Recent Progress in the Regulation of TRPC1 by Store Depletion.

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ABSTRACT

Store-operated Ca^{2+} entry (SOCE) is activated in response to depletion of the ER-Ca^{2+} stores. The ER-Ca^{2+} sensor protein, STIM1, oligomerizes when [Ca^{2+}] in the store is decreased and moves to ER/PM junctional domains where it interacts with and activates channels involved in SOCE, namely Orai and TRPC channels. Orai1 is the primary pore-forming component of the highly Ca^{2+} selective CRAC channel. It is recruited to ER/PM junctional domains by STIM1 where it is gated via interaction with a specific C-terminal domain of STIM1. Thus Orai1 and STIM1 are sufficient for generation of functional CRAC channels. Store depletion also leads to activation of relatively non-selective cation channels, referred to as SOC channels that contribute to SOCE in several other cell types. TRPC1 has been proposed as a possible candidate component of SOC channels. TRPC1 contributes to endogenous SOCE in many cells types. In these cells, TRPC1-mediated Ca^{2+} entry and cation currents are stimulated with either agonist or thapsigargin, and inhibited by low [Gd^{3+}] and 10-20 μM 2APB (conditions that block SOCE). STIM1 also associates with and gates TRPC1 via electrostatic interaction between STIM1 (684KK685) and TRPC1 (639DD640). Further, functional Orai1 is required for activation of TRPC1-SOCE and this has been associated with recruitment of a TRPC1/STIM1/Orai1 complex. However, there is ongoing debate regarding the activation of TRPC1 by store depletion as well as the role of Orai1 and STIM1 in regulating its function. This chapter will summarize recent studies and concepts regarding the contributions of Orai1 and TRPC1 to SOCE. We will discuss major unresolved questions regarding functional interaction between Orai1 and TRPC1 as well as possible mechanisms involved in the regulation of TRPC channels.

Key words: TRPC1, STIM1, Orai1, SOCE

ABBREVIATIONS

CRAC: Calcium release activated calcium current
GPCR: G protein-coupled receptor
GPI : Gylcosylphosphatidylinositol
SCID: Severe combined immune deficiency
SOCE: Store-operated calcium enrty
TG : Thapsigorgin

INTRODUCTION

Store-operated calcium entry (SOCE), first described almost two decades ago, is a ubiquitous Ca^{2+} entry pathway that is activated by the depletion of Ca^{2+} in the endoplasmic reticulum (ER)-Ca^{2+} store (Parekh, et al., 2005, Putney, 1990). Under physiological conditions, SOCE is activated as sequelae to the stimulation of membrane receptors, PIP2 hydrolysis, IP3 generation, and IP3-mediated Ca^{2+} release from the ER. However, SOCE is activated by depletion of the intracellular Ca^{2+} store per se rather than by proximal events related to PIP2 hydrolysis. SOCE also regulates a number of critical physiological functions including secretion, cell proliferation, endothelial cell migration, T cell activation and mast cell degranulation (Parekh, et al., 2005). The mechanism(s) involved in ER-plasma membrane (PM) signaling that leads to activation of SOC channels has posed a major challenge in this field until very recently when some of the critical steps involved in these processes were resolved.

Proposed Mechanisms for regulation of SOCE

Several models that have been proposed to explain activation of SOCE (Parekh, et al., 2005, Putney, et al., 2001). The most widely discussed of these are: (i) conformational coupling – a close physical association between the plasma membrane Ca^{2+} channel and an ER protein (previously proposed to be IP_3R) allows detection and relay of the luminal [Ca^{2+}] status to the surface membrane; (ii) secretion coupling - cortical ER is dynamically regulated so that it interacts with PM channels when luminal [Ca^{2+}] is low; (iii) channel recruitment - regulated trafficking and fusion of vesicles containing pre-assembled channels, and possibly accessory signaling proteins, with the PM; and (iv) diffusible messenger - a diffusible calcium influx factor, generated in response to store depletion, is released into the cytosol to activate the plasma membrane Ca^{2+} channel. While none of these models has been conclusively established, the first appears to be the most relevant one conceptually (further dis-

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cussed below). Lack of very exclusive SOCE inhibitors has contributed to the difficulty in resolving the components and mechanisms of SOCE.

