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Contribution of TRPC1 and Orai1 to Ca^{2+} entry activated 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 by the ER Ca^{2+} sensor protein, STIM1 which oligomerizes and moves to ER/PM junctional domains where it interacts with and activates channels involved in SOCE. Two types of channel activities have been described. I_{CRAC} , via Ca^{2+} release-activated Ca^{2+} (CRAC) channel, which displays high Ca^{2+} selectivity and accounts for the SOCE and cell function in T lymphocytes, mast cells, platelets, and some types of smooth muscle and endothelial cells. Orai1 has been established as the pore-forming component of CRAC channels and that interaction of Orai1 and STIM1 is sufficient for generation of the CRAC channel. Store depletion also leads to activation of relatively non-selective cation currents (referred to as I_{SOC}) that contribute to SOCE in several other cell types. TRPC channels, including TRPC1, TRPC3, TRPC4, have been proposed as possible candidate channels for this Ca^{2+} influx. TRPC1 is the best characterized channel in this regard and reported to contribute to endogenous SOCE in many cell types. TRPC1-mediated Ca^{2+} entry and cation current in cells stimulated with agonist or thapsigargin were inhibited by low $[\text{Gd}^{3+}]$ and 10–20 μM 2APB (conditions that block SOCE). Importantly, STIM1 also associates with and gates TRPC1 via electrostatic interaction between STIM1 ($^{684}\text{KK}^{685}$) and TRPC1 ($^{639}\text{DD}^{640}$). Further, store depletion induces dynamic recruitment of a TRPC1/STIM1/Orai1 complex and knockdown of Orai1 completely abrogates TRPC1 function. Despite these findings, there has been much debate regarding the activation of TRPC1 by store depletion as well as the role of Orai1 and STIM1 in SOC channel function. This chapter summarizes recent studies and concepts regarding the contributions of Orai1 and TRPC1 to SOCE. Major unresolved questions regarding functional interaction between Orai1 and TRPC1 as well as possible mechanisms involved in the regulation of TRPC channels by store depletion will be discussed.

Introduction

Store-operated calcium entry (SOCE) was first described almost two decades ago as a Ca^{2+} entry mechanism in the plasma membrane that is activated by the depletion of Ca^{2+} in the endoplasmic reticulum (ER)- Ca^{2+} store. Since then this Ca^{2+} entry pathway has been demonstrated to be a ubiquitously present in all excitable and non-excitabile cells [1–3]. Under physiological conditions, SOCE is activated in response to stimulation of membrane receptors that lead to the hydrolysis of PIP_2 , IP_3 generation, and IP_3 -mediated Ca^{2+} release from the ER via activation of the IP_3 receptor. Use of the ER Ca^{2+} pump inhibitor thapsigargin (Tg) demonstrated that SOCE activation is regulated by depletion of the intracellular Ca^{2+} store rather than proximal events associated with receptor-dependent PIP_2 hydrolysis. Thus, the name store-operated calcium entry, or capacitative Ca^{2+} entry. Although Ca^{2+} influx via specific plasma membrane store-operated calcium (SOC) channels

replenishes the ER Ca^{2+} store, it also regulates a number of critical physiological functions such as secretion, cell proliferation, endothelial cell migration, T cell activation and mast cell degranulation, etc. [2]. Identification of the mechanism(s) involved in ER-plasma membrane (PM) signaling that results in activation of SOC channels in the surface membrane as well as the channel components themselves has been a major challenge in this field, until very recently when some of these critical issues have been resolved.

Early studies established a close proximity of SOCE to the ER membrane. It was shown that since the ER lies very close to the PM Ca^{2+} entering the cells is rapidly taken up into the ER lumen via the SERCA pump [4, 5]. These functional associations between ER and PM provided the basis for several models that have been proposed to explain activation of SOCE [2, 5]. The models that have garnered most attention are: (i) *conformational coupling* – a close physical association between the PM Ca^{2+} channel and an ER protein (previously proposed to be IP_3R) allows detection and relay of the luminal $[\text{Ca}^{2+}]$ status to the surface membrane; (ii) *secretion coupling* – cortical ER is dynamically regulated so that it interacts with PM channels when luminal $[\text{Ca}^{2+}]$ 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 PM Ca^{2+} channel.

