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The TR $_{(i)}$P to Ca^{2+} signaling just got STIMy: an update on STIM1 activated TRPC channels

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1. ABSTRACT

Calcium is a ubiquitous signaling molecule, indispensable for cellular metabolism of organisms from unicellular life forms to higher eukaryotes. The biological function of most eukaryotic cells is uniquely regulated by changes in cytosolic calcium, which is largely achieved by the universal phenomenon of store-operated calcium entry (SOCE). The canonical TRPs and Orai channels have been described as the molecular components of the store-operated calcium channels (SOCC). Importantly, the ER calcium-sensor STIM1 has been shown to initiate SOCE via gating of SOCC. Since the discovery of STIM1, as the critical regulator of SOCE, there has been a flurry of observations suggesting its obligatory role in regulating TRPC and Orai channel function. Considerable effort has been made to identify the molecular details as how STIM1 activates SOCC. In this context, findings as of yet has substantially enriched our understanding on, the modus operandi of SOCE, the distinct cellular locales that organize STIM1-SOCC complexes, and the physiological outcomes entailing STIM1-activated SOCE. In this review we discuss TRPC channels and provide an update on their functional regulation by STIM1.

Keywords

Calcium signaling; SOCE; TRPC channels; STIM1; Caveolin; Lipid raft; Gene Regulation; Proliferation

2. CALCIUM SIGNALING

Calcium (Ca^{2+}) is one of the simplest and perhaps the most versatile cellular messengers. The idea that Ca^{2+} is intimately associated with cell physiology was realized over a century ago by Sidney Ringer. In 1883 he made the landmark observation that, it was a trace amount of Ca^{2+} which made isolated rat hearts placed in distilled water to beat continuously (1–2). Since this inadvertent discovery, there has been a prolific advance in the saga of Ca^{2+} signaling. In a cellular context the onset of Ca^{2+} signaling is marked primarily by an increase in the cytosolic Ca^{2+} ($(Ca^{2+})_{cyt}$), which then engages in regulating a myriad of complex biological processes such as muscle contraction, neural transmission, hormonal, peptides, and fluid secretion, gene transcription, inflammation, cell proliferation and even cell death (3–4). In order to efficiently coordinate these physiologically diverse functions, cells strategically organize an array of PM Ca^{2+} channels, pumps, buffers, exchangers, and their regulators that spatiotemporally orchestrate $(Ca^{2+})_{cyt}$ levels in response to various signaling cues. It is thus apparent that, the deliberate positioning of purpose-specific signaling molecules at precise cellular compartments would be a judicious physiological act

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of a cell. Importantly, the specificity and accurate execution of almost all of these physiologically diverse processes depend on the controlled regulation of the multifarious Ca^{2+} signals that are initiated at precise cellular microdomains and are regulated by protein-protein interactions (5–6). Thus, compartmentalization of Ca^{2+} influx pathways to specific microdomains favors efficient communication between an assortment of receptors, channels, and their modulators. In addition, such microdomains can facilitate cellular signaling by providing an elaborately organized niche to cluster a unique set of molecular components that would otherwise be physically isolated (7–9).

Distinct signaling mechanisms are shown to be present in different cell types that trigger Ca^{2+} influx (6, 10). In most cells types, both release of Ca^{2+} from intercellular ER stores as well as Ca^{2+} influx across the PM is essential to maintain a critical control of various physiological functions (4, 10–12). The critical question, however, is how do complex biological systems translate different signaling cues to choreograph the generation and homeostasis of cellular Ca^{2+} signals in order to execute appropriate physiological responses in a controlled manner. To this end a multitude of defined molecular components of Ca^{2+} influx and release pathways have been identified and studied extensively. However, in this review we mainly focus on store-operated Ca^{2+} entry (SOCE), a unique cellular mechanism that maintains Ca^{2+} homeostasis in virtually every cell. As an important aspect of SOCE, we discuss TRPC channels and their regulation by STIM1 and other regulators. Furthermore, specific cellular microdomains involved in regulating TRPC function, STIM1 localization upon stimulation, the TRPC channel gating mechanism and the physiological outcomes of channel activation are also summarized.

3. MOLECULAR COMPONENTS OF SOCE

Most cellular processes require the release of Ca^{2+} from intracellular ER-stores followed by the influx of external Ca^{2+} to sustain many physiological responses. Ca^{2+} influx across the PM is not only essential for the refilling of internal ER-stores, but also contributes toward regulation and fine-tuning of several biological processes (5, 10, 13). This very process of activating PM Ca^{2+} channels by ER store-depletion is referred to as store-operated Ca^{2+} entry (SOCE) and the channels activated are broadly classified as store-operated Ca^{2+} entry channels (SOCC) (14–17). As illustrated in Figure 1, the first step in the initiation of SOCE is the binding of agonists (hormone or growth factors) to the PM receptors (e.g.-GPCRs/RTKs). Activation of the cell surface receptor engages a cascade of signaling events that culminates in the PLC- (Phospholipase C) mediated hydrolysis of the membrane PIP2 (phosphotidylinositol-4,5- bisphosphate) resulting in the generation of membrane bound diacylglycerol (DAG) and the diffusible messenger - inositol 1,4,5-trisphosphate (IP_3). IP_3 binds to IP_3Rs that are localized in the ER and mediates the release of Ca^{2+} from intracellular ER stores, which results in activation of the PM-SOCC thereby elevating $(Ca^{2+})_{cyt}$. This increase in $(Ca^{2+})_{cyt}$ is not only essential for regulating biological functions, but is also pumped back into the ER via the SERCA pump. The remaining Ca^{2+} is then pumped out that concertedly help in lowering the $(Ca^{2+})_{cyt}$, and completing the SOCE cycle (14).

