

The dynamic complexity of the TRPC1 channelosome

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A rise in cytoplasmic $[Ca^{2+}]$ due to store-operated Ca^{2+} entry (SOCE) triggers a plethora of responses, both acute and long term. This leads to the important question of how this initial signal is decoded to regulate specific cellular functions. It is now clearly established that local $[Ca^{2+}]$ at the site of SOCE can vary significantly from the global $[Ca^{2+}]$ in the cytosol. The assembly of key Ca^{2+} signaling proteins within the domains generates such Ca^{2+} microdomains. For example, GPCR, IP_3 receptors, TRPC3 channels, the plasma membrane Ca^{2+} pump and the endoplasmic reticulum (ER) Ca^{2+} pump have all been found to be assembled in a complex and all of them contribute to the Ca^{2+} signal. Recent studies have revealed that two other critical components of SOCE, STIM1 and Orai1, are also recruited to these regions. Thus, the entire machinery for activation and regulation of SOCE is compartmentalized in specific cellular domains, which facilitates the specificity and rate of protein-protein interactions that are required for activation of the channels. In the case of TRPC1-SOC channels, it appears that specific lipid domains, lipid raft domains (LRDs), in the plasma membrane, as well as cholesterol-binding scaffolding proteins such as caveolin-1 (Cav-1), are involved in assembly of the TRPC channel complexes. Thus, plasma membrane proteins and lipid domains as well as ER proteins contribute to the SOCE- Ca^{2+} signaling microdomain and modulation of the Ca^{2+} signals per se. Of further interest is that modulation of Ca^{2+} signals, i.e., amplitude and/or frequency, can result in regulation of specific cellular functions. The emerging data reveal a dynamic Ca^{2+} signaling complex composed of TRPC1/Orai1/STIM1 that is physiologically consistent with the dynamic nature of the Ca^{2+} signal that is generated. This review will focus on the recent studies, which demonstrate critical aspects of the TRPC1 channelosome that are involved in the regulation of TRPC1 function and TRPC1-SOC-generated Ca^{2+} signals.

Introduction to the Role of TRPC Channels in Store-Operated Ca^{2+} Entry

Store-operated calcium entry (SOCE) was identified more than two decades ago as a Ca^{2+} entry mechanism that is activated in response to stimulation of calcium mobilizing receptors. It

was further established that SOCE is activated by depletion of the endoplasmic reticulum (ER)- Ca^{2+} stores per se and not due to events proximal to receptor signaling. Physiologically, this is achieved in response to stimulation of plasma membrane receptors that result in activation of phosphatidylinositol-4,5-bisphosphate (PIP_2)-specific phospholipase C (PLC), hydrolysis of PIP_2 into IP_3 and DAG, and IP_3 -mediated release of Ca^{2+} from the ER via binding to the IP_3R . SOCE occurs in both non-excitabile and excitable cells, and is vital for the control of many critical functions such as gene expression, proliferation, migration, fluid secretion, mast cell degranulation, platelet aggregation and T-cell activation. SOCE has been extensively studied in all cell types and conclusive evidence was presented early on that Ca^{2+} influx was mediated via a channel. However, the mechanism(s) involved in sensing ER calcium depletion and relaying the signal to the plasma membrane for activation of the plasma membrane calcium channels, as well as the molecular identity of the components of the channels involved in SOCE, remained elusive for a very long time.

The well-established role of *Drosophila* TRP channel in phototransduction,^{1,2} and its characterization as a Ca^{2+} -permeable channel that is activated in response to receptor-mediated PIP_2 hydrolysis, propelled the search for mammalian homologs of this channel. This led to the discovery of the superfamily of TRP channels that consists of three major subfamilies—the TRP canonical (TRPC), TRP vanilloid (TRPV) and TRP melastatin (TRPM) groups; and two minor subfamilies—TRPP (TRP channels linked to polycystic kidney disease) and TRP mucolipin (TRPML) groups.² The TRPC subfamily that consists of seven members (TRPC1-7) critically contribute to SOCE and agonist-dependent, store-independent calcium channels, in a number of different cell types.¹⁻⁵ The TRPC family is most closely related to the *Drosophila* TRP both in sequence homology and by their activation in response to receptor-coupled PIP_2 hydrolysis.^{1-3,6-8} However, it became evident that TRPCs did not account for SOCE in all cell types, especially in cells such as T lymphocytes and mast cells which display a highly Ca^{2+} -selective current called I_{CRAC} (Ca^{2+} -release activated Ca^{2+} current).⁹⁻¹¹ This current is inhibited by characteristic SOCE inhibitors, 2APB and low concentrations (1–5 μM) Gd^{3+} and La^{3+} .⁴ On the other hand both endogenously and heterologously expressed TRPC channels do not display I_{CRAC} -like properties. Instead they generate relatively non-selective cation channels in response to agonist stimulation.^{4,12} Furthermore, although all TRPC proteins have been proposed as candidates for channels mediating SOCE, a number

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of them are activated only in response to treatment with agonist and not thapsigargin and not all of them are blocked by 2APB or 1 μM Gd^{3+} . Some TRPC channels also appear to be directly activated by Ca^{2+} (e.g., TRPC4 and TRPC5 channels). Thus, only some TRPC channels fulfill all the criteria for SOCE. However, inconsistencies have been reported in the activation of these channels by store depletion, much more so in heterologously expressed systems. Studies examining effects of knockdown of endogenous proteins have yielded more consistent findings. Another complication in elucidating the role of TRPC channels in SOCE is the ability and propensity of different members of the TRPC subfamily to form homomeric or heteromeric channels. Such interactions can potentially result in the generation of a plethora of Ca^{2+} -permeable channels with a range of channel properties and activation mechanisms^{12,13} although there is not much information available regarding the endogenous status of TRPC channels. Despite the lack of such data, it was clearly shown that none of the TRPC channels, either individually or in combination, recapitulated the properties of I_{CRAC} . Thus they were excluded as potential CRAC channel component(s) and TRPC channels that were activated by store depletion were termed SOC (store-operated calcium) channels. Among the seven TRPC channels, TRPC1 has been most consistently demonstrated as a component of the SOC channel in a variety of cell types.^{14–24} The role of TRPC1 in SOCE was further established by data showing that fluid secretion from salivary glands is severely compromised in TRPC1^{-/-} mice which is associated with loss of SOCE in acinar cells.²⁵ Pancreatic acini from these mice also display loss of SOCE.²⁶ It has been suggested that heteromeric associations of TRPC1 with other TRPC channels can underlie the diversity in channel properties (as reviewed in refs. 3 and 27–29). Another interesting concept is that TRPC1 can serve as a functional link to couple store-independent TRPC channels to intracellular Ca^{2+} store depletion.³⁰ The latter role of TRPC1 is yet to be tested physiologically.

Based on the findings that TRPC channels do not contribute to the CRAC channel function, search for the molecular component(s) of the CRAC channel continued, as did studies to identify the ER Ca^{2+} sensor and regulatory mechanism for SOCE. Both of these components have been elucidated recently. The discovery of Orai1 as the essential component of CRAC channels and STIM1 as the ER Ca^{2+} sensor protein involved in regulating the plasma membrane channels have provided new areas for study in the field of SOCE. Of further interest is the finding that activation of TRPC channels following store depletion is dependent not only on STIM1 but also on Orai1 (discussed in detail below). Thus significant strides have been made in the field of calcium signaling that expand our understanding of the components and possible mechanisms that determine the regulation of TRPC channels.^{27,30–32} This review will highlight these advances with a special focus on the function and regulation of TRPC1.