The first store-operated current to be measured and the one studied in greatest detail is the calcium-release-activated calcium current (ICRAC) identified in T-lymphocytes and RBL cells. ICRAC, mediated by the CRAC channel, is a highly Ca^{2+} selective (Ca^{2+}/Na^{+} of $\geq$400) and inwardly rectifying current that is greatly increased when divalent cations are removed from the external medium. The channel is also permeable to other divalents such as Mn^{2+}, Sr^{2+}, and Ba^{2+}, which are occasionally used as surrogate cations to assess SOCE. ICRAC is activated relatively slowly by perfusion of the cell cytosol with EGTA (likely due to passive depletion of internal Ca^{2+} store) and faster when cells are stimulated with an agonist or thapsigargin (Tg) (Parekh, et al., 2005). The CRAC channel is predicted to have very low single-channel conductance ~15 femtosiemens. Application of similar methodologies in other cell types leads to activation of Ca^{2+} entry associated with relatively non-selective cation currents (Ambudkar, 2007, Parekh, et al., 2005). These currents have been historically referred to as ISOC. They have been described as store-operated channel currents since they are activated under the same conditions used for ICRAC and are inhibited by 1 μM Gd^{3+} and 10-20 μM 2APB, similar to ICRAC. Note however that in some cells, agonist stimulation also leads to activation of cation channels that are not blocked by 2APB or low Gd^{3+}. Furthermore, some channels are only activated by agonist and not by Tg, discriminating them from "store-operated" channels. Identification of SOC channels with diverse biophysical characteristics, ranging from non-selective to relatively Ca^{2+} selective, suggests the possibility that a variety of distinct channels may be involved in SOCE.

Molecular components of SOCE

TRPC1. TRPC1, the first mammalian TRPC protein to be identified, has also been most consistently demonstrated as a SOC channel component in a variety of cell types, including keratinocytes, platelets, smooth, skeletal, and cardiac muscles, DT40, HEK293, salivary gland, neuronal, intestinal and endothelial cells (for a recent review, see Ambudkar, et al., 2007). Mutations in the TRPC1 pore region alters the properties of ISOC, suggesting that TRPC1 contributes to the channel pore. Further, TRPC1 mediates sufficient Ca^{2+} entry to regulate cellular function such as KCa channel activation, proliferation and gene expression. Heteromeric associations of TRPC1 with other TRPC channels could account for the diversity in channel properties and function attributed to it (as reviewed in (Ambudkar, 2007, Ambudkar, et al., 2007)). However, TRPC channels do not contribute to endogenous CRAC channel function (exceptions to this suggestion are further discussed below). Although overexpression of TRPC1 did not result in substantial increase in SOCE, knockdown of TRPC1 has been associated with consistent decrease in endogenous SOCE in many cell types (as noted above). Further, there is severe loss of SOCE and salivary gland fluid secretion in mice lacking TRPC1 (Liu, et al., 2007), although loss of TRPC1 did not appear to affect function of platelets or cerebral artery smooth muscle cells from these mice (Dietrich, et al., 2007, Varga-Szabo, et al., 2008). These findings suggest cell type- and tissue-specific functions for TRPC1 in SOCE.

Orai1. Orai1 is a four-transmembrane domain PM protein identified in 2006 through genome-wide RNAi screening in Drosophila S2 cell for CRAC channel-associated proteins and by genetic linkage analysis in severe combined immune deficiency (SCID) patients (Feske, et al., 2006). Further studies using site-directed mutagenesis confirmed that Orai1 forms the pore of the CRAC channel (see a recent review by Prakriya (2009)). While this is now widely accepted together with the suggestion that Orai1 is present and important for SOCE in all cell types, there is little information regarding endogenous Orai1 function in all cell types. Two other members of the Orai family, Orai2 and Orai3, which have apparently distinct channel properties, are also present in these cell types. The relative levels of expression and function of these Orai channels in different cell types are far from being clearly established.

