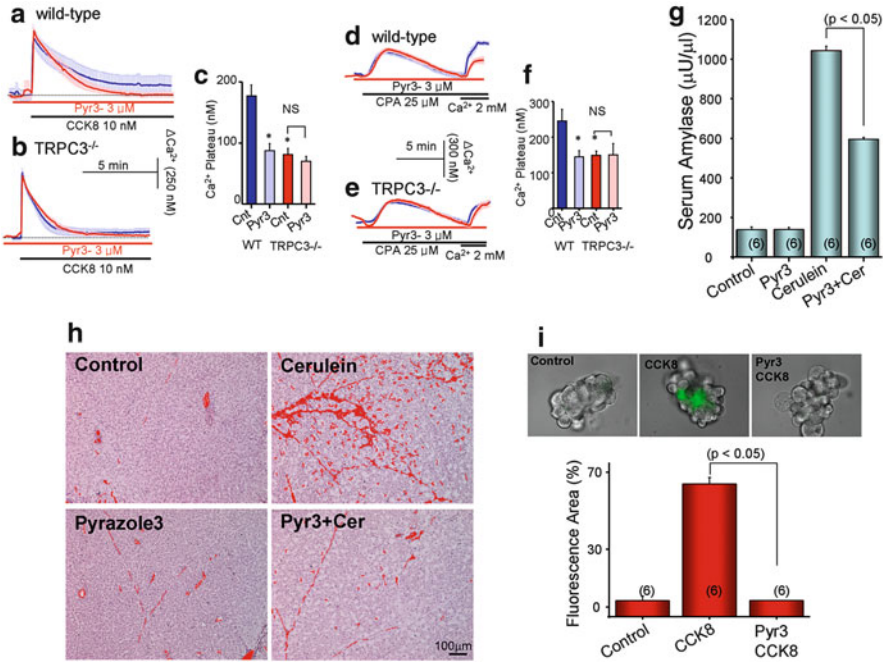

Fig. 5 Inhibition of TRPC3 protects against Ca^{2+}-dependent injury. Ca^{2+} measurements show that deletion of TRPC3 and Pyrazole 3 inhibits the same component of Ca^{2+} influx activated by receptor stimulation (**a**–**c**) or store depletion (**d**–**f**). Treating mice with Pyrazole 3 reduces the severity of cerulein-induced acute pancreatitis as assayed by the increase in serum amylase (**g**) and edema (**h**) and activation of intracellular trypsin (**i**). Results were reproduced from Smyth et al. (2012)
* In c,f denotes $p<0.05$

forms of receptor-stimulated Ca^{2+} influx. By contrast, expression of TRPC channels is more cell-specific and deletion of any of the TRPC channels results in only partial reduction in the receptor-mediated Ca^{2+} influx. Although TRPC channels also contribute to the regulation of the membrane potential by depolarizing the cells when activated, it seems that their function can be spared, at least temporarily.

Good examples for the therapeutic potential of inhibiting TRPC channels are the inhibition of TRPC3 and TRPC6 in reducing cardiac hypertrophy (Kuwahara et al. 2006; Wu et al. 2010) and of TRPC3 in ameliorating Ca^{2+}-dependent damage in the brain, pancreas, and salivary glands (Kim et al. 2009b, 2011; Hartmann et al. 2008). Analysis of patient tissues revealed increased TRPC3 and TRPC6 expression in cardiac hypertrophy that could be reproduced by overexpression of the channels in mice hearts, and hearts are protected when expression of the channels is reduced (Kuwahara et al. 2006; Wu et al. 2010; Millay et al. 2009). This suggested that pharmacological inhibition of TRPC3 should prevent cardiac hypertrophy. Pyrazole 3 is an inhibitor of TRPC3 (Kim et al. 2011; Kiyonaka et al. 2009; see Fig. 5) that is tolerated well by mice with no apparent gross side

effects when infused over a 2 weeks period (Kiyonaka et al. 2009). Inhibition of TRPC3 by Pyrazole 3 prevented cardiac hypertrophy induced by aortic banding (Kiyonaka et al. 2009). Together, the genetic and pharmacological studies suggest that inhibition of TRPC3 is a promising target in treatment of cardiac hypertrophy.