TRPC Channel Complexes

TRPC channels have the inherent ability to interact with other TRPC proteins as well as with regulatory and scaffolding proteins.^{3,33,34} All TRPC proteins have six transmembrane domains

with a pore region that is localized in the linker region between the fifth and sixth domains. Both the N and C termini are localized within the cytosol, and all TRPC proteins also have 3–4 N-terminal ankyrin repeats, a conserved C-terminal TRP domain, coiled-coiled domains and putative PIP_2 binding domains.^{1,2,6,35,36} These domains delineate regions in the channels where protein-protein interactions can occur. Indeed, mammalian TRPC channels were shown several years ago to be assembled in a supramolecular signaling complex, which included key Ca^{2+} signaling proteins as well as other signaling and scaffolding proteins. For example, all these channels have calmodulin (CaM)-binding domains and direct interaction with CaM has been reported in almost all cases, although the exact function of these is not yet clear. In addition, the channels appear to have conserved Homer1, IP_3R and caveolin-1 (Cav-1)-binding domains.^{32,37–40} Biochemical evidence has also been provided for the association of G proteins, SERCA and PMCA as well as PLC with TRPC channels. It is interesting to note that TRPCs have the ability to interact with both plasma membrane as well ER-associated proteins (these have also been confirmed in proteomic studies for TRPC3^{3,41}). In addition, some TRPC proteins also bind lipids such as PIP_2 and possibly cholesterol. The rest of this review will primarily focus on proteins associated with TRPC1 and their role in the regulation of channel function, cellular localization and targeting to the plasma membrane.

TRPC1 is localized in a wide variety of tissues and contributes to various physiological functions, including fluid secretion, endothelial cell migration and permeability, smooth and skeletal muscle function, cell proliferation, differentiation, wound healing and protection against cell death. The characteristics of the channels formed by TRPC1 are quite diverse, ranging from relatively Ca^{2+} selective to non-selective (when comparing Ca^{2+} vs. Na^+ permeability), likely due to its assembly in a heteromeric channel with other TRPC proteins.^{3,27–29,42,43} Of these, only interaction with TRPC1, TRPC4, TRPC3 or TRPC7 has been linked with generation of SOC channels. Most of the reported interactions appear to involve the N-terminus of these channels, and more specifically the coiled-coiled or ankyrin repeat regions of the protein.^{3,36,42,44,45} Furthermore, surface expression of the TRPC1 channel has also been reported to depend on its interactions with other TRPCs (e.g., TRPC4) in some instances.⁴² More detailed studies will be required to map the exact residues involved in these TRPC1-TRPC interactions and to confirm the physiological relevance of the putative heteromeric channels in native systems.

In addition to other TRPCs, interaction of TRPC1 with proteins such as Cav-1,^{37,38} β -tubulin (microtubule structures),⁴⁶ and RhoA (remodeling of the cytoskeleton)¹⁹ also determines surface expression of the channel. TRPC1 channelosome contains scaffolding proteins, such as RACK1, HOMER, IP_3R , ezrin, caveolin, junctate, ZO1, EB-50 and NHERF that determine the localization of the channel complex in the cell. Key Ca^{2+} signaling proteins such as PLC, $\text{G}_{\alpha\text{q}/11}$, IP_3R and PMCA,^{3,38,39,47–49} and depending on the cell type, neurotransmitter and growth factor receptors such as bradykinin⁵⁰ and fibroblast growth factor receptors¹⁷ are also found to be associated with the channel.

Several studies have also reported direct interaction of TRPC1 with the IP₃R.^{19,32,51} In aggregate, proteomic and biochemical studies reveal that TRPC1 is assembled in a supramolecular signaling complex with various calcium signaling proteins that include upstream components (e.g., G protein-coupled receptors, G-proteins and PLC) and downstream components (e.g., IP₃Rs and [Ca²⁺]_i), regulatory proteins such as SERCA and PMCA).^{33,41,52}

Proteomic analysis of affinity purified TRPC1 complex has led to the identification of a number of proteins that are involved in vesicle trafficking and membrane fusion events, such as vesicle-associated SNARE proteins, dynamin, clathrin, PI3K, rac, RhoA and Cav-1. A number of cytoskeletal components and actin-interacting proteins were also detected within TRPC1 channelosome. While cytoskeletal remodeling associated with the activation of TRPC1 channelosomes has not yet been described, PIP₂ hydrolysis leads to localized cytoskeletal changes. Since the TRPC channelosome also contains proteins from the ER and plasma membrane, it has been suggested that TRPC1 is segregated into specific Ca²⁺ signaling complexes components that are associated with the ER as well as the plasma membrane. The proteins in both membranes are held together by direct protein-protein interactions or via association with a scaffolding protein. This implies that the ER and plasma membrane are in close apposition to each other at the site where this complex is localized. TRPC1 has a conserved Cav-1 binding domains in the N-terminus and two putative domains on the C-terminus.^{37,38,53,54} Based on the detergent solubility of TRPC1 and its association with Cav-1, a cholesterol-binding protein that resides in the lipid raft domains, it was proposed that TRPC1 channels are localized within these functionally distinct plasma membrane domains. An important aspect of this suggestion is that the architecture of the domain might facilitate the specificity as well as rate of protein-interactions required for channel activation. The domain can also provide a platform for the assembly and dynamic interactions within the complex.

Localization of the TRPC Channelosome in Calcium Signaling Microdomains

The process by which cells coordinate signals generated intracellularly with the activation of different calcium channels in the plasma membrane is vital to ensure the efficiency and efficacy of cellular signaling. This precise coordination is achieved by segregating proteins into complexes in distinct cellular or membrane domains. Assembly of the protein complex allows the plasma membrane calcium channel to be located in close proximity to its regulator(s) and/or effector(s). Furthermore, segregating these protein complexes into microdomains allows for the generation of compartmentalized Ca²⁺ signals which can be locally decoded for the regulation of downstream effectors.^{33,55,56} The early observations that SOCE-dependent refill of internal Ca²⁺ stores occurred with minimal diffusion of Ca²⁺ in the cytosol suggested the Ca²⁺ entering the cell is rapidly sequestered into the ER by the activity of the ER Ca²⁺ pump (SERCA).^{4,57,58} This led to the hypothesis that the ER was localized in close proximity to the plasma membrane, which could potentially contribute to the

ER-PM signaling required for the activation of SOCE. It has now been shown that local Ca²⁺ signals generated by CRAC channels can be decoded for activation of specific gene transcription.⁵⁹⁻⁶¹

Consistent with the findings that TRPC1 is assembled in microdomains several studies have provided conclusive evidence that SOCE occurs within spatially segregated Ca²⁺ signaling microdomains.^{12,34,48,60-62} Thus, it is reasonable to suggest that downstream effector and regulatory proteins might also be scaffolded in close vicinity to the channels. Proteins involved in generating the intracellular calcium signals as well as those regulating the level of [Ca²⁺]_i in the cell are tightly associated with each other and localized in a functional complex within this region, e.g., PMCA interacts with TRPC1 and has also been shown to regulate CRAC channel function. The microdomain provides the spatial and structural architecture to facilitate the specificity and optimal rate of protein-protein interactions required for the generation of active channels and for transduction of the Ca²⁺ signals for cellular regulation. Another example of such an interaction is between Homer, IP₃R and TRPC1, which leads to regulation of TRPC1 function. HOMER mediates the interaction between TRPC1 and IP₃R and disruption of this interaction is associated with TRPC1 activation by ER-Ca²⁺ depletion.³⁹ RhoA, which associates with TRPC1, is also involved in TRPC1-IP₃R interactions and regulation of SOCE.¹⁹