STIM1. The mechanism involved in transmitting the signal of store depletion to the channels located on the PM remained elusive until the stromal interaction molecule (STIM) was identified (Liou, et al., 2005, Roos, et al., 2005) as the key regulatory protein for SOCE . STIM has two human homologs, STIM1 and STIM2,

both of which are suggested to be ER proteins. They have a short N-terminal domain which resides in the ER lumen, a single transmembrane domain and a relatively large cytosolic C-terminal domain. The N-terminal domain has an EF-hand domain that binds Ca^{2+} within the range of [Ca^{2+}] in the ER of resting cells (Liou, et al., 2005), with the Ca^{2+} affinity of STIM1 is reported to be higher than that of STIM2 (Brandman, et al., 2007). However, the function of STIM2 is poorly understood while substantial information has accumulated regarding STIM1. When [Ca^{2+}] in the ER lumen is decreased, the Ca^{2+} bound to STIM1 dissociates leading to conformational changes (which have not yet been fully characterized) in the protein. The net result is that STIM1 monomers oligomerize and translocate to specific ER-PM junctional regions where they aggregate into puncta (Zhang, et al., 2005). It is proposed that STIM1 interacts with and activates channels involved in SOCE at these locations. Both TRPC1 and Orai1 interact with and are activated by STIM1 in response to Ca^{2+} store depletion (Ambudkar, et al., 2007, Li, et al., 2007, Worley, et al., 2007). The EF-hand domain, SAM motif, coiled-coil domains and proline-rich region are important in mediating the oligomerization and translocation of STIM1 to the ER-PM domains (Li, et al., 2007, Park, et al., 2009, Worley, et al., 2007). The lysine-rich C-terminal end of STIM1 (referred to as the polybasic tail) has been proposed to be involved in puncta formation in ER-PM junctions, but not in STIM1 oligomerization or Orai1 activation per se (Park, et al., 2009). While the polybasic tail region does not directly gate CRAC channels, STIM1(684KK685) in this region interacts with and gates TRPC1. Under physiological conditions, the polybasic tail region of STIM1 is critical for activation of CRAC as well as TRPC1 channels within the native signaling microdomain in the cell. The polybasic tail region contains a consensus sequence which could mediate interaction of the protein with PIP_2 in the plasma membrane (Heo, et al., 2006, Liou, et al., 2007, Walsh, et al., 2010). Such an interaction could enable anchoring of STIM1 in the plasma membrane and thus facilitate its association with the channels and activation of SOCE, although a recent study (Korzeniowski, et al., 2009) has questioned the possible role of PIP2. Several other studies show that lipid raft domains (that are also enriched in PIP_2) could serve as platforms for recruitment and anchoring STIM1/channel complexes in the cell periphery (Alicia, et al., 2008, Pani, et al., 2008), although this concept has also been questioned in a recent study (DeHaven, et al., 2009). The interesting role of lipid rafts in SOCE has been recently reviewed (Ambudkar, et al., 2010, Pani, et al., 2009b) and will be briefly discussed later in this chapter.

Channels mediating SOCE

CRAC channels. While knockdown of Orai1 or STIM1 lead to dramatic reduction or complete elimination of SOCE and ICRAC, studies in several cell types reveal that co-expression of Orai1 with STIM1 induces a large increase in SOCE and generates substantial ICRAC (Hogan, et al., 2010). Further, mutations in conserved negatively charged residues in the transmembrane region of Orai1 alter the Ca^{2+} selectivity of the CRAC channel (Hogan, et al., 2010) and expression of the Orai1 mutant associated with SCID does not result in generation of ICRAC in cells when co-expressed with STIM1. Expression of EF hand mutant of STIM1 (D76A) induces constitutive CRAC activity. Together these findings provide strong evidence that Orai1 is the essential pore-forming unit of CRAC channels and that STIM1 serves as the regulator of the channel and sensor of store depletion. Orai2 or Orai3 when expressed with STIM1 in HEK293 cells generate store-operated channels with different magnitudes and channel characteristics, leading to the speculation that native CRAC channels may involve combinations of Orai proteins (Hogan, et al., 2010). However, their exact roles in SOCE have not yet been conclusively demonstrated in any cell type.