It was recently shown that inhibition of TRPC3 reduces brain injury caused by hemorrhage-related brain inflammation. Intracerebral hemorrhage caused by rupture of blood vessels in the brain results in leakage of thrombin into the parenchyma and activation of neuronal and astrocytes TRPC3. Activated Ca^{2+} influx causes excessive Ca^{2+} influx that is associated with inflammation and neuronal injury (Qureshi et al. 2009). Application of Pyrazole 3 intracerebroventricularly and intraperitoneally to mice after induction of intracerebral hemorrhage to inhibit TRPC3 activation significantly reduced brain injury (Munakata et al. 2013). It should be of interest to determine whether inhibition of TRPC3 reduces brain damage due to other inflammation modalities.

Acute pancreatitis and Sjögren's syndrome are inflammatory diseases that damage the pancreas and salivary glands (Sah et al. 2012; Alevizos and Illei 2010). A common nodal point in all models of these diseases is an excess Ca^{2+} influx (Cheng et al. 2012; Petersen and Sutton 2006). Salivary glands and pancreatic acini express high level of TRPC3 (Bandyopadhyay et al. 2005; Kim et al. 2006), and TRPC3 mediates significant portion of receptor- and store-mediated Ca^{2+} influx (Kim et al. 2009b). The therapeutic potential of inhibiting TRPC3 in these diseases was tested by genetic and pharmacological inhibition of TRPC3 (Kim et al. 2011). Part of these findings is illustrated in Fig. 5 (reproduced from Kim et al. 2009b). Figure 5a–f shows similar inhibition of receptor- and store-mediated Ca^{2+} influx by deletion of TRPC3 and by Pyrazole 3 and that Pyrazole 3 has no effect on the Ca^{2+} signal in *Trpc3*$^{-/-}$ cells, indicating that in vivo Pyrazole 3 primarily inhibits TRPC3. Inhibition of TRPC3 by Pyrazole 3 reduced all indices of acute pancreatitis, including serum amylase (Fig. 5g), edema (Fig. 5h), and intracellular activation of trypsin (Fig. 5i). Pyrazole 3 similarly inhibited all Ca^{2+}-dependent acinar cells injury tested in the parotid glands (Kim et al. 2009b).

The results with Pyrazole 3 in the three model diseases illustrate the usefulness and relative safety of inhibiting TRPC channels activity as a therapeutic approach to diseases caused by excessive Ca^{2+} influx, in particular inflammatory diseases. It is hoped that as more and more potent and selective inhibitors of TRPC channels develop, the full benefit of inhibiting their function will be explored and will be used to treat human diseases.

References

Alevizos I, Illei GG (2010) MicroRNAs in Sjogren's syndrome as a prototypic autoimmune disease. Autoimmun Rev 9(9):618–621

Almassy J, Won JH, Begenisich TB, Yule DI (2012) Apical Ca2+-activated potassium channels in mouse parotid acinar cells. J Gen Physiol 139(2):121–133

Antigny F, Koenig S, Bernheim L, Frieden M (2013) During post-natal human myogenesis, normal myotube size requires TRPC1 and TRPC4 mediated Ca2+ entry. J Cell Sci 126 (Pt 11):2525–2533

Jha A, Ahuja M, Maléth J, Moreno CM, Yuan JP, Kim MS, Muallem S (2013) The STIM1 CTID domain determines access of SARAF to SOAR to regulate Orai1 channel function. J Cell Biol 202(1):71–79

Balghi H et al (2011) Enhanced Ca2+ entry due to Orai1 plasma membrane insertion increases IL-8 secretion by cystic fibrosis airways. FASEB J 25(12):4274–4291

Bandyopadhyay BC et al (2005) Apical localization of a functional TRPC3/TRPC6-Ca2+-signaling complex in polarized epithelial cells. Role in apical Ca2+ influx. J Biol Chem 280 (13):12908–12916

Blair NT, Kaczmarek JS, Clapham DE (2009) Intracellular calcium strongly potentiates agonist-activated TRPC5 channels. J Gen Physiol 133(5):525–546

Cheng KT, Liu X, Ong HL, Swaim W, Ambudkar IS (2011) Local Ca entry via Orai1 regulates plasma membrane recruitment of TRPC1 and controls cytosolic Ca signals required for specific cell functions. PLoS Biol 9(3):e1001025

Cheng KT et al (2012) STIM1 and STIM2 protein deficiency in T lymphocytes underlies development of the exocrine gland autoimmune disease, Sjogren's syndrome. Proc Natl Acad Sci U S A 109(36):14544–14549

Darbellay B et al (2010) Human muscle economy myoblast differentiation and excitation-contraction coupling use the same molecular partners, STIM1 and STIM2. J Biol Chem 285 (29):22437–22447