In addition to protein components, plasma membrane lipids also significantly contribute to the organization and function of Ca²⁺ signaling microdomains and TRPC1 channelosome. For example, PIP₂, PIP₃, cholesterol and sphingolipids are concentrated in biochemically distinct plasma membrane lipid domains called the lipid raft domains (LRDs) or caveolar LRDs, referring to LRDs that contain the cholesterol-binding protein Cav-1.^{12,53,63,64} The importance of lipid rafts in calcium signaling has been highlighted in several recent reviews.^{12,53,63} Key calcium signaling protein components (e.g., EGFR receptors, G-proteins, PMCA pumps, Homer and PKC) and related cellular events (e.g., receptor-mediated turnover of PIP₂) have been localized to caveolar LRDs.^{12,53} Importantly, SOCE has been proposed to occur within LRDs, including caveolin-containing LRDs, as disruption of these domains attenuates SOCE.¹² Studies showing that TRPC1 channel function require intact LRDs have been reported in human salivary gland cells,^{37,38,65} C2C12 skeletal myoblasts,⁶⁶ striated muscle (as recently reviewed by Sabourin et al.⁶⁷ polymorphonuclear neutrophils,⁶⁸ endothelial cells,⁶⁹ human platelets,⁷⁰ THP-1 monocytic cells⁷¹ and mouse spermatogenic cells and sperm).⁷² There are strong data to show that TRPC1 is regulated within lipid raft domains. In resting cells, TRPC1 is partitioned in both raft and non-raft fractions³⁸ while following Ca²⁺ store depletion, there is a significant increase in TRPC1 in the lipid raft fraction.⁶⁵ Furthermore, STIM1, a key activator of TRPC1, was also shown to associate with LRDs where it interacts with TRPC1 and gates the channel, consistent with demonstration that TRPC1-SOCE occurs in LRD. Disruption of LRDs attenuates STIM1 association with LRDs, as well as its interaction with TRPC1, leading to reduced SOCE.⁶⁵ It is interesting to hypothesize that following stimulation of the cells LRDs might contribute to the critical molecular reassembly and

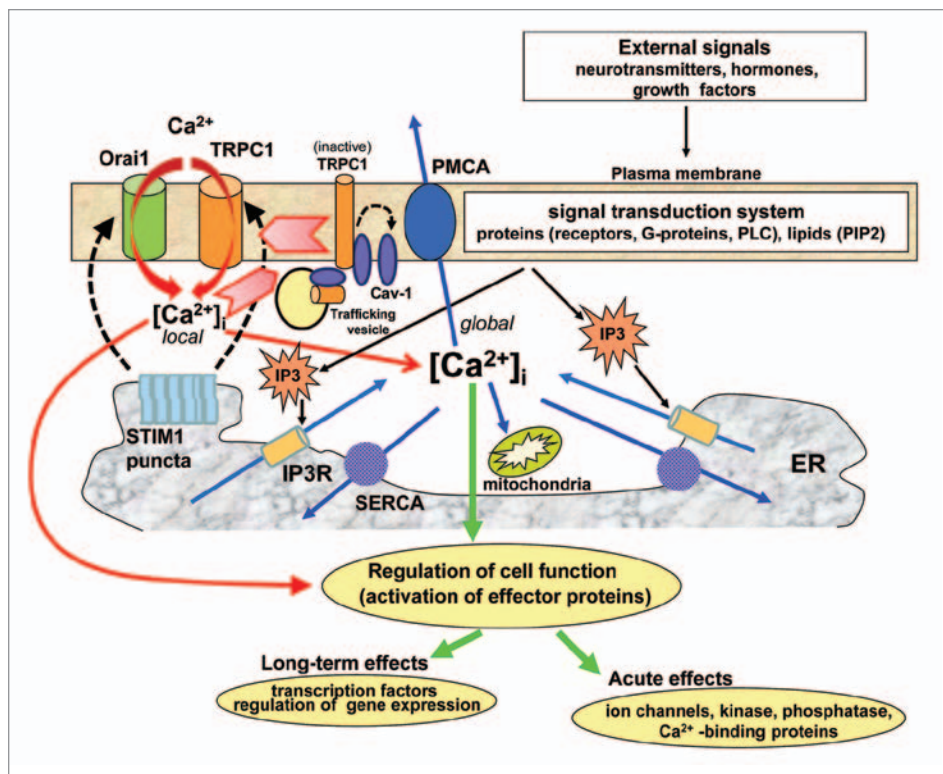


Figure 1. Model showing the various molecular components associated with the store-operated calcium entry pathway. The figure depicts the Ca^{2+} signaling microdomain related to the activation, regulation and modulation of SOCE and the resultant changes in $[\text{Ca}^{2+}]_i$ in the cytosol. Both local and global changes can result as a result of activation of SOCE and these can lead to long-term as well as acute effects on cell function. Major regulatory pathways are shown by black dashed lines while Ca^{2+} fluxes are indicated by red arrows. Large light red arrows indicate TRPC1 trafficking of TRPC1-vesicles and status of Cav-1-TRPC1 interaction in “inactive” and active TRPC1-SOC channel. Further details of each component are discussed in the text.

reorganization of TRPC1 channelosomes that are required for activation and maintenance of an activated SOC channel.

Lipid composition of the plasma membrane can also elicit effects via rearrangement of the cytoskeleton and/or regulation of vesicle trafficking. One concept that has gained much attention is that vesicle fusion or channel targeting does not randomly occur in all regions of the plasma membrane but involves precise spatio-temporal resolution. Such targeting is critical in the generation of active channels at the exact localization in the cell where they can impact regulation of specific cellular functions. It has been suggested that the lipid composition of LRD functions as a platform to coordinate events associated with cytoskeletal remodeling and PIP_2 metabolism.⁷³ Considerable data have been provided to suggest that vesicle fusion events occur within this domain based on the detection of SNARE proteins associations with LRDs.⁶³ Alternatively, lipid modification of proteins as well as their interaction with PIP_2 via PH domains can target their localization into lipid rafts. Thus, it is significant that several TRPCs, including TRPC1, are associated with LRDs and have PIP_2 -binding domains. The relatively high concentrations of PIP_2 in LRD as well the presence of proteins involved in PIP_2 metabolism, protein scaffolding and cytoskeletal remodeling, could direct the

targeting to and clustering of TRPC channels at the plasma membrane, as well as regulation of channel activity. It is reasonable to hypothesize that assembly and regulation of TRPC channels within specialized cellular domains determines the generation and precise regulation of distinct Ca^{2+} signaling microdomains. The amplitude and duration of these localized intracellular calcium signals will be determined by mechanisms generating the signal (Ca^{2+} internal release and entry channels, as well as trafficking and regulation of these channels) and those involved in decay of the signal (channel inactivation, Ca^{2+} pumping via SERCA and PMCA, buffering and diffusion) (see Fig. 1). Such local $[\text{Ca}^{2+}]_i$ changes are likely to be important in the regulation of cellular functions, both acute (activation of ion-channels, secretion, etc) and/or long-term (activation of transcription, proliferation, etc.).