Orai1 and STIM1 interact within very specific signaling regions of the cells. Most of the data have been obtained from studies with Orai1 and STIM1 tagged with fluorescent proteins, whereas the localization of native STIM1 and Orai1 in resting and stimulated cells has not yet been described. It is also important to note that the introduction of a fluorescent protein tag at the N-terminus of STIM1 prevents its PM insertion, which might induce artifacts that have not yet been identified and ruled out. Based on the currently reported studies, it is widely accepted that STIM1 is translocated into the ER/PM junctional domains in response to store depletion. Orai1 is located primarily in the plasma mem-

brane and displays a diffused pattern under resting conditions and upon ER-Ca^{2+} store depletion, rapidly clusters and co-localizes with STIM1 puncta within ER-PM microdomains (Hogan, et al., 2010). Two hydrophobic amino acid residues located in the C-terminal coiled-coil motif of Orai1, L273 and L276, are essential for interaction of the channel with STIM1 (Hogan, et al., 2010, Prakriya, 2009). Several independent studies have identified a critical Orai1-interaction domain in STIM1 (Kawasaki, et al., 2009, Muik, et al., 2009, Park, et al., 2009, Yuan, et al., 2009) that is involved in gating Orai1. Furthermore, another domain called the CRAC Modulatory Domain located within the C-terminus of STIM1 (Mullins, et al., 2009) appears to mediate fast Ca^{2+}-dependent inactivation of Orai1 by binding to Ca^{2+}. Interestingly, a calmodulin (CaM) binding site was recently identified in the N-terminus of Orai1 (68-91aa) and eliminating CaM binding abrogated Orai1 inactivation (Mullins, et al., 2009), suggesting that CaM synergistically acts with STIM1 to mediate the Ca^{2+}-dependent fast inactivation of Orai1 channel. Thus, STIM1 interaction with Orai1 accounts for several important regulatory mechanisms associated with CRAC channels including their gating in response to store depletion as well as Ca^{2+}-dependent inactivation.

SOC channels. STIM1 also interacts with and modulates the activity of TRPC1 (Cheng, et al., 2008, Huang, et al., 2006, Ong, et al., 2007). As is the case with CRAC channels, knockdown of STIM1 reduces endogenous TRPC1 function (Ca^{2+} entry and cation currents) stimulated by store depletion, while co-expression of TRPC1 and STIM1 increases SOCE. Immunoprecipitation of STIM1 with TRPC1 increases upon stimulation of cells either with an agonist or Tg (Huang, et al., 2006, Ong, et al., 2007, Yuan, et al., 2007, Zeng, et al., 2008). As seen for Orai1, the D76A mutant of STIM1 induces constitutive activation of TRPC1. Two regions of STIM1 are suggested to interact with TRPC1: the ERM (ezrin/radixin/-moesin) domain (Huang, et al., 2006) which is appears to interact with TRPC1 but is not involved in gating the channel and the STIM1 polybasic tail (684KK685) residues which interacts electrostatically with the negatively charged residues in the C-terminus of TRPC1 (639DD640), resulting in gating of the channel (Zeng, et al., 2008). Thus, STIM1 is a versatile ER sensor that regulates different channels that respond to store depletion. Importantly, specific domains of STIM1 are involved in regulation of these channels.