Characteristics of SOCE

Experimental methods for activation and assessment of SOCE

Typical reagents used for activation of SOCE include agonists that stimulate PIP_2 hydrolysis and IP_3 -mediated Ca^{2+} release from ER, e.g. CCh, bradykinin, angiotensin and ATP, as well as agents that lead to passive depletion of the ER Ca^{2+} store such as Tg and BHQ (SERCA inhibitors), TPEN (low affinity ER-permeant Ca^{2+} chelator), and ionomycin. Measurements of SOCE are made either using fluorescent Ca^{2+} indicator dyes or electrophysiologically using whole cell patch clamp techniques. Agents stimulating SOCE are usually added to the bathing solution of dye-loaded cells. In the case of electrophysiological recordings, critical inclusions in the the pipette solution are EGTA (to suppress store reloading and Ca^{2+} -dependent inactivation of the channel), $\text{ATP}+\text{Mg}^{2+}$ (to suppress activation of TRPM7), or IP_3 as required (the technique has been discussed in great detail [1, 6]). Despite the lack of very exclusive SOCE inhibitors, a few have been established as suitable inhibitors of SOCE. These include Gd^{3+} (1–5 μM) or 2-aminoethylidiphenyl borate (2APB, 10–20 μM). Thus, the current criteria used to identify SOCE are (i) activation of Ca^{2+} entry and calcium currents by agonists, Tg, or IP_3 (included in the pipette solution for current measurements); and (ii) inhibition of both by 1–5 μM Gd^{3+} and 10–20 μM 2APB.

Characteristics of the currents associated with SOCE

The first store-operated current to be measured and the one studied in greatest detail, is the calcium-release-activated calcium current (I_{CRAC}) identified in T-lymphocytes and RBL cells [1]. I_{CRAC} , mediated by the CRAC channel, is a highly Ca^{2+} selective ($\text{Ca}^{2+}/\text{Na}^+$ of 400) and inwardly rectifying current which is greatly increased when divalent cations are removed from the external medium. The channel is also permeable to other divalent such as Mn^{2+} , Sr^{2+} , and Ba^{2+} , which are occasionally used as surrogate cations to assess SOCE. I_{CRAC} 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 Tg (see [1, 2, 6] for detailed description of I_{CRAC} properties). The CRAC channel is predicted to have very low single-channel conductance ~15 femtosiemens (fS) [7].

Application of similar methodologies in other cell types leads to activation of Ca^{2+} entry associated with relatively non-selective cation currents [2, 4, 8–10]. These currents have been historically referred to as I_{SOC} . They have been described as store-operated channel currents since they are activated under the same conditions used for I_{CRAC} and are inhibited by $1\mu\text{M}$ Gd^{3+} and $10\text{--}20\mu\text{M}$ 2APB, similar to I_{CRAC} . 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.

Proposed molecular components of SOCE

TRPC Channels

The long search for channel mediating SOCE first led to the identification of mammalian transient receptor potential (TRP) channels. Members of the TRPC (TRP Canonical) subfamily were proposed as candidate channels for SOCE based on their activation by stimuli that lead to PIP_2 hydrolysis. The TRPC subfamily consists of 7 members (TRPC1–7) that displaying diverse properties, modes of regulation and physiological functions. They are also suggested to be assembled as homomeric or heteromeric channels, although there is little information regarding the status of endogenous TRPC channels [11, 12]. While it is generally accepted that TRPC channels are activated downstream of agonist-stimulated PIP_2 hydrolysis, there is considerable discrepancy regarding their activation mechanisms. Many studies suggest that TRPC channels do not have an apparent contribution to endogenous CRAC channel function (exceptions to this suggestion are further discussed below). Therefore, TRPC channels activated in response to store depletion, have been referred to as SOC channels, to distinguish them from CRAC channels [11–14]. TRPC1, the first mammalian TRPC protein to be identified, has 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 [15–26]. Mutations in the TRPC1 pore region alters the properties of I_{SOC} suggesting that TRPC1 contributes to the channel pore. Further, TRPC1 mediates sufficient Ca^{2+} entry to regulate cellular function such as K_{Ca} 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 [8, 13]). 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 (noted above). We have reported severe loss of SOCE and salivary gland fluid secretion in mouse lacking TRPC1 [19], although loss of TRPC1 did not appear to affect function of platelets or cerebral artery smooth muscle cells from these mice [27, 28]. These findings suggest cell type- and tissue-specific function for TRPC1 in SOCE. Other studies have also previously linked TRPC4 and TRPC3 to SOCE [2, 26, 29–31]. Notably, TRPC4-mediated current was highly Ca^{2+} selective and displayed inward rectification similar to that of I_{CRAC} [32].