New Components of TRPC1 Channelosome

As noted above, the identification of Orai1 and STIM1 about six years ago invigorated the field of calcium signaling.^{31,74} Two critical and central proteins involved in SOCE were identified within a short span of time that paved the way for exciting new studies. The first STIM1, a single transmembrane domain protein located in the ER membrane, with cytosolic C-terminus and a luminal N-terminus containing an EF-hand domain that functions as a sensor domain for $[\text{Ca}^{2+}]_{\text{ER}}$.⁷⁵⁻⁷⁸ Further it has been established that STIM1 is the primary regulator of SOCE involved in the gating of SOC and CRAC channels. Under resting conditions, STIM1 shows a diffuse pattern of localization in the ER. Following store depletion Ca^{2+} binding to the EF-hand domain is disrupted, causing STIM1 to multimerize and translocate to the ER-PM junctional regions (in the cell periphery where ER is apposed to the plasma membrane) where it aggregates (referred to as punctae). STIM1 interacts with and gates plasma membrane channels (Orai1 and TRPC1) leading to activation of SOCE within the same domain.⁷⁹⁻⁸¹ The C-terminal region of STIM1 (aa 233–685) is believed to span the region between the ER and PM and be associated either directly with lipid components in the plasma membrane or proteins which helps to anchor protein. The relatively high concentration of STIM1 that is generated at these specific ER-PM junctional domains facilitates recruitment of Orai1 and TRPC1 to these sites. The second component, Orai1, was identified by RNAi screens of

Drosophila S2 cells as well as genetic linkage analysis in severe combined immune deficiency (SCID) patients who express an Orai1 mutant (Orai1R91W) that is associated with loss of CRAC channel activity and defective calcium signaling in T lymphocytes and accounts for the immunodeficiency in the individuals. Furthermore, overexpression of Orai1 with STIM1 recapitulates almost all the native properties of the CRAC channel. Orai1 is a four transmembrane domain that has been shown by mutational analysis to form the pore of the CRAC channel and determine the high Ca^{2+} selectivity of the channel.^{31,82,83} Thus, with the discovery of STIM1 and Orai1 the elusive CRAC channel components were finally identified. Another member of the STIM family, STIM2, shares significant homology to STIM1 but appears to have different properties and functions than STIM1. The exact function of STIM2 and its interaction with either TRPC1 or Orai1 is not clear. Similarly two other Orai proteins have also been identified, Orai2 and Orai3. The exact function of these has not yet been established although it appears that their properties are not exactly alike.

A major advancement in the field of TRPC channels was the finding that STIM1 also interacts with and regulates activation of TRPC1 in response to Ca^{2+} store depletion.^{24,30,84-86} Knockdown of STIM1 prevents activation of TRPC1 while expression of constitutively active STIM1 mutants leads to activation of the channel. Further, there is an increase in TRPC1-STIM1 association upon store depletion, which is reversed following store refilling. STIM1 also reportedly associates with a number of other TRPC channels, including TRPC3 and TRPC6, and regulate the activity of these channels in response to internal Ca^{2+} store depletion. However, this study also suggested a central role for TRPC1 in the activation of these other TRPCs by STIM1. It was shown that the apparent regulation of these TRPC channels by STIM1 is due to their heteromeric interaction with TRPC1 and that STIM1 primarily interacts with and regulates TRPC1. Initially, the ezrin/radixin/moesin (ERM) domain of STIM1 was reported to be the domain involved in the interaction with TRPC1, while the lysine-rich region in the tail of STIM1 C-terminus (commonly known as the polybasic tail/motif) was proposed to mediate STIM1 aggregation at the ER-PM junctional domains^{87,88} and activate TRPC1-SOC channels.³⁰ However, subsequent studies revealed the presence of an Orai1-interacting region, SOAR, within this ERM domain.^{89,90} Expression of the SOAR induced activation of endogenous Orai1 in the absence of store depletion (note that TRPC1 is not activated by this domain). Furthermore, in resting, unstimulated cellular conditions, the Orai1-gating domain in STIM1 is occluded by intramolecular interactions within STIM1 that are modified by store depletion. This results in unmasking of this domain and subsequent gating of Orai1 channel by STIM1.⁹¹ An important study demonstrated that STIM1 gates TRPC1 via an electrostatic interaction between the positively charged amino acids in the STIM1 polybasic tail (⁶⁸⁴KK⁶⁸⁵) and the negatively charged ones in the C-terminus of TRPC1 (⁶³⁹DD⁶⁴⁰).⁸⁵ Thus, distinct domains in the STIM1 C-terminus are involved in gating Orai1 and TRPC1. Notably, the polybasic tail region of STIM1 also contains a putative consensus sequence that has been suggested to mediate its interaction with the plasma

membrane phospholipids.^{87,92} This raises the possibility that this region may serve to anchor STIM1 in the plasma membrane, thereby facilitating its subsequent association with other proteins including the Orai1 and TRPC1. Furthermore, it has been proposed that PIP_2 -rich plasma membrane domains, which include LRDs, are involved in the regulation of SOCE by STIM1 as disruption of lipid rafts alters the interactions of STIM1 with plasma membrane channels and activation of TRPC1-mediated SOCE. The latter finding is consistent with all previous studies demonstrating the localization of TRPC1, and its regulation, within LRD.

Adding to the complexity of store-dependent activation of TRPC1, several studies showed that Orai1 is also required for TRPC1 function in SOCE.^{59,93,94} Orai1 associates with TRPC1 following store depletion and this interaction is required for TRPC1 activation. In fact all three proteins, STIM1, Orai1 and TRPC1 form a complex following store depletion, which critically depends on the translocation and aggregation of STIM1 in the ER/PM junctional domains. More importantly, several studies showed that functional Orai1 channel is required for the activation of TRPC1 by STIM1, ruling out the possibility that the CRAC channel functions merely as a scaffold for TRPC1. Thus, the newly identified components of TRPC1 channelosome, Orai1 and STIM1, critically and dynamically impact channel activation. In addition, proteomic analysis of immunopurified TRPC channel complex has provided novel putative interacting partners for TRPC1 (as well as TRPC3).^{41,52} In particular several proteins are involved in membrane trafficking such as SNAPs, SNARES, GTPases, dynamin, clathrin-coated vesicle associated proteins. Some regulatory proteins such as phosphates and kinases were also identified as were cytoskeletal and microtubule components. Functional role for these proteins remains to be established.

Dynamic Reorganization of the TRPC1 Channelosome During Activation of SOCE

An important component of TRPC1 channelosome that is required for plasma membrane localization of the channel is Cav-1. This component binds to an N-terminal region of TRPC1 (aa 271–349) which when mutated disrupts the plasma membrane localization of TRPC1, resulting in a significant decrease in Ca^{2+} influx upon store depletion.^{37,69,95} Subsequent studies have revealed two putative Cav-1 binding domains in the C-terminus of TRPC1: aa 626–635, the function of which is not known^{37,38,53} and aa 781–789, which binds to the Cav-1 scaffolding domain and appears to be involved in channel activation.⁵⁴ Paradoxically, overexpression of Cav-1 leads to a reduction in SOCE.^{37,53} A recent study demonstrated that in resting cells Cav-1 sequestered the inactive channel within the PM region.⁹⁵ Following stimulation TRPC1 dissociates from Cav-1 and binds to STIM1, which also results in activation of TRPC1-SOC channel. Thus, it has been suggested that Cav-1 determines the tonic activity of TRPC1 and can compete against endogenous STIM1. Indeed, inhibition of SOCE induced by overexpression of Ca_v1 was reversed by simultaneously expressing higher levels of STIM1 (relative to that of Cav-1).^{53,95} Even more

interesting is the apparent shift in state of TRPC1 clustering in ER-PM junctional domains. Prior to activation, the channel is clustered with Cav-1 is dissociated from the channel whereas after stimulation it aggregates with STIM1. Whether Orai1 impacts this reorganization is not very well established. However, these data provide evidence for a critical rearrangement of the TRPC1 channelosome that is essential for channel activation.