A very important and somewhat contentious aspect of store-dependent regulation of TRPC1 is the requirement of functional Orai1. Knockdown of endogenous Orai1 or transfection of cells with functionally defective Orai1 mutants (R91W, E106Q) attenuates the increase in SOCE induced by TRPC1-STIM1 overexpression (Cheng, et al., 2008, Kim, et al., 2009). Similar experimental maneuvers abrogate endogenous SOCE in HSG cells, which is significantly dependent on endogenous TRPC1 (Ong, et al., 2007). It has been suggested that there is dynamic assembly of a TRPC1\STIM1/Orai1 complex in response to store depletion (Cheng, et al., 2008, Huang, et al., 2006, Kim, et al., 2009, Ong, et al., 2007, Zeng, et al., 2008). Together, these data highlight the critical contributions of STIM1, Orai1, and TRPC1 to SOCE mediated by SOC channels. While STIM1 appears to be involved in gating TRPC1-SOC channels, the functional interaction between TRPC1 and Orai1 that is required for TRPC1 activation is not known. Several possible scenarios have been suggested and are discussed below.

TRPC1 and SOC channel diversity

Only some TRPCs consistently display store-dependent regulation while others have been suggested to primarily contribute to agonist-stimulated non-store-operated Ca^{2+} entry mechanisms. TRPC1, the first mammalian TRPC protein to be identified, is widely expressed in neuronal as well as non-neuronal tissues including muscle (Ambudkar, 2007, Montell, 2005, Parekh, et al., 2005). Interestingly, TRPC1 forms diverse SOCs ranging from relatively selective to non-selective (Ca^{2+} versus Na^{+}) (Brough, et al., 2001, Liu, et al., 2005). Some TRPC1-SOCs display strong anomalous mole-fraction behavior for Ca^{2+}/Na^{+} permeability which render them more permeable to Ca^{2+} under physiological conditions. Such diversity could be a result of different channels generated by homomeric or heteromeric interactions of TRPC1 with other TRPC monomers. Although there is no conclusive evidence so far of a native homomeric TRPC1 channel, selective interactions of TRPC1 with other TRPCs, e.g. TRPC4/TRPC5 or TRPC3/TRPC7 have been reported (see (Ambudkar, et al., 2007)). TRPC1–

TRPC1 multimers are generated by interaction of the N-terminal coiled–coiled domain interactions (Engelke, et al., 2002) while TRPC1–TRPC3 heteromers are formed by interaction of the first ankyrin repeat region in TRPC1with an as yet unknown region in the TRPC3 N-terminus (Liu, et al., 2005). The interaction of TRPC1 with TRPC4, TRPC3, or TRPC7 has been linked with generation of SOC channels (Ambudkar, et al., 2007, Yuan, et al., 2007). These studies provide a molecular basis for the diversity seen in TRPC-associated SOC channels. The voluminous amount of data reported in the last 8–10 years which describe function of heterologously expressed TRPC, and the controversies in those observations due to inherent problems associated with such expression systems will not be discussed here.

As discussed above, a considerable amount of data provide strong evidence that TRPC1 is a component of SOC channels in a large variety of cells. However, the fact that none of the TRPCs generate ICRAC has been a source of concern. The exceptions are TRPC4 in aortic endothelial and smooth muscle cells (Freichel, et al., 2004, Freichel, et al., 2001) and keratinocytes (Fatherazi, et al., 2006), TRPC1 in DT40 cells (Mori, et al., 2002), and TRPC3 in T lymphocytes (Philipp, et al., 2003) where ICRAC-like currents have been recorded following store depletion. While it has been suggested that Orai1 and STIM1 interact with and contribute to TRPC1-SOC channel function (Ong, et al., 2007), it is quite possible that other TRPCs might also associate with these two proteins. An interesting study demonstrated that Orai1 can also interact with TRPC3 and TRPC6 to mediate store-dependent regulation of these channels (Liao, et al., 2008). We have proposed earlier, as have Muallem and colleagues, that TRPC1 is the STIM1 binding component in TRPC-containing SOC channel complexes. While TRPC2 and TRPC7 can also link PM SOC to ER, TRPC7 is not found in majority of the SOC channels and TRPC2 is not expressed in human cells. Thus a reasonable hypothesis for how diverse SOC channels are regulated by the same intracellular signal (ER-Ca^{2+} store depletion) is that TRPC1 links the PM SOC channel to the ER-Ca^{2+} sensor, STIM1. We suggest that TRPC1 and STIM1 can provide a molecular basis for TRPC-dependent SOCE. TRPC1, together with other TRPC monomers, forms the pore-forming component of the SOC channel while STIM1 serves as the ER-Ca^{2+} sensor that relays the ER "signal" to the channel. Our suggestion does not exclude the involvement of the other proteins (such as e.g. IP_3R, HOMER, and Caveolin-1) that have been shown to contribute to regulation of TRPC function.