Demuro A et al (2011) Subunit stoichiometry of human Orai1 and Orai3 channels in closed and open states. Proc Natl Acad Sci U S A 108(43):17832–17837

Derler I et al (2009) A Ca2(+)release-activated Ca2(+) (CRAC) modulatory domain (CMD) within STIM1 mediates fast Ca2(+)-dependent inactivation of ORAI1 channels. J Biol Chem 284 (37):24933–24938

Di A, Malik AB (2010) TRP channels and the control of vascular function. Curr Opin Pharmacol 10(2):127–132

Dietrich A et al (2005) Increased vascular smooth muscle contractility in TRPC6-/- mice. Mol Cell Biol 25(16):6980–6989

Engh A, Somasundaram A, Prakriya M (2012) Permeation and gating mechanisms in store-operated CRAC channels. Front Biosci 17:1613–1626

Feske S et al (2006) A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. Nature 441(7090):179–185

Feske S, Picard C, Fischer A (2010) Immunodeficiency due to mutations in ORAI1 and STIM1. Clin Immunol 135(2):169–182

Freichel M et al (2005) Functional role of TRPC proteins in native systems: implications from knockout and knock-down studies. J Physiol 567(Pt 1):59–66

Frischauf I et al (2009) Molecular determinants of the coupling between STIM1 and Orai channels: differential activation of Orai1-3 channels by a STIM1 coiled-coil mutant. J Biol Chem 284(32):21696–21706

Grigoriev I et al (2008) STIM1 is a MT-plus-end-tracking protein involved in remodeling of the ER. Curr Biol 18(3):177–182

Hartmann J et al (2008) TRPC3 channels are required for synaptic transmission and motor coordination. Neuron 59(3):392–398

Hawkins BJ et al (2010) S-glutathionylation activates STIM1 and alters mitochondrial homeostasis. J Cell Biol 190(3):391–405

Hong JH et al (2011) Polarized but differential localization and recruitment of STIM1, Orai1 and TRPC channels in secretory cells. Traffic 12(2):232–245

Hou X, Pedi L, Diver MM, Long SB (2012) Crystal structure of the calcium release-activated calcium channel Orai. Science 338(6112):1308–1313

Huang GN et al (2006) STIM1 carboxyl-terminus activates native SOC, I(crac) and TRPC1 channels. Nat Cell Biol 8(9):1003–1010

Jeon JP et al (2012) Selective Galphai subunits as novel direct activators of transient receptor potential canonical (TRPC)4 and TRPC5 channels. J Biol Chem 287(21):17029–17039

Ji W et al (2008) Functional stoichiometry of the unitary calcium-release-activated calcium channel. Proc Natl Acad Sci U S A 105(36):13668–13673

Kasai H, Augustine GJ (1990) Cytosolic Ca2+ gradients triggering unidirectional fluid secretion from exocrine pancreas. Nature 348(6303):735–738

Kim JY et al (2006) Homer 1 mediates store- and inositol 1,4,5-trisphosphate receptor-dependent translocation and retrieval of TRPC3 to the plasma membrane. J Biol Chem 281(43):32540–32549

Kim MS et al (2009a) Native store-operated Ca2+ influx requires the channel function of Orai1 and TRPC1. J Biol Chem 284(15):9733–9741

Kim MS et al (2009b) Deletion of TRPC3 in mice reduces store-operated Ca2+ influx and the severity of acute pancreatitis. Gastroenterology 137(4):1509–1517

Kim MS et al (2011) Genetic and pharmacologic inhibition of the Ca2+ influx channel TRPC3 protects secretory epithelia from Ca2+ -dependent toxicity. Gastroenterology 140(7):2107–2115, 2115.e2101–2104

Kiselyov K, Shin DM, Muallem S (2003) Signalling specificity in GPCR-dependent Ca2+ signalling. Cell Signal 15(3):243–253

Kiselyov K, Wang X, Shin DM, Zang W, Muallem S (2006) Calcium signaling complexes in microdomains of polarized secretory cells. Cell Calcium 40(5–6):451–459

Kiyonaka S et al (2009) Selective and direct inhibition of TRPC3 channels underlies biological activities of a pyrazole compound. Proc Natl Acad Sci U S A 106(13):5400–5405

Korzeniowski MK, Manjarres IM, Varnai P, Balla T (2010) Activation of STIM1-Orai1 involves an intramolecular switching mechanism. Sci Signal 3(148):ra82