The concept of SOCE (also known as capacitative Ca^{2+} entry) was introduced about three decades back (18–19); however, the molecular identity of the ion channels and the regulators facilitating SOCE has just begun (20). Early genetic screens in *Drosophila* identified spontaneous gene mutations involved in phototransduction pathway, which eventually lead to the discovery of TRP channels (21). Mammalian homologs of the *Drosophila trp* genes have been suggested as candidate SOCC components. The TRPC (canonical TRP) family of ion channels constitutes a major subclass of the seven TRP subfamilies identified thus far. (22–27). TRPC1 was the first member of the TRPC family to

be cloned and has been studied extensively (28–29). In addition to TRPC1, other TRPCs have also been shown to be regulated by store depletion *per se*. In addition, recent findings have also suggested that Orai proteins, which appear to be the molecular component of the elusive CRAC (Ca^{2+} release-activated Ca^{2+} entry) channels, to function as SOCC, however, this is predominantly seen in immune cells (10, 30–33). Moreover, TRPC channels have been shown to interact with Orai1, suggesting that both these channels can be the components of the illusive SOCE channel especially in non-immune cells.

One of the important aspects of SOCE that was poorly understood is the molecular mechanism (s) by which the status of the ER Ca^{2+} store is communicated to the PM channels, to initiate Ca^{2+} influx. Thus, identification of the working mechanism of SOCE has been a continuous scientific pursuit for over two decades. Multiple possibilities were put forward to provide a reasonable explanation to the question of how the status of ER stores is communicated to the PM Ca^{2+} channels (14–15, 34–36). For instance it has been suggested that, for SOCE activation the signal from the ER stores is transmitted to the PM Ca^{2+} channels by either a conformational-coupling mechanism, or via a diffusible messenger CIF (Ca^{2+} influx factor), which transits information about store-depletion, and activates the PM Ca^{2+} channels. In addition, store-depletion dependent recruitment of vesicle associated SOCC channel to the PM has also been proposed as a possible mechanism. However, none of these proposed models have either been refuted or have gotten universal acceptance.

A recent seminal finding in the field of store-operated Ca^{2+} signaling has now solved the enigma of ER-PM communications during activation of SOCE. In mid-2005, two groups – one led by Tobias Meyer (Stanford University) and the other jointly led by Michael Cahalan (University of California at Irvine) and Kenneth Stauderman (Torrey Pines Therapeutics Inc., La Jolla, CA), independently identified the signaling molecule that was involved in linking ER store-depletion to the activation of PM-SOCC. Using large-scale RNA-interference screens on cellular signaling proteins, they identified STIM proteins (stromal interaction molecule 1 and 2) as the most important regulators of SOCE (37–38). Identification of STIM, as the missing link involved in transmitting the ER store-depletion message to the PM-SOCC, gave an answer for the most sought after query in store-operated Ca^{2+} signaling biology. STIM1 and STIM2 are both single-pass transmembrane proteins with N-terminal Ca^{2+} binding EF-hands (canonical and hidden) and SAM (sterile alpha motif) domain located either extracellular or in the ER lumen. On the other hand the C-terminus domain of STIM1 is localized in the cytoplasm and is shown to contain the ERM (ezrin-radixin-moesin) and coiled-coil protein interacting domains (39–40). STIM1 has been shown to be glycosylated and associate with the PM (41–43), however the precise role of PM-STIM1 in SOCE remains unclear (44). In addition, STIM1 is shown to be phosphorylated (41), predominantly at serine residues, and this appears to be important for SOCE regulation during cell division (45–46). An updated structure of STIM1 showing critical molecular domains identified thus far is shown in Figure 2. Similarly STIM2, which is about 45 percent identical with STIM1, was also identified as a second modulator; however recent reports indicate that STIM2 is primarily involved in maintaining basal Ca^{2+}, rather than initiating SOCE (44).

Functionally, STIM1 in the ER responds to the changes in ER Ca^{2+} levels via reversible binding of Ca^{2+} with its EF-hand domain. Following depletion of ER Ca^{2+}, STIM1 undergoes homotypic interactions and forms oligomeric clusters (puncta) at the ER-PM junctions. The STIM1 puncta at the peripheral ER can thus physically associate with PM-SOCC thereby activating SOCE (37, 47–52). Although the identification of the ER Ca^{2+} sensor, STIM1, has revolutionized our understanding of SOCE, the question on what is the importance of STIM1 puncta and how are they stabilized and recruited to sites of ER in apposition to the PM is still an unsolved mystery. Whether, the composition of the PM at

these junctional sites influence the specificity of STIM1 recruitment is not known. Thus, information on the identity of the ER-PM junction sites, at sub-plasma membrane regions, where STIM1 clusters translocate and associate with the PM-SOCC to activate SOCE is warranted. Nevertheless, identification of STIM1 as a critical regulator of TRPC as well as the Orai channels has provided novel insight into their activation paradigms.

4. ACTIVATION MODALITIES OF NON-SELECTIVE TRPC CHANNELS

TRPC channels function as non-selective cation channels and conduct relatively large Na^+, Ca^{2+}, Ba^{2+} or Sr^{2+} inward currents (53). Although TRPC channels can be classified into two different groups depending on their mode of activation (store vs receptor mediated activation), under physiological condition all TRPC channels are activated by G-protein coupled mechanism. Several studies, using various cells, have shown that TRPC1, C4, and sometimes C5 can be activated by store depletion *per se* (54–55). While, the second group comprising of TRPC3, C6, and C7 can be activated by second messenger system. Also, it has been shown that the C3/C6/C7 subfamily can be gated by DAG (26, 54–55). Thus, this group forms a distinct subfamily of DAG-sensitive cation channels, coupling receptor/PLC-signaling pathways to cation entry. While there is no doubt that all members of the TRPC3/C6/C7 subfamily can principally be activated by DAG, it is still contentious issue as to whether DAG can be regarded as the physiological activator of the native channel. As deduced from pharmacological inhibition of DAG lipase and DAG kinase, it appears that endogenously generated DAG is sufficient for channel activation (17), however; this needs to be verified in other cell types. Most notably, receptor agonists and DAG did not display additive effects of C3 and C6 current amplitudes, suggesting that the same TRPC channels are activated by DAG and by PLC-linked receptors and that DAG may be the decisive second messenger generated by PLC. However, so far a direct interaction of DAG with these TRPC proteins has not been demonstrated.