Current concepts regarding SOC channels

The presence and function of CRAC channels has yet to be confirmed in all cell types, although it has been established as the primary SOCE pathway in T lymphocytes, mast cells, and several other cell types (Hogan, et al., 2010, Liu, et al., 2004, Prakriya, 2009). While there are substantial data on the key aspects of channel assembly and activation of CRAC channels, the mechanism involved in TRPC-SOC channels has yet to be completely resolved. Despite the relatively large number of studies which demonstrate that TRPC1 is involved in SOCE there is continued skepticism regarding the relevance of TRPC1. A major reason for this is that TRPC1-/- mice do not display loss of function in all cell types (Dietrich, et al., 2007, Varga-Szabo, et al., 2008). Further, heterologous expression of TRPC1 does not consistently result in a substantial increase in SOCE. In this context it is important to note that mutations causing loss of Orai1 function, as in SCID patients, does not lead to functional defects in all the tissues (Feske, et al., 2006). Further, when expressed Orai1 is alone, i.e. without co-expression of STIM1, there is no marked increase in SOCE. In fact expression of Orai1 alone often results in a small decrease in function. Since most previous studies with TRPC1 did not take into account the possible requirement for STIM1, these studies need to be carefully re-evaluated. The more important and relevant question is regarding the requirement of functional Orai1 for store depletion-dependent activation of TRPC1. Since there are no conclusive data elucidating a possible mechanism for this as yet, major debate has ensued over how TRPC1 and Orai1 can simultaneously contribute to SOCE.

Several possible mechanisms have been proposed to explain the functional interactions between Orai1 and TRPC1, although none of this have been convincingly supported or refuted by experimental evidence: (1) Orai1 and TRPC1 generate distinct CRAC and SOC channels; (2)

TRPC1 and Orai1 interact to form one channel; (3) Orai1 is a critical modulator of TRPC1 channels; with Orai1 either directly or indirectly activating TRPC1; and (4) TRPC1 regulates Orai1 function.

Lipid Rafts and SOCE

The importance of lipid raft domains (LRDs) in calcium signaling has been recently reviewed quite extensively (Ambudkar, et al., 2010, Pani, et al., 2009b). Lipid rafts are dynamic assemblies of plasma membrane lipids that are enriched in sphingolipid, cholesterol and glycosylphosphatidylinositol anchored proteins, and are found in virtually every eukaryotic cell. The presence of these lipids, as well as the interaction of cholesterol with neighboring hydrocarbon chains, gives the rafts a more rigid liquid-ordered state that is distinct from the surrounding disordered phospholipid bilayer. Lipid raft heterogeneity and stability can be achieved by lipid-lipid, protein-lipid and protein-protein interactions. Such heterogeneity allows for the association and assembly of various proteins involved in the cell signaling machinery, such as receptor tyrosine kinases and G protein-coupled receptors, establishment of cell polarity, cholesterol transport, potocytosis, and endocytosis (Ambudkar, et al., 2010, Pani, et al., 2009b).