As discussed above, Orai1 is a critical component of the active TRPC1-channelosome. Association of TRPC1 not only with STIM1 but also Orai1 determines activation of TRPC1 following depletion of ER-Ca²⁺ stores, although the latter association is likely not to be a direct protein-protein interaction. In cells such as salivary gland and pancreatic acini as well as other cell types, Orai1, TRPC1 and STIM1 contribute to SOCE.^{47,84,96} In these cells, TRPC1 generates channels with characteristics of I_{SOC} while endogenous I_{CRAC} is not detected under normal conditions of store depletion. These studies raise the question as to whether Orai1 and TRPC1 form a heteromeric channel or whether the two proteins constitute two distinct channels that function within a single Ca²⁺ signaling microdomain. This critical question was addressed in a recent study which demonstrated that TRPC1 and Orai1 are components of distinct STIM1-gated channels.⁵⁹ In cells that typically express SOC channel activity upon activation, expression of a STIM1 mutant that can gate Orai1 but not TRPC1 results in generation of I_{CRAC} in cells typically displaying I_{SOC}. This suggested that these cells have an underlying CRAC channel activity that is masked by the suppression of the function of endogenous TRPC1. Furthermore, expression of SOAR in these cells resulted in CRAC channel activation in the absence of store depletion and this function did not involve TRPC1. Finally this study revealed that Orai1-mediated Ca²⁺ influx triggers recruitment of TRPC1 into the plasma membrane where it is gated by STIM1. Based on this finding, it will be important to assess the involvement of the vesicle fusion proteins identified by the proteomic analysis of TRPC1 channelosome in the trafficking of TRPC1 during SOCE. In aggregate, this study established that the Orai1/STIM1-CRAC channel is likely to be the first channel to be activated following ER-Ca²⁺ store depletion and that extracellular Ca²⁺ entry via Orai1 triggers insertion of TRPC1 channel within the same plasma membrane domain where it is gated by STIM1.⁵⁹ As discussed above, this is accompanied by a reorganization of the TRPC1/Cav-1 complex to result in the TRPC1/STIM1/Orai1 signaling complex. Importantly, while Ca²⁺ entry via Orai1 is sufficient for regulation of some cellular functions, e.g. NFAT activation, the additional Ca²⁺ entry mediated by TRPC1 is required for activation of K_{Ca} channels. The complex dissociates once ER-Ca²⁺ stores are refilled. Under this condition, STIM1 leaves the ER-PM junctional domains and relocates in the ER and Orai1 is also predicted to move out of the puncta regions.

While Cav-1 binding on the N-terminus of TRPC1 plays a crucial role in determining the plasma membrane localization and retention of TRPC1, function of the putative Cav-1 binding domains on the C-terminus has not yet been established. It is interesting to note that the first C-terminal Cav-1 binding domain overlaps with the site of STIM1 gating, which lies in

between the two putative Cav-1 binding domains. Based on the finding that when Cav-1 is dissociated from the channel STIM1 binds to and gates TRPC1 following store depletion, it is interesting to speculate that this domain might have a role in the regulation of TRPC1 by STIM1. In addition to STIM1 and Cav-1, the C-terminus of TRPC1 also contains the binding site for Homer 1 (aa 644–650), which lies just downstream of the STIM1 gating site. Homer 1 functions as a regulator of TRPC1 channel activity, as disruption of the TRPC1-Homer 1 association results in spontaneous channel activity.³⁹ Homer 1 also mediates the association of TRPC1 with IP₃R to form a TRPC1/Homer/IP₃R complex, and suppresses the activity of both TRPC1 as well as IP₃R. It has been suggested that the association of Homer 1 with TRPC1 prevents STIM1 access to the C-terminal gating site on TRPC1.³² Whether and how Cav-1, Homer and STIM1 coordinately regulate the same TRPC1-SOC channel remains to be determined. Alternately, Cav-1 and Homer regulation of the channel could depend on the cell type or particular cellular localization; e.g. Cav-1 is not uniformly distributed in polarized epithelial cells.

There is an increasing amount of data showing the requirement of intact LRDs for the formation of TRPC1 channelosomes, several recent studies have suggested a link between Orai1 function with lipid rafts and possibly Cav-1. It has been shown that phospholipids in the plasma membrane significantly influences Orai1-mediated Ca²⁺ entry or STIM1 translocation.^{97,98} Also Orai1 has been shown to actively recycle between the endosomal compartment and the plasma membrane during resting physiological conditions. Following depletion of the ER-Ca²⁺ stores, Orai1 is actively trafficked to the plasma membrane from the endosomal compartments, which results in increased surface expression of Orai1. Cell division also alters Orai1 trafficking. During meiosis, Orai1 is internalized through an endocytic pathway mediated and regulated by Cav-1. A Cav-1-binding site has been mapped to the N-terminus of Orai1, although both the cytoplasmic N- and C-termini are required for its internalization. More intriguingly, STIM1 puncta can form intracellularly and interact with Orai1 in the endosomes.⁹⁹ Lutz Birnbaumer and co-workers had previously proposed that the recruitment of Orai1:TRPC:STIM1 complexes into the LRDs determines their function as SOC channels, as these same complexes would function as receptor-operated channels when localized outside lipid rafts.¹⁰⁰ With the identification of Cav-1 binding domains on both TRPC1 and Orai1 channels, as well as the implications of LRDs in the trafficking of both channels, the key step to understanding their channel functions would be to elucidate how and what protein-protein association(s) determine the assembly of a dynamic active complex comprising of Orai1, TRPC1 and STIM1 (including possibly STIM2) in the ER-PM junctions and subsequent dissociation of this complex once the stores are refilled. Thus, the data available until now demonstrate that SOCE is accompanied by a specific, spatiotemporally-determined reorganization of the channels as well as the regulator(s) of this process. This complex can be remodeled depending on the functional exigency of the cells as well as the physiological function of different cell types. Future studies should focus on establishing the physiological relevance and localization of this mechanism in various cell and tissue types.

Concluding Remarks

In conclusion, the TRPC1 channel is localized with other components of SOCE (e.g., STIM1, Orai1, Cav-1, etc.) within the calcium signaling microdomains. Generation of the TRPC1 channelosome is dynamic as rearrangement occurs prior to and after activation. Prior to activation by STIM1, TRPC1 (or TRPC1-containing vesicles) likely recycle within the plasma membrane domain, scaffolding to Cav-1 in the plasma membrane for short periods of time. In response to stimulation the status of the channel changes to a stable STIM1-TRPC1 active channel that is closely associated with a STIM1-Orai1-CRAC channel. Thus, recruitment of TRPC1 into the Ca²⁺ signaling microdomain also leads to dynamic modulation of the spatial patterning of local Ca²⁺ signals generated by Orai1. Activity of TRPC1 can modify the duration and/or amplitude of local [Ca²⁺]_i which results in the regulation of specific downstream functions. Both acute and long term remodeling of the TRPC1 channel complexes have been observed. For example, increases in TRPC1

expression have been associated with anti-apoptotic events, cell proliferation, exposure to cytokines such as TNF α , and during muscular hypertrophy. While similar changes in Orai1 or STIM1 expression under specific conditions have not yet been reported, it is reasonable to propose that the TRPC1 channel together with STIM1 and Orai1 can control and determine the plasticity of cellular calcium homeostasis by modulating, either acutely or long-term, local intracellular calcium signals. Furthermore, the SOCE-specific Ca²⁺ microdomains provide a compartmentalized but dynamic platform for the regulation of specific Ca²⁺-dependent cellular functions. The interaction of TRPC1 with various proteins in the plasma membrane and those involved in mediating events upstream and downstream of the signaling cascade facilitates the generation and determines the regulation of the TRPC1 channelosome. Future studies directed towards resolving the spatio-temporal assembly of the TRPC1 channel complex will provide significant insights into the dynamic complexity and plasticity of such complexes in generating, maintaining and regulating intracellular calcium signaling.