Kuwahara K et al (2006) TRPC6 fulfills a calcineurin signaling circuit during pathologic cardiac remodeling. J Clin Invest 116(12):3114–3126

Lee MG et al (1997) Polarized expression of Ca2+ channels in pancreatic and salivary gland cells. Correlation with initiation and propagation of [Ca2+]i waves. J Biol Chem 272(25):15765–15770

Lee KP et al (2009) Molecular determinants of fast Ca2+-dependent inactivation and gating of the Orai channels. Proc Natl Acad Sci U S A 106(34):14687–14692

Lee KP et al (2010a) An endoplasmic reticulum/plasma membrane junction: STIM1/Orai1/TRPCs. FEBS Lett 584(10):2022–2027

Lee KP, Yuan JP, So I, Worley PF, Muallem S (2010b) STIM1-dependent and STIM1-independent function of transient receptor potential canonical (TRPC) channels tunes their store-operated mode. J Biol Chem 285(49):38666–38673

Lee KP, Choi S, Hong JH, Ahuja M, Graham S, Ma R, So I, Shin DM, Muallem S, Yuan J (2014) Molecular determinants mediating gating of transient receptor potential canonical (TRPC) channels by stromal interaction molecule 1 (STIM1). J Biol Chem [Epub ahead of print]. PMID: 24464579

Li M, Chen C, Zhou Z, Xu S, Yu Z (2012) A TRPC1-mediated increase in store-operated Ca2+ entry is required for the proliferation of adult hippocampal neural progenitor cells. Cell Calcium 51(6):486–496

Liao Y et al (2007) Orai proteins interact with TRPC channels and confer responsiveness to store depletion. Proc Natl Acad Sci U S A 104(11):4682–4687

Liao Y et al (2008) Functional interactions among Orai1, TRPCs, and STIM1 suggest a STIM-regulated heteromeric Orai/TRPC model for SOCE/Icrac channels. Proc Natl Acad Sci U S A 105(8):2895–2900

Liao Y et al (2009) A role for Orai in TRPC-mediated Ca2+ entry suggests that a TRPC:Orai complex may mediate store and receptor operated Ca2+ entry. Proc Natl Acad Sci U S A 106(9):3202–3206

Liou J et al (2005) STIM is a Ca2+ sensor essential for Ca2+-store-depletion-triggered Ca2+ influx. Curr Biol 15(13):1235–1241

Liou J, Fivaz M, Inoue T, Meyer T (2007) Live-cell imaging reveals sequential oligomerization and local plasma membrane targeting of stromal interaction molecule 1 after Ca2+ store depletion. Proc Natl Acad Sci U S A 104(22):9301–9306

Lis A et al (2007) CRACM1, CRACM2, and CRACM3 are store-operated Ca(2+) channels with distinct functional properties. Curr Biol 17(9):794–800

Liu X, Bandyopadhyay BC, Singh BB, Groschner K, Ambudkar IS (2005) Molecular analysis of a store-operated and 2-acetyl-sn-glycerol-sensitive non-selective cation channel. Heteromeric assembly of TRPC1-TRPC3. J Biol Chem 280(22):21600–21606

Liu X et al (2007) Attenuation of store-operated Ca2+ current impairs salivary gland fluid secretion in TRPC1(-/-) mice. Proc Natl Acad Sci U S A 104(44):17542–17547

McCarl CA et al (2009) ORAI1 deficiency and lack of store-operated Ca2+ entry cause immunodeficiency, myopathy, and ectodermal dysplasia. J Allergy Clin Immunol 124(6):1311–1318, e1317

McNally BA, Somasundaram A, Yamashita M, Prakriya M (2012) Gated regulation of CRAC channel ion selectivity by STIM1. Nature 482(7384):241–245

McNally BA, Somasundaram A, Jairaman A, Yamashita M, Prakriya M (2013) The C- and N-terminal STIM1 binding sites on Orai1 are required for both trapping and gating CRAC channels. J Physiol 591(Pt 11):2833–2850

Millay DP et al (2009) Calcium influx is sufficient to induce muscular dystrophy through a TRPC-dependent mechanism. Proc Natl Acad Sci U S A 106(45):19023–19028

Muallem S, Schoeffield MS, Fimmel CJ, Pandol SJ (1988) Agonist-sensitive calcium pool in the pancreatic acinar cell. II. Characterization of reloading. Am J Physiol 255(2 Pt 1):G229–G235