SOCE has been shown to occur within distinct LRDs including caveolin-containing LRDs. Caveolins are primarily found in the caveolae, which are flask-shaped invaginations found abundantly in the plasma membrane of many cell types. Among the three members of be caveolin family, Caveolin-1 (Cav-1) has been most extensively studied and shown to associate with and regulate the activities of many signaling proteins via a 20-amino acid segment (aa 82-101) in the caveolin scaffolding domain (CSD). Interaction with the CSD of Cav-1 either maintains the signaling protein in an inactive state until a stimulus is presented or terminates signal transmission after activation, allowing for precise regulation of the cellular signaling events (see recent review by Ambudkar et al. (2010)). LRDs are proposed to exert their effects by acting as a scaffold for the assembly and organization of the calcium signaling machinery within these domain, rather than a direct effect on channel function per se (as reviewed by Ambudkar et al. (2010) and Pani et al. (2009)). Such scaffolding increases the probability and rate of specific protein-protein interaction(s) between these signaling proteins that are critical in the regulation and compartmentalization of the Ca^{2+} entry signal. SOC channels are localized within the lipid rafts and perturbations in the integrity of LRDs severely impacted channel function in many cells types, including human salivary gland cells, polymorphonuclear neutrophils, endothelial cells and human platelets. Furthermore, TRPC1 and Orai1 have been shown to colocalize with STIM1 within lipid rafts (Alicia, et al., 2008, Martin, et al., 2009, Pani, et al., 2008). The TRPC channels have conserved Cav-1 binding domains located within the N-and C-termini (Pani, et al., 2009b). The N-terminal caveolin-binding motif (aa 322 and 349) binds to the CSD. TRPC1 co-immunoprecipitates with Cav-1 in human salivary gland (Lockwich, et al., 2000) and pulmonary artery endothelial cells (Kwiatek, et al., 2006). The N-TRPC1-Cav-1 interaction determines the localization of TRPC1 in the PM. Cav-1 binds the inactive channel and when overexpressed, competes against channel activation by store depletion by STIM1 (Pani, et al., 2009a). Expression of higher levels of STIM1 relative to Cav-1 was sufficient to induce recovery of TRPC1 function (Pani, et al., 2009a). Furthermore, STIM1 (684KK685) interaction with TRPC1 which gates the channel electrostatistically also induces dissociation of the channel from Cav1. Considering the increasing complexity of the assembly of TRPC1 with other signaling proteins within the LRDs, more studies are required delineate how protein-protein association changes as TRPC1 progresses through the different stages of assembly (trafficking, insertion into the plasma membrane and subsequent activation following store depletion). Lutz Birnbaumer and co-workers suggested that the recruitment of Orai:TRPC:STIM1 complexes into the LRDs is required for store-dependent regulation but these same complexes would function as receptor-operated channels when localized outside lipid rafts (Liao, et al., 2009). However, this interesting concept needs to be further evaluated.

Conclusions

After more than two decades of intense efforts, recent studies have lead to the identification of novel channels and regulatory proteins that determine the mechanism of SOCE. STIM1 appears to be the central molecule with multiple

targets, such as Orais and TRPC channels, as well as proteins involved in protein synthesis, adenylyl cyclase, and cell adhesion. The physiological relevance of these different functions of STIM1 and whether they are all associated with SOCE needs to considered within the context of specific cell types. Emerging studies also reveal that SOCE is associated with highly specialized microdomains in the cells where local Ca^{2+} signaling events near the plasma membrane leads to significant regulation of cell function within the cytosol and nucleus. It is now well established that SOCE is required for a number of critical cell functions, not only for refilling of intracellular calcium stores. The change in local and global Ca^{2+} signals as a result of SOCE have very significant and different outcomes on the regulation of downstream cellular functions including secretion and gene regulation etc. Future studies in addition to focusing on the more didactic aspects of the structure and properties of ion channels involved in SOCE, should also be directed towards examining the relevance of other components that might be activated either concurrently with or downstream from SOCE activation and assess the impact of these on local and global Ca^{2+} signaling and on cell function.

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