References

1. Minke H, Cook B. TRP channel proteins and signal transduction. *Physiol Rev* 2002; 82:429-72.
2. Montell C. The TRP superfamily of cation channels. *Sci STKE* 2005; 272:3.
3. Ambudkar IS, Ong HL. Organization and function of TRPC channelosomes. *Pflugers Arch* 2007; 455:187-200.
4. Parekh AB, Putney JW Jr. Store-operated calcium channels. *Physiol Rev* 2005; 85:757-810.
5. Venkatachalam K, van Rossum DB, Patterson RL, Ma HT, Gill DL. The cellular and molecular basis of store-operated calcium entry. *Nat Cell Biol* 2002; 4:263-72.
6. Montell C. Physiology, phylogeny and functions of the TRP superfamily of cation channels. *Sci STKE* 2001; 2001:re1.
7. Montell C, Birnbaumer L, Flockerzi V. The TRP channels, a remarkably functional family. *Cell* 2002; 108:595-8.
8. Venkatachalam K, Montell C. TRP channels. *Annu Rev Biochem* 2007; 76:387-417.
9. Penner R, Matthews G, Neher E. Regulation of calcium influx by second messengers in rat mast cells. *Nature* 1988; 334:499-504.
10. Matthews G, Neher E, Penner R. Second messenger-activated calcium influx in rat peritoneal mast cells. *J Physiol* 1989; 418:105-30.
11. Lewis RS, Cahalan MD. Mitogen-induced oscillations of cytosolic Ca²⁺ and transmembrane Ca²⁺ current in human leukemic T cells. *Cell Regul* 1989; 1:99-112.
12. Ambudkar IS, Ong HL, Singh BB. Molecular and functional determinants of Ca²⁺ signaling microdomains. In: Sitaramayya A, Ed. *Signal Transduction: Pathways, Mechanisms and Diseases*. New York: Springer 2010; 237-53.
13. Latorre R, Zaelzer C, Brauchi S. Structure-functional intimacies of transient receptor potential channels. *Q Rev Biophys* 2009; 42:201-46.
14. Beech DJ. Emerging functions of 10 types of TRP cationic channel in vascular smooth muscle. *Clin Exp Pharmacol Physiol* 2005; 32:597-603.
15. Cai S, Faterazi S, Presland RB, Belton CM, Roberts FA, Goodwin PC, et al. Evidence that TRPC1 contributes to calcium-induced differentiation of human keratinocytes. *Pflugers Arch* 2006; 452:43-52.
16. Dietrich A, Chubakov V, Kalwa H, Rost BR, Gudermann T. Cation channels of the transient receptor potential superfamily: their role in physiological and pathophysiological processes of smooth muscle cells. *Pharmacol Ther* 2006; 112:744-60.
17. Fiorio Pla A, Maric D, Brazer SC, Giacobini P, Liu X, Chang YH, et al. Canonical transient receptor potential 1 plays a role in basic fibroblast growth factor (bFGF)/FGF receptor-1-induced Ca²⁺ entry and embryonic rat neural stem cell proliferation. *J Neurosci* 2005; 25:2687-701.
18. Liu X, Wang W, Singh BB, Lockwich T, Jadlovec J, O'Connell B, et al. Trp1, a candidate protein for the store-operated Ca²⁺ influx mechanism in salivary gland cells. *J Biol Chem* 2000; 275:3403-11.
19. Mehta D, Ahmed GU, Paria BC, Holinstat M, Voyno-Yasenetskaya T, Tirupathi C, et al. RhoA interaction with inositol-1,4,5-triphosphate receptor and transient receptor potential channel-1 regulates Ca²⁺ entry. *J Biol Chem* 2003; 278:33492-500.
20. Mori Y, Wakamori M, Miyakawa T, Hermosura M, Hara Y, Nishida M, et al. Transient receptor potential 1 regulates capacitative Ca²⁺ entry and Ca²⁺ release from endoplasmic reticulum in B lymphocytes. *J Exp Med* 2002; 195:673-81.
21. Rao JN, Platoshyn O, Golovina VA, Liu L, Zou T, Marasa BS, et al. TRPC1 functions as a store-operated Ca²⁺ channel in intestinal epithelial cells and regulates early mucosal restitution after wounding. *Am J Physiol Gastrointest Liver Physiol* 2006; 290:782-92.
22. Tirupathi C, Ahmed GU, Vogel SM, Malik AB. Ca²⁺ signaling, TRP channels and endothelial permeability. *Microcirculation* 2006; 13:693-708.
23. Vandebrouck C, Martin D, Colson-Van Schoor M, Debaix H, Gailly P. Involvement of TRPC in the abnormal calcium influx observed in dystrophic (*mdx*) mouse skeletal muscle fibers. *J Cell Biol* 2002; 158:1089-96.
24. Yuan JP, Zeng W, Huang GN, Worley PF, Muallem S. STIM1 heteromultimerizes TRPC channels to determine their function as store-operated channels. *Nat Cell Biol* 2007; 9:636-45.
25. Liu X, Cheng KT, Bandyopadhyay BC, Pani B, Dietrich A, Paria BC, et al. Attenuation of store-operated Ca²⁺ current impairs salivary gland fluid secretion in TRPC1(-/-) mice. *Proc Natl Acad Sci USA* 2007; 104:17542-7.
26. Hong JH, Li Q, Kim MS, Shin DM, Feske S, Birnbaumer L, et al. Polarized but differential localization and recruitment of STIM1, Orai1 and TRPC channels in secretory cells. *Traffic* 2011; 12:232-45.
27. Ambudkar IS. TRPC1: a core component of store-operated calcium channels. *Biochem Soc Trans* 2007; 35:96-100.
28. Ambudkar IS, Ong HL, Liu X, Bandyopadhyay B, Cheng KT. TRPC1: the link between functionally distinct store-operated calcium channels. *Cell Calcium* 2007; 42:213-23.
29. Zagranichnaya TK, Wu X, Villereal ML. Endogenous TRPC1, TRPC3 and TRPC7 proteins combine to form native store-operated channels in HEK-293 cells. *J Biol Chem* 2005; 280:29559-69.
30. Huang GN, Zeng W, Kim JY, Yuan JP, Han L, Muallem S, Worley PF. STIM1 carboxyl-terminus activates native SOC, I(crac) and TRPC1 channels. *Nat Cell Biol* 2006; 8:1003-10.
31. Hogan PG, Lewis RS, Rao A. Molecular basis of calcium signaling in lymphocytes: STIM and ORAI. *Annu Rev Immunol* 2010; 28:491-533.
32. Yuan JP, Lee KP, Hong JH, Muallem S. The closing and opening of TRPC channels by Homer1 and STIM1. *Acta physiologica* 2011.
33. Ambudkar IS. Ca²⁺ signaling microdomains:platforms for the assembly and regulation of TRPC channels. *Trends Pharmacol Sci* 2006; 27:25-32.
34. Kiselyov K, Wang X, Shin DM, Zang W, Muallem S. Calcium signaling complexes in microdomains of polarized secretory cells. *Cell Calcium* 2006; 40:451-9.
35. Pedersen SF, Owsianik G, Nilius B. TRP channels: an overview. *Cell Calcium* 2005; 38:233-52.
36. Schaefer M. Homo- and heteromeric assembly of TRP channel subunits. *Pflugers Arch* 2005; 451:35-42.
37. Brazer SC, Singh BB, Liu X, Swaim W, Ambudkar IS. Caveolin-1 contributes to assembly of store-operated Ca²⁺ influx channels by regulating plasma membrane localization of TRPC1. *J Biol Chem* 2003; 278:27208-15.
38. Lockwich TR, Liu X, Singh BB, Jadlovec J, Weiland S, Ambudkar IS. Assembly of Trp1 in a signaling complex associated with caveolin-scaffolding lipid raft domains. *J Biol Chem* 2000; 275:11934-42.
39. Yuan JP, Kiselyov K, Shin DM, Chen J, Shcheynikov N, Kang SH, et al. Homer binds TRPC family channels and is required for gating of TRPC1 by IP₃ receptors. *Cell* 2003; 114:777-89.
40. Zhu MX, Tang J. TRPC channel interactions with calmodulin and IP₃ receptors. *Novartis Found Symp* 2004; 258:44-58.
41. Lockwich T, Pant J, Makusky A, Jankowska-Stephens E, Kowalak JA, Markey SP, Ambudkar IS. Analysis of TRPC3-interacting proteins by tandem mass spectrometry. *J Proteome Res* 2008; 7:979-89.