Orai Channels

Orai1 is a four-transmembrane domain plasma membrane 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. Knockdown of Orai1 decreased CRAC channel function in S2 cells, while a single point mutation in Orai1 (R91W) was associated with loss of I_{CRAC} and defect in the Ca^{2+} signaling of T-lymphocytes obtained from SCID patients [33]. Further studies using site-

directed mutagenesis revealed that Orai1 forms the pore of the CRAC channel [34]. This concept is now widely accepted as is the suggestion that Orai1 is present and is important for SOCE in all cell types. However, data on the endogenous Orai1 function in all cell types are not currently available. Two other members of the Orai family, Orai2 and Orai3, which have apparently distinct channel properties, are also present in cells. The relative levels of expression and function of these Orai channels are far from being clearly established.

STIM proteins

The mechanism involved in transmitting the signal of store depletion to the channels located on the plasma membrane remained elusive until the stromal interaction molecule (STIM) was identified by the Stauderman [35] and Meyer [36] groups in 2005. STIM has two human homologs, STIM1 and STIM2, which are primarily localized in the ER membrane. They are single transmembrane protein molecules with a relatively large cytosolic C-terminal domain. The N-terminus lies within the ER lumen and has a Ca^{2+} binding site which serves as a calcium sensor in the ER [36]. The available data suggest that when the $[\text{Ca}^{2+}]$ in the ER lumen decreases, the Ca^{2+} bound to the EF hand domain of STIM1 dissociates leading to conformational changes in the protein. The net result is that STIM1 monomers oligomerize and translocate to specific ER-PM junctional regions where they aggregate into puncta [37]. 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 [13, 38, 39]. The role of STIM2 has not yet been conclusively established and will not be discussed in this review.

Within the past few years, the structure of STIM1 has been closely scrutinized to define the mechanism(s) involved in its association with, and activation of OraIs, and TRPC proteins. Mutations in the EF hand domain induce constitutive activation of SOCE [36, 37]. Further the EF hand domain, SAM motif, coiled-coil domain and proline-rich region are important in mediating the oligomerization and translocation of STIM1 to critical ER-PM junctional regions where SOCE is regulated [38–40]. The lysine-rich C-terminal end of STIM1 (referred to as polybasic tail) has been proposed to be involved in activation of SOCE. Deletion of the polybasic tail resulted in loss of SOCE likely due to loss of STIM1 puncta formation in ER-PM junctions, although STIM1 oligomerization per se is not affected [40]. The polybasic tail region does not directly gate CRAC channels although STIM1^(684KK685) in this domain of STIM1 is involved in gating TRPC1. The polybasic tail region contains a consensus sequence which could mediate interaction of the protein with PIP_2 in the plasma membrane [41–43]. Such an interaction could enable anchoring of STIM1 in the plasma membrane and thus facilitate its association with the channels and activation of SOCE. However, the suggested role of PIP_2 binding to and anchoring STIM1 puncta in ER/PM junctional regions has been contradicted [44]. 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 [45–47]. This concept has also been questioned [48]. The interesting role of lipid rafts in SOCE will not be discussed in this chapter although this has been recently reviewed [49, 50].

STIM1/Orai1 and CRAC channels

Studies in several cell types reveal that expression of Orai1 alone reduces endogenous SOCE while expression of STIM1 alone does not change SOCE. Co-expression of Orai1 with STIM1 induces a large increase in SOCE and generates substantial I_{CRAC} [51]. Furthermore, mutations in conserved negatively charged residues in the TM region of Orai1 alter the Ca^{2+} selectivity of the CRAC channel [34, 51, 52] while expression of the Orai1 mutant associated with SCID does not lead to generation of I_{CRAC} 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. Expression of Orai2 or Orai3 with STIM1 in HEK293 cells shows that both homologs are capable of constituting store-operated channels with different magnitudes and channel characteristics, leading to the speculation that native CRAC channels may involve combinations of Orai proteins [51]. Although expression of either Orai1 homologue lead to partial recovery of I_{CRAC} in cells from SCID patients, their exact roles in SOCE have not yet been conclusively demonstrated in any cell type.