Muik M et al (2008) Dynamic coupling of the putative coiled-coil domain of ORAI1 with STIM1 mediates ORAI1 channel activation. J Biol Chem 283(12):8014–8022

Muik M et al (2011) STIM1 couples to ORAI1 via an intramolecular transition into an extended conformation. EMBO J 30(9):1678–1689

Muik M, Schindl R, Fahrner M, Romanin C (2012) Ca(2+) release-activated Ca(2+) (CRAC) current, structure, and function. Cell Mol Life Sci 69(24):4163–4176

Mullins FM, Park CY, Dolmetsch RE, Lewis RS (2009) STIM1 and calmodulin interact with Orai1 to induce Ca2+-dependent inactivation of CRAC channels. Proc Natl Acad Sci U S A 106(36):15495–15500

Munakata M et al (2013) Transient receptor potential canonical 3 inhibitor Pyr3 improves outcomes and attenuates astrogliosis after intracerebral hemorrhage in mice. Stroke 44 (7):1981–1987

Ng LC et al (2012) TRPC1 and Orai1 interact with STIM1 and mediate capacitative Ca(2+) entry caused by acute hypoxia in mouse pulmonary arterial smooth muscle cells. Am J Physiol Cell Physiol 303(11):C1156–C1172

Nilius B, Owsianik G (2011) The transient receptor potential family of ion channels. Genome Biol 12(3):218

Nilius B, Owsianik G, Voets T, Peters JA (2007) Transient receptor potential cation channels in disease. Physiol Rev 87(1):165–217

Ong HL et al (2007) Dynamic assembly of TRPC1/STIM1/Orai1 ternary complex is involved in store operated calcium influx: evidence for similarities in SOC and CRAC channel components. J Biol Chem 282(12):9105–9116

Ong HL, Jang SI, Ambudkar IS (2012) Distinct contributions of Orai1 and TRPC1 to agonist-induced [Ca(2+)](i) signals determine specificity of Ca(2+)-dependent gene expression. PLoS One 7(10):e47146

Palty R, Raveh A, Kaminsky I, Meller R, Reuveny E (2012) SARAF inactivates the store operated calcium entry machinery to prevent excess calcium refilling. Cell 149(2):425–438

Pandol SJ, Schoeffield MS, Fimmel CJ, Muallem S (1987) The agonist-sensitive calcium pool in the pancreatic acinar cell. Activation of plasma membrane Ca2+ influx mechanism. J Biol Chem 262(35):16963–16968

Parekh AB, Putney JW Jr (2005) Store-operated calcium channels. Physiol Rev 85(2):757–810

Park CY et al (2009) STIM1 clusters and activates CRAC channels via direct binding of a cytosolic domain to Orai1. Cell 136(5):876–890

Pedersen SF, Owsianik G, Nilius B (2005) TRP channels: an overview. Cell Calcium 38(3–4):233–252

Penna A et al (2008) The CRAC channel consists of a tetramer formed by Stim-induced dimerization of Orai dimers. Nature 456(7218):116–120

Petersen OH, Sutton R (2006) Ca2+ signalling and pancreatitis: effects of alcohol, bile and coffee. Trends Pharmacol Sci 27(2):113–120

Petersen OH, Sutton R, Criddle DN (2006) Failure of calcium microdomain generation and pathological consequences. Cell Calcium 40(5–6):593–600

Qureshi AI, Mendelow AD, Hanley DF (2009) Intracerebral haemorrhage. Lancet 373 (9675):1632–1644

Rao JN et al (2012) Polyamines regulate intestinal epithelial restitution through TRPC1-mediated Ca(2)+ signaling by differentially modulating STIM1 and STIM2. Am J Physiol Cell Physiol 303(3):C308–C317

Riccio A et al (2009) Essential role for TRPC5 in amygdala function and fear-related behavior. Cell 137(4):761–772

Roos J et al (2005) STIM1, an essential and conserved component of store-operated Ca2+ channel function. J Cell Biol 169(3):435–445

Sah RP, Garg P, Saluja AK (2012) Pathogenic mechanisms of acute pancreatitis. Curr Opin Gastroenterol 28(5):507–515

Selvaraj S et al (2012) Neurotoxin-induced ER stress in mouse dopaminergic neurons involves downregulation of TRPC1 and inhibition of AKT/mTOR signaling. J Clin Invest 122(4):1354–1367

Shaw PJ, Feske S (2012) Physiological and pathophysiological functions of SOCE in the immune system. Front Biosci (Elite Ed) 4:2253–2268