42. Hofmann T, Schaefer M, Schultz G, Gudermann T. Subunit composition of mammalian transient receptor potential channels in living cells. *Proc Natl Acad Sci USA* 2002; 99:7461-6.
43. Liu X, Bandyopadhyay BC, Singh BB, Groschner K, Ambudkar IS. Molecular analysis of a store-operated and 2-acetyl-sn-glycerol-sensitive non-selective cation channel. Heteromeric assembly of TRPC1-TRPC3. *J Biol Chem* 2005; 280:21600-6.
44. Lepage PK, Lussier MP, Barajas-Martinez H, Bousquet SM, Blanchard AP, Francoeur N, et al. Identification of two domains involved in the assembly of transient receptor potential canonical channels. *J Biol Chem* 2006; 281:30356-64.
45. Villereal ML. Mechanism and functional significance of TRPC channel multimerization. *Semin Cell Dev Biol* 2006; 17:618-29.
46. Bollimuntha S, Cornatzer E, Singh BB. Plasma membrane localization and function of TRPC1 is dependent on its interaction with beta-tubulin in retinal epithelium cells. *Vis Neurosci* 2005; 22:163-70.
47. Lee KP, Yuan JP, Hong JH, So I, Worley PF, Muallem S. An endoplasmic reticulum/plasma membrane junction: STIM1/Orai1/TRPCs. *FEBS Lett* 2010; 584:2022-7.
48. Ambudkar IS, Bandyopadhyay BC, Liu X, Lockwich TP, Paria B, Ong HL. Functional organization of TRPC-Ca²⁺ channels and regulation of calcium microdomains. *Cell Calcium* 2006; 40:495-504.
49. Lockwich T, Singh BB, Liu X, Ambudkar IS. Stabilization of cortical actin induces internalization of transient receptor potential 3 (Trp3)-associated caveolar Ca²⁺ signaling complex and loss of Ca²⁺ influx without disruption of Trp3-inositol trisphosphate receptor association. *J Biol Chem* 2001; 276:42401-8.
50. Delmas P. Assembly and gating of TRPC channels in signalling microdomains. *Novartis Found Symp* 2004; 258:75-89.
51. Tang J, Lin Y, Zhang Z, Tikunova S, Birnbaumer L, Zhu MX. Identification of common binding sites for calmodulin and IP₃ receptors on the carboxyl-termini of TRP channels. *J Biol Chem* 2001; 276:21303-10.
52. Lockwich T, Makusky A, Kowalak JA, Markey SP, Ambudkar IS. Proteomic analysis of TRP channels. In: Zhu MX, Ed. *TRP Channels*. CRC Press 2011; 113-34.
53. Pani B, Singh BB. Lipid rafts/caveolae as microdomains of calcium signaling. *Cell Calcium* 2009; 45:625-33.
54. Kwiatek AM, Minshall RD, Cool DR, Skidgel RA, Malik AB, Tiruppathi C. Caveolin-1 regulates store-operated Ca²⁺ influx by binding of its scaffolding domain to transient receptor potential channel-1 in endothelial cells. *Mol Pharmacol* 2006; 70:1174-83.
55. Berridge MJ, Bootman MD, Roderick HL. Calcium signalling: dynamics, homeostasis and remodelling. *Nat Rev Mol Cell Biol* 2003; 4:517-29.
56. Kiselyov K, Kim JY, Zeng W, Muallem S. Protein-protein interaction and function TRPC channels. *PLoS Arch* 2005; 451:116-24.
57. Liu X, Rojas E, Ambudkar IS. Regulation of KCa current by store-operated Ca²⁺ influx depends on internal Ca²⁺ release in HSG cells. *Am J Physiol* 1998; 275:571-80.
58. Mogami H, Tepikin AV, Petersen OH. Termination of cytosolic Ca²⁺ signals: Ca²⁺ reuptake into intracellular stores is regulated by the free Ca²⁺ concentration in the store lumen. *EMBO J* 1998; 17:435-42.
59. Cheng KT, Liu X, Ong HL, Swaim W, Ambudkar IS. Local Ca entry via Orai1 regulates plasma membrane recruitment of TRPC1 and controls cytosolic Ca signals required for specific cell functions. *PLoS Biol* 2011; 9:1001025.
60. Parekh AB. Ca²⁺ microdomains near plasma membrane Ca²⁺ channels: impact on cell function. *J Physiol* 2008; 586:3043-54.
61. Parekh AB. Local Ca²⁺ influx through CRAC channels activates temporally and spatially distinct cellular responses. *Acta Physiol* 2009; 195:29-35.
62. Li Q, Luo X, Muallem S. Functional mapping of Ca²⁺ signaling complexes in plasma membrane microdomains of polarized cells. *J Biol Chem* 2004; 279:27837-40.
63. Isshiki M, Anderson RG. Function of caveolae in Ca²⁺ entry and Ca²⁺-dependent signal transduction. *Traffic* 2003; 4:717-23.
64. Simons K, Toomre D. Lipid rafts and signal transduction. *Nat Rev Mol Cell Biol* 2000; 1:31-9.
65. Pani B, Ong HL, Liu X, Rausser K, Ambudkar IS, Singh BB. Lipid rafts determine clustering of STIM1 in endoplasmic reticulum-plasma membrane junctions and regulation of store-operated Ca²⁺ entry (SOCE). *J Biol Chem* 2008; 283:17333-40.
66. Formigli L, Sassoli C, Squecco R, Bini F, Martinesi M, Chellini F, et al. Regulation of transient receptor potential canonical channel 1 (TRPC1) by sphingosine 1-phosphate in C2C12 myoblasts and its relevance for a role of mechanotransduction in skeletal muscle differentiation. *J Cell Sci* 2009; 122:1322-33.
67. Sabourin J, Cognard C, Constantin B. Regulation by scaffolding proteins of canonical transient receptor potential channels in striated muscle. *J Muscle Res Cell Motil* 2009; 30:289-97.
68. Kannan KB, Barlos D, Hauser CJ. Free cholesterol alters lipid raft structure and function regulating neurotrophil Ca²⁺ entry and respiratory burst: correlations with calcium channel raft trafficking. *J Immunol* 2007; 178:5253-61.
69. Murata T, Lin MI, Stan RV, Bauer PM, Yu J, Sessa WC. Genetic evidence supporting caveolae microdomain regulation of calcium entry in endothelial cells. *J Biol Chem* 2007; 282:16631-43.
70. Brownlow SL, Sage SO. Transient receptor potential protein subunit assembly and membrane distribution in human platelets. *Thromb Haemostasis* 2005; 94:839-45.
71. Berthier A, Lemaire-Ewing S, Prunet C, Monier S, Athias A, Bessede G, et al. Involvement of a calcium-dependent dephosphorylation of BAD associated with the localization of Trpc-1 within lipid rafts in 7-keto-cholesterol-induced THP-1 cell apoptosis. *Cell Death Differ* 2004; 11:897-905.