Several mechanisms have been shown to activate/inactivate TRPC channels. N-terminus of IP_3Rs is shown to interact with TRPC channels, which can activate TRPC1, TRPC3, and TRPC4 channels (56–57). In contrast, calmodulin is also shown to interact with TRPC channels and is involved in Ca^{2+}-dependent feedback inhibition of TRPC channels by acting as a Ca^{2+} sensor (58–59). Interestingly, IP_3Rs peptides compete with calmodulin for binding to TRPC channels (60), and addition of calmodulin prolongs the interval between Ca^{2+} release from intracellular stores and activation of Ca^{2+} influx (59–60). Additionally, since calmodulin binding affinity increases with elevated Ca^{2+}, this mechanism may be important for certain adaptations and be influenced by cytosolic Ca^{2+} or release of Ca^{2+} from intercellular stores and might also be critical for allowing STIM1 to interact with TRPC channels. Phosphorylation has also been identified as a possible regulator of TRPC channels. TRPC1 is known to be phosphorylated by PKC and is important for Ca^{2+} entry in human endothelial cells (61). In contrast, TRPC3 activity is shown to be inhibited by both PKC and PKG (62). PKG inactivates TRPC3 by phosphorylating TRPC3 at Thr-11 and Ser-263, whereas, PKC inactivates TRPC3 by phosphorylation on Ser-712. On the other hand, Src kinases were found to be essential for the activation of TRPC3 (63); however no direct evidence with regard to phosphorylation of TRPC3 channel has been established. Similarly, Fyn (member of the Src family kinase) and CaMK II-dependent phosphorylation has been shown to regulate TRPC6 activity (64–65). Besides these mechanisms other proteins are also shown to be critical for the activation of TRPC channels. For example, Homer and caveolin1 (Cav1) has been shown to negatively regulate TRPC1 channels (66–67), whereas, STIM1 positively activate TRPC1 channels (68). Similarly, RyRs interaction with TRPC channels is shown to be essential for its activation (69). Although, it has been shown that Homer antibodies efficiently immunoprecipitate TRPC1-TRPC4 complexes, it was not equivalently able to precipitate using IP_3Rs antibodies (70). These results suggest

that when different subunits form a functional channel the mode of their regulation could be completely different. Moreover, since Homer and Cav1 could bind to different TRPC channels, intermolecular interactions of the TRPC channels could influence Homer and Cav1 binding, thereby modulating the channel activity differently. In addition, since these proteins bind at the C-terminus of TRPC channel, there could be competition between their interactions and several factors may decide as to which protein will interact with TRPC channels and regulate Ca^{2+} influx accordingly. Overall, these results suggest that diverse regulatory mechanism could be present, which can regulate these TRPC channels and depending on the need and the complexity of the cell, the cell may decide as to which regulatory protein TRPC channels will interact, in order to modulate Ca^{2+} influx. Thus, more research is needed to confirm these interactions within more dynamic conditions involving activation/inactivation of these TRPC channels and quantify the association of each protein with regard to channel activity.

TRPC proteins contain several assembly domains that could be important in regulating protein-protein interactions. N-terminal cytosolic domain of TRPC channels contains coiled-coil domains and several (3–4) ankyrin repeats, which are one of the most common protein–protein interaction motifs. Structural information on ankyrin domains includes a 33-amino-acid sequence representing a highly conserved helix–turn–helix structure that can be important for self multimerization, and also can play an essential role in protein-protein interactions. Importantly, deletion of the ankyrin domains in several TRPCs not only loses their ability to form tetramers, but also had issues with regard to membrane targeting and activation of TRPC channels. For example, deletion of TRPC3 ankyrin domains has been shown to decrease its PM localization (60, 71). Similarly, deletion of the first ankyrin domain in TRPC5, failed to form homo or heteromultimers thereby leading to non-functional channels (72). Although the amino acids involved in these interactions are not yet identified, point mutations within this region in TRPV6 have been shown to abolish channel assembly and resulted in non-functional channels, indicating that similar mechanisms can also exist in the canonical TRPs as well. Besides, the ankyrin domains, a coiled-coil region is also present at both the N and C-terminus of TRPC channels. Importantly, the N-terminal coiled-coil region is found to be essential for TRPC1 assembly and for interaction of *Drosophila* TRPL and TRPγ (73). Also, tetramerization of TRPC4 or TRPC6 channels requires both the coiled-coil motif and the ankyrin repeats (74).

In *Drosophila*, the TRP channels have been shown to be associated in a signalplex, which provides a unique platform to modulate TRP channel activity (26, 75). The key component of the *Drosophila* signalplex is INAD scaffold, which exhibits five PDZ domains and interacts with multiple proteins to orchestrate phototransduction. Similarly, mammalian TRPC channels not only interact among themselves to form homomeric and heteromeric channels, but have also been shown to interact with multiple proteins (55, 76). However, the scaffold protein similar to INAD has not yet been identified. Additionally, it is not clear if all these proteins interaction are constitutive or are dynamic. Thus, identification of interplay between these signalplex proteins can be essential for regulating the physiological function exhibited by these TRPC channels. Furthermore, alterations in their interactions can potentially inhibit or amplify channel function leading to pathological conditions. Recent research has indicated that TRPC channels are assembled into lipid raft domains, which are not only critical for the function of TRPC channels, but are also important in the assembly and retention of TRPC1 at the PM (55, 67, 76–80). Interestingly, mutations in the *Drosophila* INAD protein not only hamper TRP function, but also alter the localization of its interacting proteins (81). Thus, it can be anticipated that disruption of the mammalian TRPC signalplex will also lead to altered localization of TRPC channels and thus interfere in its ability to functionally interact with critical regulatory molecules.