Since Orai1 and STIM1 appear to interact within very specific regions of the cells, the recruitment of these proteins to these locations has created much interest and speculation. The currently available data have been obtained from studies with Orai1 and STIM1 tagged with fluorescent proteins. It is important to note that localization of native STIM1 and Orai1 in resting and stimulated cells has not yet been described and that introduction of a fluorescent protein tag at the N-terminus of STIM1 prevents its plasma membrane insertion. However, the currently reported data using TIRF microscopy demonstrate that STIM1 is translocated into the ER/PM junctional domains in response to store depletion. On the other hand, Orai1 is located primarily in the plasma membrane and displays a diffused pattern under resting conditions. Upon ER Ca^{2+} store depletion, Orai1 rapidly clusters and co-localizes with STIM1 puncta within ER-PM microdomains [53, 54]. Two hydrophobic amino acid residues located in the C-terminal coiled-coil motif of Orai1, L273 and L276, have identified to be essential in Orai1-STIM1 interaction. Physical association between Orai1 and STIM1 during activation of SOCE has been further substantiated by FRET measurements [51, 55]. These data also suggest that both STIM1 and Orai1 undergo conformational changes that are associated with CRAC channel activation. Several independent studies have identified a critical Orai1-interaction domain in STIM1 [40, 56–58] that is involved in gating Orai1. Furthermore, another domain (CMD, the CRAC Modulatory Domain) within the C-terminus of STIM1 [59] 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). Eliminating CaM binding abrogated Orai1 inactivation [59] 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. Interestingly, the inhibitory effect of 2-APB on SOCE has now been ascribed to disruption of STIM1 puncta.

TRPC1/Orai1/STIM1 and SOC channels

STIM1 also interacts with and modulates the activity of TRPC1 and other members of the TRPC channel family [60–62]. Knockdown of STIM1 in several cell types reduces endogenous TRPC1 function (Ca^{2+} entry and cation currents) stimulated by store depletion. Furthermore, co-expression of TRPC1 and STIM1 increases SOCE. STIM1 immunoprecipitates with TRPC1 and this association increases upon stimulation of cells either with an agonist or thapsigargin [61–65]. As in the case of Orai1, the D76A mutant of STIM1 also induces constitutive activation of TRPC1. Two regions of STIM1 are suggested to interact with TRPC1: the ERM (ezrin/radixin/moesin) domain [61] which is involved in the interaction with TRPC1, and the STIM1 polybasic tail ($^{684}KK^{685}$) residues which interacts electrostatically with the negatively charged residues in the C-terminus of TRPC1 ($^{639}DD^{640}$), resulting in gating of the channel [65]. Thus, STIM1 has distinct gating mechanisms for TRPC1 and Orai1.

A very important and intriguing aspect of store-dependent regulation of TRPC1 is the requirement of functional Orai1. Knocking down endogenous Orai1 or transfection of cells with functionally defective Orai1 mutants (R91W, E106Q) attenuate the increase in SOCE induced by TRPC1-STIM1 overexpression [60, 66]. Similar experimental maneuvers abrogate endogenous SOCE in HSG cells, which is significantly dependent on endogenous TRPC1 [62]. Physical proximity and association of Orai1 and TRPC1 has been suggested in studies demonstrating the dynamic assembly of a TRPC1-STIM1-Orai1 complex in response to store depletion [60–62, 65–67]. Together, these data highlight the critical contributions of STIM1, Orai1, and TRPC1 to SOCE. While the role of STIM1 in regulation of TRPC1 has been now resolved, the exact functional interaction between TRPC1 and Orai1 is not yet known. A study in human platelets cells suggested that Orai1 mediates the communication between STIM1 and TRPC1, since in the absence of Orai1, TRPC1 functions as a store-independent channel [67]. Another study suggests that Orai1 acts as a regulatory subunit of TRPC channels based on the findings that Orai1 physically interacts with the N- and C- termini of TRPC3 and TRPC6 to transform store-insensitive channels into store-operated channels [68]. Further studies will be required to elucidate the exact contribution of Orai1 to TRPC1-SOC function.

Interesting findings also suggest that TRPC1 might be required for CRAC channel function. Low levels of expression of either Orai1 or TRPC1 with STIM1 fail to sustain SOCE or I_{CRAC} , whereas when the three proteins are expressed together at the same low levels, a normal SOCE response was obtained [66]. These findings are in contrast to previous data that knockdown of TRPC1, TRPC3 or expression of STIM1 mutants that fail to gate TRPC channels do not affect I_{CRAC} and that Orai1+STIM1-CRAC channels assemble functionally without contribution from TRPC channels in cells such as T lymphocytes and mast cells. Furthermore, loss of SOCE in cells following knockdown of TRPC channels or in cells isolated from TRPC-knockout mice demonstrate lack of compensation of function by the residual Orai and STIM1 proteins [19]. Thus, although TRPC1 and Orai1 could be mutually dependent, they distinctly contribute to SOCE and regulation of specific cellular functions. It is also possible that native SOCE components and signaling could vary in different cell types depending on the specific physiological functions that are regulated by SOCE.