72. Trevino CL, Serrano CJ, Beltran C, Felix R, Darszon A. Identification of mouse *trp* homologs and lipid rafts from spermatogenic cells and sperm. *FEBS Lett* 2001; 509:19-25.
73. Meiri KF. Lipid rafts and regulation of the cytoskeleton during T cell activation. *Philos T Roy Soc B* 2005; 360:1663-72.
74. Putney JW Jr. Recent breakthroughs in the molecular mechanism of capacitative calcium entry (with thoughts on how we got here). *Cell Calcium* 2007; 42:103-10.
75. Liou J, Kim ML, Heo WD, Jones JT, Myers JW, Ferrell JE Jr, Meyer T. STIM is a Ca²⁺ sensor essential for Ca²⁺-store-depletion-triggered Ca²⁺ influx. *Curr Biol* 2005; 15:1235-41.
76. Roos J, DiGregorio PJ, Yeromin AV, Ohlsen K, Lioudyno M, Zhang S, et al. STIM1, an essential and conserved component of store-operated Ca²⁺ channel function. *J Cell Biol* 2005; 169:435-45.
77. Smyth JT, Dehaven WJ, Jones BF, Mercer JC, Trebak M, Vazquez G, Putney JW Jr. Emerging perspectives in store-operated Ca²⁺ entry: Roles of Orai, Stim and TRP. *Biochim Biophys Acta* 2006; 1763:1147-60.
78. Soboloff J, Spassova MA, Tang XD, Hewavitharana T, Xu W, Gill DL. Orai1 and STIM reconstitute store-operated calcium channel function. *J Biol Chem* 2006; 281:20661-5.
79. Luik RM, Wu MM, Buchanan J, Lewis RS. The elementary unit of store-operated Ca²⁺ entry: local activation of CRAC channels by STIM1 at ER-plasma membrane junctions. *J Cell Biol* 2006; 174:815-25.
80. Ong HL, Liu X, Tsaneva-Atanasova K, Singh BB, Bandyopadhyay BC, Swaim WD, et al. Relocalization of STIM1 for activation of store-operated Ca²⁺ entry is determined by the depletion of subplasma membrane endoplasmic reticulum Ca²⁺ store. *J Biol Chem* 2007; 282:12176-85.
81. Xu P, Lu J, Li Z, Yu X, Chen L, Xu T. Aggregation of STIM1 underneath the plasma membrane induces clustering of Orai1. *Biochem Biophys Res Commun* 2006; 350:969-76.
82. Gwack Y, Srikanth S, Feske S, Cruz-Guillory F, Oh-hora M, Neems DS, et al. Biochemical and functional characterization of Orai proteins. *J Biol Chem* 2007; 282:16232-43.
83. Prakriya M, Feske S, Gwack Y, Srikanth S, Rao A, Hogan PG. Orai1 is an essential pore subunit of the CRAC channel. *Nature* 2006; 443:230-3.
84. Ong HL, Cheng KT, Liu X, Bandyopadhyay BC, Paria BC, Soboloff J, et al. Dynamic assembly of TRPC1-STIM1-Orai1 ternary complex is involved in store-operated calcium influx. Evidence for similarities in store-operated and calcium release-activated calcium channel components. *J Biol Chem* 2007; 282:9105-16.
85. Zeng W, Yuan JP, Kim MS, Choi YJ, Huang GN, Worley PF, Muallem S. STIM1 gates TRPC channels, but not Orai1, by electrostatic interaction. *Mol Cell* 2008; 32:439-48.
86. Li J, Sukumar P, Milligan CJ, Kumar B, Ma ZY, Munsch CM, et al. Interactions, functions and independence of plasma membrane STIM1 and TRPC1 in vascular smooth muscle cells. *Circ Res* 2008; 103:97-104.
87. Liou J, Fivaz M, Inoue T, Meyer T. Live-cell imaging reveals sequential oligomerization and local plasma membrane targeting of stromal interaction molecule 1 after Ca²⁺ store depletion. *Proc Natl Acad Sci USA* 2007; 104:9301-6.
88. Li Z, Lu J, Xu P, Xie X, Chen L, Xu T. Mapping the interacting domains of STIM1 and Orai1 in Ca²⁺ release-activated Ca²⁺ channel activation. *J Biol Chem* 2007; 282:29448-56.
89. Yuan JP, Zeng W, Dorwart MR, Choi YJ, Worley PF, Muallem S. SOAR and the polybasic STIM1 domains gate and regulate Orai channels. *Nat Cell Biol* 2009; 11:337-43.
90. Park CY, Hoover PJ, Mullins FM, Bachhawat P, Covington ED, Raunser S, et al. STIM1 clusters and activates CRAC channels via direct binding of a cytosolic domain to Orai1. *Cell* 2009; 136:876-90.
91. Korzeniewski MK, Manjarres IM, Varnai P, Balla T. Activation of STIM1-Orai1 involves an intramolecular switching mechanism. *Sci Signal* 2010; 3:82.
92. Heo WD, Inoue T, Park WS, Kim ML, Park BO, Wandless TJ, Meyer T. PI(3,4,5)P₃ and PI(4,5)P₂ lipids target proteins with polybasic clusters to the plasma membrane. *Science* 2006; 314:1458-61.
93. Kim MS, Zeng W, Yuan JP, Shin DM, Worley PF, Muallem S. Native store-operated Ca²⁺ influx requires the channel function of Orai1 and TRPC1. *J Biol Chem* 2009; 284:9733-41.
94. Ong HL, Cheng KT, Liu X, Ambudkar IS. Recent progress in the regulation of TRPC1 by store depletion. *Cell Membranes and Free Radical Research* 2010; 2:79-86.
95. Pani B, Ong HL, Brazer SC, Liu X, Rausser K, Singh BB, Ambudkar IS. Activation of TRPC1 by STIM1 in ER-PM microdomains involves release of the channel from its scaffold caveolin-1. *Proc Natl Acad Sci USA* 2009; 106:20087-92.
96. Cheng KT, Liu X, Ong HL, Ambudkar IS. Functional requirement for Orai1 in store-operated TRPC1-STIM1 channels. *J Biol Chem* 2008; 283:12935-40.
97. Walsh CM, Chvanov M, Haynes LP, Petersen OH, Tepikin AV, Burgoyne RD. Role of phosphoinositides in STIM1 dynamics and store-operated calcium entry. *Biochem J* 2010; 425:159-68.
98. Korzeniewski MK, Popovic MA, Szentpetery Z, Varnai P, Stojilkovic SS, Balla T. Dependence of STIM1/Orai1-mediated calcium entry on plasma membrane phosphoinositides. *J Biol Chem* 2009; 284:21027-35.
99. Yu F, Sun L, Machaca K. Constitutive recycling of the store-operated Ca²⁺ channel Orai1 and its internalization during meiosis. *J Cell Biol* 2010; 191:523-35.
100. Liao Y, Plummer NW, George MD, Abramowitz J, Zhu MX, Birnbaumer L. 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 USA* 2009; 106:3202-6.