Recent studies have demonstrated an obligatory role of STIM1 in activating TRPC channels and regulating SOCE (details provided in Table 1). In this context STIM1 activation of TRPC1 channel has been extensively studied. Physiologically, the interaction between STIM1 and TRPC1 is dynamic and reversible and is regulated by the status of ER Ca^{2+} stores in a way that, upon agonist-mediated store-depletion the STIM1-TRPC1 interaction is increased. However, following agonist clearance and subsequent refilling of the ER stores the STIM1-TRPC1 complex dissociate to a resting state (79). Although the precise control of this interaction cycle is not established, the kinetics underlying the homomultimeric clustering of STIM1 appears to be a prime driving force. STIM1 has been shown to associate with TRPC1 in a complex containing Orai1; however, independently their regulation by STIM1 is distinct, since functional interaction site on STIM1 is different (82–83). STIM1 binds to native TRPC channels as efficiently as it binds with ectopically expressed channels. With regard to STIM-TRPC associations, there seems to be a preference. STIM1 interacts with TRPC1, C2, C4 and C5 but doesn't seem to interact with TRPC3, C6 and C7. It has been suggested that, although STIM1 doesn't directly bind to certain TRPC channels it can still be able to influence their function indirectly since, native SOCCs can constitute heteromeric TRPC assemblies. This distinction in the activation mechanism of TRPC channels could explain as why TRPC1/C4/C5 are deemed as candidates for store depletion, whereas TRPC3/C6/C7 are mainly believed to be dependent on second messenger system. Similarly, TRPC1 has been shown to functionally interact with Orai1 channels and Orai1 channels have been shown to be regulators of TRPC channels (84–85). In addition, SOCE via the TRPC channels has been shown to be both Orai1 dependent and Orai1-independent (86–87), suggesting that positioning of specific channels along with precise interactions with other molecules at cellular compartments could co-ordinate Ca^{2+} entry. Overall, these results suggest that depending on the cell type and the need of Ca^{2+} signal, these interactions could have additive effect on the regulation of TRPC channels and that Ca^{2+} channels are more complex that requires multiple proteins for the generation of Ca^{2+} currents. In addition, compartmentalization of these key proteins could also play a significant role in these functional interactions and many of the TRPC interacting proteins have been shown to be partitioned in both lipid raft and non-lipid raft fractions (76).

The STIM1-TRPC interaction is shown to be mediated by the ERM domain present in the cytosolic region of STIM1 and deletion of the ERM domain has been shown to suppress SOCE. Interestingly, however, the binding and gating of TRPC channels by STIM1 seems to be dissociated. The poly-Lys (poly-K) region in STIM1 C-terminus is shown to be required for TRPC channel gating but is dispensable for STIM1-TRPC interactions. Deletion of this poly-Lys region impairs the ability of STIM1 to gate native TRPC1 channels and initiate SOCE. Further studies lead to the identification of the $^{684}KK^{685}$ residues within the STIM1 poly-K region to be imperative for TRPC1 gating. This gating was shown to be facilitated by electrostatic associations between the $STIM1^{684}KK^{685}$ and $TRPC1^{639}DD^{640}$ which was proved by well thought out charge-swap experiments. These negatively charged aspartates is conserved in other TRPCs as well, thus it can be reasoned that the electrostatic association will be a common step in the STIM1-mediated gating (direct or indirect) of other TRPC channels. Indeed, with the exception to TRPC7, this commonality (presence of the conserved DD domain) exists in the STIM1-mediated activation of TRPC channels.

5. MEMBRANE RAFTS/CAVEOLAE AND CA^{2+} SIGNALING

The PM is spatially organized into multiple microdomains which constitute unique signaling nodes to efficiently relay external signals. The concept of lipid rafts was initially developed to convey the idea that these membrane microdomains form small, but discrete membrane

platforms which function as signaling organizers (8, 88–91). A sub category of the lipid-rich membrane rafts are 'caveolae', which form omega-shaped membrane invaginations ranging from 50–200nm in diameter. This structural uniqueness of caveolae enables specific set of PM signaling complex to reach deeper into the cytosol thereby giving an added advantage for PM-organellar crosstalk. Thus, these invaginations can facilitate interactions between proteins that are cytosolic as well as located in separate organelles (e.g.-ER and mitochondria) (92), thereby mediating a communication between separate membrane compartments (e.g.-PM with ER), that would otherwise be several microns apart (7, 90–91, 93–94). Membrane rafts have been efficiently shown to be involved in organization of cell signaling machinery, including G protein-coupled receptor (GPCR) and receptor tyrosine kinase (RTK) pathways (95–99). Additionally, membrane rafts have now been suggested to play a significant role in many biological processes, including signal transduction pathways, apoptosis, viral infections, cell adhesion and migration, synaptic transmission, organization of the cytoskeleton, and plasma membrane protein sorting during both exocytosis and endocytosis (7, 94, 100). Importantly, since many of the above physiological process are also know to require Ca^{2+}, it can be postulated that lipid rafts can potentially regulate these processes by modulating Ca^{2+} signaling.