TRPC-generated SOC channels - Problems and Perspectives

The currently available data support the conclusion that Orai1 and STIM1 are sufficient for the generation of CRAC channel activity following store depletion. Although the presence and function of CRAC channels has yet to be confirmed in all cell types, it has been established as the primary SOCE pathway in T lymphocytes, mast cells, and several other cell types [51, 55, 69]. The domains of interactions between STIM1 and Orai1 that are involved in key aspects of channel assembly and activation have also been revealed. On the other hand, the mechanism involved in TRPC-SOC channels has yet to be completely resolved. In this section we will primarily discuss the role of TRPC1 in SOCE as more detailed studies have been done with this channel. Scepticism regarding the relevance of TRPC1 continues despite increasing number of studies which demonstrate that TRPC1 is involved in SOCE, is regulated by STIM1, and triggers SOCE-dependent Ca^{2+} signaling mechanisms in cells, including activation of cell proliferation as well as NFAT and NF- κ B signaling pathways. The role of TRPC1 in SOCE has been questioned because TRPC1 $^{-/-}$ mice do not display loss of function in all cell types. 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 affect all the tissues. Further, expression of Orai1 alone, in the absence of STIM1 does not result in an increase in SOCE. Thus, it is important that studies with TRPC1 be revisited keeping in mind the recently revealed regulation of the channel by STIM1. Major

conundrum also arises from the findings that activation of TRPC1 channel by store-depletion also requires functional Orai1. Given the lack of conclusive data elucidating a possible mechanism for this, major debate has ensued over how TRPC1 and Orai1 can simultaneously contribute to SOCE as well as the suggestion that TRPC1 and Orai1 can contribute to the same channel.

Experimental observations supporting the role for TRPC1 in SOCE and requirement of Orai1 and STIM1 for TRPC1-SOC channel function can be summarized as follows:

1. $[Ca^{2+}]$ measurements in cells stimulated by agonists, thapsigargin, BHQ, and TPEN have demonstrated activation of Ca^{2+} entry that is blocked by 1–5 μM Gd^{3+} and 10–20 μM 2APB, hallmarks of SOCE and CRAC channels.
2. Whole cell patch clamp under conditions used for CRAC channel measurements [6] such as inclusion of IP_3 in the pipette solution or stimulation of cells with Tg or agonist leads to generation of cation currents ranging from relatively selective (Ca:Na of 40:1 [10, 69]) to non-selective [70]. These currents are also completely blocked by low $[Gd^{3+}]$ and 2APB.
3. Knockdown of TRPC1 reduces SOCE and I_{SOC} .
4. Activation of TRPC1-SOCE regulates NF- κ B [71] and NFAT [72, 73].
5. STIM1 knockdown reduces endogenous TRPC1-dependent SOCE.
6. Overexpression of STIM1+TRPC1 in HSG cells and HEK293 cells leads to larger increase in SOCE than that seen with expression of TRPC1 alone. The Ca^{2+} entry is blocked completely by low $[Gd^{3+}]$ and 2APB.
7. C-terminal residues of STIM1(⁶⁸⁴KK⁶⁸⁵) activate TRPC1 via electrostatic interaction. Expression of STIM1(⁶⁸⁴EE⁶⁸⁵) mutant exerts dominant negative effect on SOCE, although it can gate Orai1.
8. Activation of endogenous and overexpressed TRPC1 requires functional Orai1, knockdown of endogenous Orai1 or expression of functionally-defective mutants leads to attenuation of TRPC1-SOCE, both endogenous and that induced by expression of STIM1+TRPC1 in HSG and HEK293 cells [60].
9. Co-immunoprecipitation and TIRF experiments show that TRPC1/Orai1/STIM1 associate following store depletion.