Shin HY et al (2010) A role of canonical transient receptor potential 5 channel in neuronal differentiation from A2B5 neural progenitor cells. PLoS One 5(5):e10359

Shinde AV et al (2013) STIM1 controls endothelial barrier function independently of Orai1 and Ca2+ entry. Sci Signal 6(267):ra18

Smyth JT, Beg AM, Wu S, Putney JW Jr, Rusan NM (2012) Phosphoregulation of STIM1 leads to exclusion of the endoplasmic reticulum from the mitotic spindle. Curr Biol 22(16):1487–1493

Stathopulos PB, Zheng L, Li GY, Plevin MJ, Ikura M (2008) Structural and mechanistic insights into STIM1-mediated initiation of store-operated calcium entry. Cell 135(1):110–122

Sundivakkam PC et al (2012) The Ca(2+) sensor stromal interaction molecule 1 (STIM1) is necessary and sufficient for the store-operated Ca(2+) entry function of transient receptor potential canonical (TRPC) 1 and 4 channels in endothelial cells. Mol Pharmacol 81(4):510–526

Takahashi N, Mori Y (2011) TRP channels as sensors and signal integrators of redox status changes. Front Pharmacol 2:58

Thorn P, Lawrie AM, Smith PM, Gallacher DV, Petersen OH (1993) Local and global cytosolic Ca2+ oscillations in exocrine cells evoked by agonists and inositol trisphosphate. Cell 74 (4):661–668

Vig M et al (2006) CRACM1 is a plasma membrane protein essential for store-operated Ca2+ entry. Science 312(5777):1220–1223

Wu X, Eder P, Chang B, Molkentin JD (2010) TRPC channels are necessary mediators of pathologic cardiac hypertrophy. Proc Natl Acad Sci U S A 107(15):7000–7005

Xu SZ et al (2008) TRPC channel activation by extracellular thioredoxin. Nature 451(7174):69–72

Yang X, Jin H, Cai X, Li S, Shen Y (2012) Structural and mechanistic insights into the activation of Stromal interaction molecule 1 (STIM1). Proc Natl Acad Sci U S A 109(15):5657–5662

Yao H, Duan M, Yang L, Buch S (2012) Platelet-derived growth factor-BB restores human immunodeficiency virus Tat-cocaine-mediated impairment of neurogenesis: role of TRPC1 channels. J Neurosci 32(29):9835–9847

Yu F, Sun L, Courjaret R, Machaca K (2011) Role of the STIM1 C-terminal domain in STIM1 clustering. J Biol Chem 286(10):8375–8384

Yu F, Sun L, Hubrack S, Selvaraj S, Machaca K (2013) Intramolecular shielding maintains STIM1 in an inactive conformation. J Cell Sci 126(Pt 11):2401–2410

Yuan JP, Zeng W, Huang GN, Worley PF, Muallem S (2007) STIM1 heteromultimerizes TRPC channels to determine their function as store-operated channels. Nat Cell Biol 9(6):636–645

Yuan JP et al (2009) SOAR and the polybasic STIM1 domains gate and regulate Orai channels. Nat Cell Biol 11(3):337–343

Yule DI, Ernst SA, Ohnishi H, Wojcikiewicz RJ (1997) Evidence that zymogen granules are not a physiologically relevant calcium pool. Defining the distribution of inositol 1,4,5-trisphosphate receptors in pancreatic acinar cells. J Biol Chem 272(14):9093–9098

Zeng W et al (2008) STIM1 gates TRPC channels, but not Orai1, by electrostatic interaction. Mol Cell 32(3):439–448

Zhang SL et al (2006) Genome-wide RNAi screen of Ca(2+) influx identifies genes that regulate Ca(2+) release-activated Ca(2+) channel activity. Proc Natl Acad Sci U S A 103(24):9357–9362

Zhang ZY, Pan LJ, Zhang ZM (2010) Functional interactions among STIM1, Orai1 and TRPC1 on the activation of SOCs in HL-7702 cells. Amino Acids 39(1):195–204

Zheng L, Stathopulos PB, Li GY, Ikura M (2008) Biophysical characterization of the EF-hand and SAM domain containing Ca2+ sensory region of STIM1 and STIM2. Biochem Biophys Res Commun 369(1):240–246

Zheng H et al (2013) Differential roles of the C and N termini of Orai1 protein in interacting with stromal interaction molecule 1 (STIM1) for Ca2+ release-activated Ca2+ (CRAC) channel activation. J Biol Chem 288(16):11263–11272