Ca^{2+} signals are generated across wide spatial and temporal ranges that are efficiently coordinated through organization of specific Ca^{2+}-channels, pumps, buffers, exchangers and protein scaffolds into common microdomains. Membrane rafts serve as such a microdomain wherein highly specific signaling events can be efficiently executed with precision. Involvement of lipid rafts/caveolae in Ca^{2+} regulation was identified over three decades back (101–102), but direct evidence was only shown recently (67, 80). The first evidence that identified the involvement of caveolae in Ca^{2+} homeostasis was observed in muscle cells where the SR was localized immediately underneath the plasma membrane and was in close proximity to caveolae (101). Soon after, other investigators identified that caveolae can effectively increase intracellular Ca^{2+}, which may activate the contractile apparatus to produce a sustained vasoconstriction (103–104). Histochemical methods further confirmed that Ca^{2+} was found in the lumina of caveolae, suggesting the importance of caveolae in Ca^{2+} signaling (105). Further, X-ray spectral analysis confirmed that Ca^{2+}-peak (corresponding to increases in $(Ca^{2+})_{cyt}$) can be found within two different cellular compartments: in small invaginations of the sarcolemma, which is caveolae, and in the intrafibrillar sarcoplasmic reticulum (106). Additionally, PMCA pumps as well as IP_3 -regulated Ca^{2+} channels were also shown to be localized in caveolae (107–109). Although these initial studies performed in mid-1990 provided clues that caveolae are important for Ca^{2+} signaling, not much research was performed to understand the mechanism or role of caveolae in Ca^{2+} influx *per se*. It was Anderson's group that initially showed that agonist-stimulated Ca^{2+} signal originated in specific areas of the plasma membrane that were enriched in Caveolin1 (Cav1) (110–111). Interestingly, not only G-protein coupled receptors but also $G_{q/11}$, phospholipase C, IP_3Rs, and SOCE channels, are now known to be present in lipid raft domains. The ability to concentrate most proteins of the SOCE cascade in a single microdomain suggests that proteins are grouped together to effectively coordinate Ca^{2+} signaling. Importantly, direct interaction between TRPC1 and Cav1 had been shown to have a direct role in regulating Ca^{2+} influx (23, 112).

Mammalian TRPC channels have been shown to associate with multiple proteins including STIM1, Gαq/11, PMCA, SERCA, IP_3Rs, Homer, CaM, GPCRs, receptor tyrosine kinases, RyRs, PLCγ, PLCβ, NCX1, Na^+/K^+/ATPase, NHERF and Cav1, which are known to influence TRPC channel function (12, 24, 55, 113–116). Although a number of such TRPC interacting proteins have been identified, none of them have been shown to be a mammalian TRPC scaffold. Nonetheless, since most of TRPC interacting proteins are also known to be localized in lipid rafts, they together can presumably provide a unique platform/scaffold to

coordinate Ca^{2+} entry. Lipid rafts are also dynamic entities, where small rafts merge into bigger platforms (117–118), which fits into the possibility of providing a preference for protein-protein interactions. Thus, lipid raft microdomains can in a dynamic, spatiotemporal fashion, facilitate direct physical, or functional, coupling between molecular components that are critical in the activation or inactivation of Ca^{2+} entry channels. In addition to its role of clustering related signaling molecules, Cav1 proteins can also regulate trafficking of various receptors/mediators to the PM (119). It is now evident that TRPC1 channels are assembled into lipid raft/caveolar microdomains, where they interact with Cav1. Importantly, all functional mammalian TRPC proteins have putative Cav1 binding domains at both their N and C terminus (67, 76, 78). The N-terminal domain of TRPC1 which interacts with Cav1 has been shown to be critical for its plasma membrane retention. However, the significance of the putative C-terminal binding domains remains to be explored. Studies performed with the expression of a mutant Cav1 (lacking its protein scaffolding and membrane anchoring domains) or *cav1* gene knockout has been shown to disrupt the plasma membrane localization of TRPC1 leading to a significant decrease in Ca^{2+} influx upon store depletion (67, 80). Additionally, loss of membrane raft domains in non-excitable cells has been shown to decrease SOCE (67, 78, 120–122), indicating that these domains can explicitly regulate several Ca^{2+} channels, thereby differentially regulating Ca^{2+} entry. Thus, it can be reasonably proposed that the Cav1 containing membrane raft domains will be the organizers of the mammalian TRPC1 signalplex, analogous to the INAD scaffold of *Drosophila* TRP.

ER localized STIM1 undergoes clustering and translocation to the sub-plasma membrane regions of the cells where it displays a punctate localization (43, 47). The site of these puncta have been proposed to be the region of the cell where functional interaction between ER and PM occurs resulting in the activation of SOCE via plasma membrane channels. Indeed SOCE has been shown to occur at sites coincident with the STIM1 peripheral clusters (48–49, 51). STIM1-dependent clustering of the CRAC channel component, Orai1, in the plasma membrane requires STIM1 puncta and is coincident with the location of the puncta (48–49, 51). Similarly, the PM- SOCC component, TRPC1, is also co-localized with STIM1 clusters (123–124). These observations suggest that in order to mediate SOCE, STIM1 needs to be targeted to specific regions of the cell where the likelihood of its interaction with PM-SOCC will be high. Thus, the site of peripheral STIM1 clusters is critical for the regulation of SOCE. However, what determines the location of the STIM1 clusters in the ER-PM junctional regions and whether these represent specific sites in the cell is not yet known. Several studies including ours suggest that PM lipid raft domains determine the peripheral clustering of STIM1 and regulation of TRPC1-mediated SOCE (76, 79, 121, 125). Depletion of ER Ca^{2+} stores increases the association of STIM1 with lipid raft domains. Further, this association appears to be critical for the activation dependent translocation of STIM1-punctae to membrane raft domains at the ER-PM junctional region of the cells. In addition, disruption of the lipid raft domains severely attenuates stimulation-dependent association of TRPC1 and STIM1. Coincident with this, raft disrupted cells also displayed reduced SOCE. Importantly, $STIM1^{D76A}$ mutant was constitutively clustered in the cell periphery co-incident with membrane raft domains and the sub-plasma membrane localization of $STIM1^{D76A}$ was also dependent on lipid raft domains integrity (79). Furthermore, disruption of lipid rafts decreases $STIM1^{D76A}$ –mediated constitutive Ca^{2+} entry and its interaction with TRPC1 (67, 78, 80, 112, 126).