A major problem facing the field currently is that of reconciling the data demonstrating that two distinct channels contribute to SOCE in some cells with the concept that SOCE is mediated via a single ubiquitously present channel, namely CRAC, in all cell types. Although further studies will be required to completely resolve the exact molecular mechanism that is involved in regulation of TRPC/Orai1/STIM1-channels, we will discuss currently proposed mechanisms:

- ***Orai1 and TRPC1 generate distinct CRAC and SOC channels.*** In this case, the larger TRPC channel activity could mask an underlying I_{CRAC} activity. However, residual I_{CRAC} has not been reported following reduction of I_{SOC} induced by knockdown of TRPC1. Assuming (as predicted by most of the currently available data) that CRAC channels do not need TRPC proteins, detection of I_{CRAC} would depend on how effectively TRPC expression has been knocked down and provided no other TRPC or cation channels are activated under these conditions.
- ***TRPC1 and Orai1 interact to form one channel:*** This model suggests that both channels contribute to the same channel pore. Conclusive experimental evidence supporting this suggestion is presently lacking.

- ***Orai1 is a critical modulator of TRPC1 channels.*** This model proposes that the pore of the SOCE channel is formed by TRPC1 and that Orai1 behaves like other tetraspanins [68] to regulate channel activity directly as a subunit of the channel. Although interesting there are not enough data available presently to support this hypothesis. Furthermore, the ion channel properties of Orai1 have been studied in great detail which provides strong evidence that Orai1 generates CRAC channel. It is also important to note that cells which display an endogenous I_{CRAC} signature following store depletion do not demonstrate any apparent contribution of TRPC1 to the current.
- ***Orai1 can indirectly regulate activation of TRPC channels.*** This model predicts that Orai1-CRAC channel activity initiates downstream signals that lead to activation of TRP channels. An example of this type of regulation has been recently demonstrated in the case of TRPC4 and TRPC5 which are directly activated by increase in $[Ca^{2+}]_i$ that results from activation of Orai1-CRAC channel. While these data were obtained from overexpression systems, TRPC5 activation was also seen in neuronal cells following activation of voltage dependent calcium channels and thus is not “Orai1-specific” and more importantly not “store-depletion dependent”. However, it is clear that not all TRPC channels that are activated following SOCE and show dependence on Orai1 are directly activated by Ca^{2+} (e.g. TRPC1 and TRPC3). Furthermore, TRPC1 activation in response to store depletion is determined by STIM1. Thus, other mechanisms triggered via Orai1-mediated Ca^{2+} entry such as TRPC channel recruitment, phosphorylation/dephosphorylation, or regulation by calmodulin can be proposed as possible modes for regulation of TRPCs channels. These latter would be consistent with all the reported data regarding activation of TRPC1 following store depletion. If Orai1-CRAC were the primary channel leading to activation of TRPC1, then TRPC1 would contribute to the total Ca^{2+} entry activated by store depletion. Further, TRPC1 function would be blocked under the conditions that block Orai1 function. Another possibility that cannot be presently ruled out is that direct interaction between the two channels is involved in regulation of TRPC1. Thus, while the role for Orai1 in TRPC1-SOCE could be an indirect one, STIM1 gates TRPC1 following store depletion. Based on this, it is accurate to describe TRPC1 as a “store-dependent” channel. Further studies will be required to establish which of these possible modes of interaction between Orai1 and TRPC1 prevails in cells.

Conclusions

After more than two decades of intense efforts to determine the mechanisms and molecular components of SOCE, recent studies have lead to identification of novel channels and regulatory proteins for this Ca^{2+} entry pathway. More importantly, the critical physiological role of SOCE and the impact of its dysfunction of this mechanism in disease is also rapidly emerging. Exciting new studies demonstrate that SOCE is associated with highly specialized microdomains in the cells where local Ca^{2+} signaling events lead to significant regulation of cell function. There is considerable agreement that SOCE is required for a number of critical cell functions and is not restricted to the purpose of refilling of intracellular calcium stores. STIM1 appears to be central molecule in SOCE which could serve several functions. This is demonstrated by the findings that it has 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 be considered within the context of specific cell types. The complexity of the Ca^{2+} signaling mechanisms associated with SOCE is evident in T and B lymphocytes or mast cells. In these cell types, stimulation of cell surface receptors leads to

the recruitment of large and distinct signaling complexes of which Orai1/STIM1-CRAC is a key component. Multiple signaling mechanisms are co-ordinately initiated within the complex. Many of these are regulated by changes in local $[Ca^{2+}]_i$ resulting from CRAC-mediated Ca^{2+} entry. In B cells, $[Ca^{2+}]_i$ increases mediated by CRAC channels are modified by the concurrent activation of other channels that either directly provide Ca^{2+} or modulate CRAC channel activity by regulating membrane potential [74]. The resulting modulation of local and global Ca^{2+} signals 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. This will provide important clues to understand and appreciate the *raison d'être* for the complexity and diversity in SOCE.

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