Ca^{2+} store depletion induces oligomerization of STIM1 which has been reported to occur prior to puncta formation in the cell periphery (37, 43, 47). The latter likely requires additional mechanisms for translocation and targeting of STIM1 oligomers to specific ER-plasma membrane junctional regions where STIM1 can interact with SOCE channels in the surface membrane (47, 49, 51). The coiled-coil domain in the C-terminus of STIM1 is

reported to be crucial for its aggregation while the amino acids, 425–671, which contain a serine-proline-rich region, appear to be important for the correct targeting of the STIM1 cluster to the cell periphery after Ca^{2+} store-depletion. The polycationic region in the C-terminal tail of STIM1 also appears to help STIM1 targeting to PM region but is not essential for oligomerization after Ca^{2+} store depletion (40, 52, 127). Thus, aggregation of STIM1 that occurs in response to a decrease in ER- (Ca^{2+}) and its translocation to the sub-plasma membrane region can be dissociated, although the latter is dependent on the former. These findings provide an important insight into the mechanism that is involved in the store-dependent regulation of TRPC1 channels by STIM1. Based on these findings, it can be suggested that caveolar microdomains in the PM can provide a unique platform for clustering and interaction of STIM1 and TRPC1 in the ER-plasma membrane junctions (see proposed model in Figure 3). Additionally, lipid rafts can anchor STIM1 and thus determine its localization in specific ER-PM junctions where it can functionally interact with plasma membrane channels and regulate SOCE. Furthermore, since caveolar lipid rafts have been shown to be relatively stable membrane domains they can serve as precise micro-compartments to facilitate the dynamic interactions between specific proteins in the ER and the surface membrane that are involved in regulation of SOCE.

Studies from our lab had identified a subtle molecular rearrangement in TRPC1-Cav1-STIM1 interaction that is indispensable for TRPC1-mediated Ca^{2+} entry. TRPC1 interaction with Cav1 precisely occurs at the membrane raft microdomains that regulate the PM association of TRPC1. Interestingly, Cav1 targets TRPC1 to same PM microdomains where the peripheral STIM1 clusters are organized in response to Ca^{2+} store depletion. Additionally, activation dependent raft recruitment of TRPC1 was severely impaired following Cav1 silencing. Consistent with the impaired caveolar association of TRPC1, SOCE was also significantly attenuated. In contrast, increased expression of Cav1 also inhibited SOCE, suggesting that Cav1 scaffolds TRPC1 in ER-PM domains but suppress its function. Importantly, it was further shown that Ca^{2+} store depletion increased the association of endogenous TRPC1 and STIM1, but decreased that of TRPC1 and Cav1. Additionally, only expression of STIM1 at higher levels, relative to Cav1 resulted in the dissociation of TRPC1-Cav1 complex and recovery of SOCE. These findings suggest that when STIM1 relocates into ER-PM junctional regions, in response to Ca^{2+} store-depletion, it associates with TRPC1 and mediates release of TRPC1 from Cav1 thus regulating the activation of TRPC1-SOCE. In addition, since Orai1 has been shown to interact with TRPC1 and STIM1, it could be speculative that Orai1 could also be present in these lipid rafts.

The positively charged ^{684}KK^{685} in the C terminus of STIM1 has been recently shown to gate TRPC1 channels via electrostatic interaction with the negatively charged ^{639}DD^{640} in the C terminus of TRPC1 (39). Importantly, reverse charged mutant of TRPC1, TRPC1 (DD to KK), which inhibits TRPC1-mediated Ca^{2+} entry also inhibited TRPC1-Cav1 dissociation (68). Interestingly, expression of a charge swapped mutant STIM1 (KK to EE), which would electrostatically complement the TRPC1 (DD to KK) mutant, resulted in the dissociation of TRPC1 from its scaffold – Cav1, following store depletion. As a consequence of TRPC1-Cav1 dissociation, a relative recovery in SOCE was also observed. These findings demonstrate that the same amino acid residues of STIM1 that are involved in gating of TRPC1 are also critical for mediating release of TRPC1 from Cav1, suggesting that molecular rearrangement involving TRPC1 (i.e. store-depletion induced association of TRPC1-STIM1 and dissociation of TRPC1-Cav1) is imperative for the activation of the channel by STIM1. Although the exact mechanism is not yet established, it can be hypothesized that STIM1 interaction with TRPC1 can induce a conformational change in TRPC1 that can result in its dissociation from Cav1 thereby allowing STIM1-mediated gating of TRPC1. Consistent with this, the Cav1 interacting domains have been found to

overlap within the STIM1 gating domains and, further studies will be required to understand the structural details of this functional regulation. Overall, these findings suggest that Cav1 acts as a scaffold to retain inactive TRPC1 in the PM regions in juxtaposition to ER where STIM1 aggregates following store-depletion (see proposed model in Figure 3). STIM1-dependent activation of TRPC1-SOCE involves dissociation of TRPC1 from Cav1 and association with STIM1. Targeting of the channels and STIM1 to the same microdomains ensure the specificity and rate of interaction between these proteins that are essential for activation of SOCE. Importantly, interaction between STIM1 and TRPC1 has been shown to be mediated via the C-terminal domain. Furthermore, the C-terminal caveolin1 binding site overlaps with the STIM1 gating domains, indicating that a tight interplay between these two proteins will be critical for the regulation of TRPC1 channels.

6. PHYSIOLOGICAL SIGNIFICANCE OF STIM1 REGULATED TRPC FUNCTION

The role of TRPCs and STIM1 in regulating Ca^{2+} entry is well established; however the exact physiological role of these channels in regulating biological function is still not yet determined. Increase in $(Ca^{2+})_{cyt}$ within the cytosol is essential for cellular activities such as cell proliferation, gene expression, secretion, migration, and adhesion. Since, STIM1 and TRPC1 are key regulators of Ca^{2+} signaling, it can be anticipated that they will be critical for these biological functions (see Figure 4 for an illustration). Consistent with this, inhibition of STIM1, or deletion of TRPC1 is shown to reduce Ca^{2+} influx and frequency of Ca^{2+} oscillations in pancreatic acinar cells, which were essential for enzyme and fluid secretion (128). Importantly, TRPC1 knockout mice, also exhibited a significant decrease in SOCE along with severe loss of salivary gland fluid secretion (129). Although in this report, the role of STIM1 was not determined, TRPC1 has been shown to interact with STIM1 especially in salivary gland cells and is critical for its activation (68, 79, 124). Altogether, these results suggest that TRPC1-STIM1 association could regulate fluid and enzymatic secretion at least in the acinar cells of both salivary and pancreatic glands

In addition, to secretion, the role of TRPC1 and STIM1 in regulating cell migration and probably proliferation is more widely accepted. Vascular smooth muscle cells (VSMCs) retain the capacity for plasticity that enables switching to a non-contractile modulated phenotype that is important for blood vessel formation and vascular adaptation, which is as also dependent on Ca^{2+} entry. Interestingly, extracellular application of antibodies that specifically bound to STIM1 inhibited Ca^{2+} entry and cell migration, but not proliferation. STIM1 was shown to functionally, interact with TRPC1, and TRPC1 contributed to Ca^{2+} entry and cationic current. Importantly, TRPC1-containing channels were important for both cell proliferation as well as migration. These data suggest a complex situation in which STIM1 and TRPC1 are important for cell migration, and TRPC1 can have functions that are independent with at least PM STIM1 (130–131). Additionally, in another report it was shown that silencing of STIM1 suppressed phosphorylation of cAMP-responsive element binding protein (CREB) and cell growth (131–132). Consistent with this it has been shown that STIM1-induced Ca^{2+} influx viaTRPC1 regulates NFkB activation and cell proliferation (68). Importantly, STIM1 translocation to the plasma membrane promotes intestinal epithelial cell (IEC) migration after wounding by enhancing TRPC1-mediated Ca^{2+} signaling and provides new insight into the mechanism of intestinal epithelial restitution (133). Together these results suggest that STIM1, along with TRPC1 is an essential component of SOCE and are involved in cell proliferation (130–132). Similarly, endothelial progenitor cells (EPCs) also express TRPC1, STIM1 and Orai1 and inhibition of SOCE impaired proliferation of EPCs (134). Although in this study a direct interaction between TRPC1 and STIM1 was not observed, but since TRPC1 is essential in endothelial cell function, it can be hypothesized that Ca^{2+} entry via TRPC1 can increase cell proliferation. If

this is true then, it can be regarded as a novel target to enhance the regenerative outcome of cell-based therapy.

NRK fibroblasts also express TRPC1, C5, C6, Orai1 and STIM1, and that the levels of their expression were dependent upon the growth stage of the cells (135). Similarly, Endothelin-1 (ET-1) treatment for 48h enhanced TRPC1 expression, SOCE, and transcription factor activation without upregulating STIM1. However, knockdown of STIM1 suppressed these effects, thereby preventing a hypertrophic response (136). These results suggest that STIM1 plays an essential role in the development of cardiomyocyte hypertrophy. Additionally, TRPC6-overexpressing Huh-7 cells proliferated 80 percent faster than did untransfected control cells and their SOCE amplitude was also significantly higher. In contrast, proliferation rate and SOCE amplitude was significantly decreased in TRPC6-knockdown cells, suggesting that TRPC6 or a channel that multimerise with TRPC6 is essential for cell proliferation of Huh-7 cells. Interestingly, SOCE was also reduced by STIM1 and Orai1 knockdowns, suggesting possible cooperation between these proteins in these cells. Consistent with this TRPC6 expression was decreased in isolated hepatocytes from healthy patients, but highly expressed in tumor samples, which strongly support a role for TRPC6 channels in liver oncogenesis (137).

Mast cell degranulation is dependent on Ca^{2+}influx through "Ca^{2+} channels" that can also convey Sr^{2+}. The release of histamine from rat peritoneal mast cells, whether supported by Ca^{2+} or Sr^{2+}, was effectively blocked by low concentrations of La^{3+}. Although STIM1 and Orai1 are being shown to be essential for the activation of mast cells, knockdown of TRPC5, substantially reduces influx of Ca^{2+} as well as degranulation in RBL-2H3 cells (138). Moreover, overexpression of Orai1 with STIM1 promotes constitutive influx of Ca^{2+} but not of Sr^{2+}, whereas overexpression of TRPC5 with STIM1 promotes constitutive influx of both ions. These data suggest that Sr^{2+}-permeable TRPC5 acts in conjunction with Orai1 and STIM1 to allow Sr^{2+} and other divalent ions to permeate and support degranulation in mast cells. Furthermore, ectopic expression of Fyn or TRPC1, in Fyn null mast cells restored Ca^{2+} responses and increased mast cell degranulation, suggesting that TRPC1 participates in Ca^{2+} influx required for mast cell degranulation. This demonstrates that in addition to a role described previously for Orai1 in promoting mast cell degranulation, nonselective cation channels also participate in promoting the exocytotic response (139). Consistent with this, STIM1 and TRPC1 have also been implicated in thrombin- and ADP-induced platelet aggregation (140), probably through the regulation of Ca^{2+} entry, which might become targets for the development of therapeutic strategies to treat platelet hyperactivity and thrombosis disorders). Additionally, in PASMCs Ca^{2+} entry was mediated by the TRPC1 channel through activation of STIM1, which may be an important model for future identification of SOCs in PASMCs and they may be useful targets for the development of new drugs to treat pulmonary hypertension (141). Although these examples indicate that TRPCs and STIM1 interactions are beneficial for cellular system, but could be harmful in conditions such as metabolic syndrome, where abnormally elevated adrenal TRPC expression may underlie increased plasma epinephrine and heart rate. The excess of plasma catecholamines and increased heart rate are risk factors for cardiovascular disease. Thus, TRPCs are also potential therapeutic targets in the fight against cardiovascular disease (142). Also, increased in cell proliferation due to increased activity of STIM1-TRPC1, could potentially lead to cancer and more research is needed to verify the possible role of TRPC1-STIM1 function in this regard.

7. CONCLUSION AND PERSPECTIVE

Ca^{2+} entry via the SOCE mechanism is essential for maintaining cellular functions. Intracellular Ca^{2+} stores communicate with the plasma membrane SOCE channels to

orchestrate cellular Ca^{2+} homeostasis that regulate fundamental biological functions. Although STIM1 has been shown to be the regulators for both TRPCs and Orai channels and this process is essential for maintaining intracellular Ca^{2+} stores as well as influencing vital physiological processes, the exact biological function of many of these channels have not yet been fully explored. Furthermore, results using knockout mouse models, differ significantly from human cells and cells obtained from patient populations, where Orai1 was not found to be critical for T cell activation in humans but not in mice. This could be due to differential expression of certain Ca^{2+} channels (including TRPCs) and their regulators in a given cell type, which could not only dictate the composition of the SOCE channel, but can also be essential in their regulation. Additionally, STIM1 has been shown to form oligomers, but the significance of these oligomers is still not yet known. One possibility can be that STIM1 needs to form oligomers in order to influence ER restructuring to form ER-PM junctions. The second possibility may be that oligomeric STIM1 can initiate protein-protein interactions needed to functionally gate and regulate SOCE channels. In addition, Orai1 has been shown to interact with TRPC channels, but the significance of this interaction is not clear and more research is needed to decipher the role of individual SOCE channels. Also, nothing is known about the role of PM STIM1, one possibility can be that PM STIM1 can be present in lipid rafts and since STIM1 is known to multimerize, it can assist in establishing peripheral STIM1 puncta. Overall, although recent findings have helped in understanding the mechanisms of SOCE, future studies are still needed to completely evaluate the role of these functional interactions and finally use this information for therapeutic interventions.

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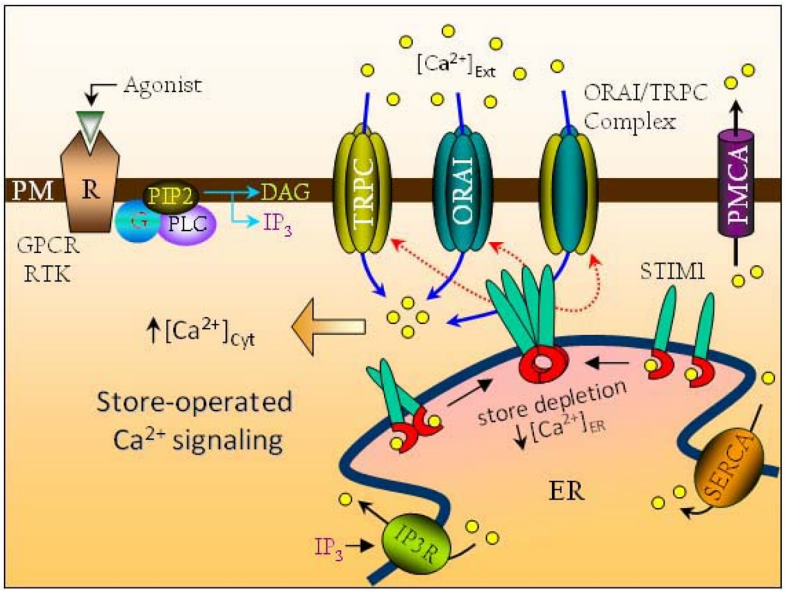

Figure 1.

STIM1 activated SOCE. The current molecular concept of SOCE is STIM1-mediated activation of PM-SOCC (TRPC/Orai channels). Agonist-induced receptor (GPCR/RTK) activation results in PLC-mediated hydrolysis of PIP2, generating the diffusible and membrane-bound cellular messengers – DAG (diacylglycerol) and IP_3 respectively. IP_3 binds to its receptor (IP_3Rs) in the ER depleting the ER Ca^{2+} stores. This leads to STIM1 oligomerization by interaction of mono- or dimeric STIM1 molecules. The STIM1 oligomeric-clusters thus formed are subsequently recruited to ER-PM juxtaposed sites allowing STIM1 to physically activate the TRPC and Orai channels to bring about Ca^{2+} entry. This raises the $(Ca^{2+})_{cyt}$ which results in store-operated Ca^{2+} signaling and influences a variety of cellular functions. To complete the SOCE cycle, $(Ca^{2+})_{cyt}$ is sequestered back to the ER by the SERCA pump and/or extruded to the cells' exterior by PMCA. The membrane-associated lipid messenger - DAG also has the ability to activate select TRPC channels independent of ER-stores and presumably STIM1 as well.

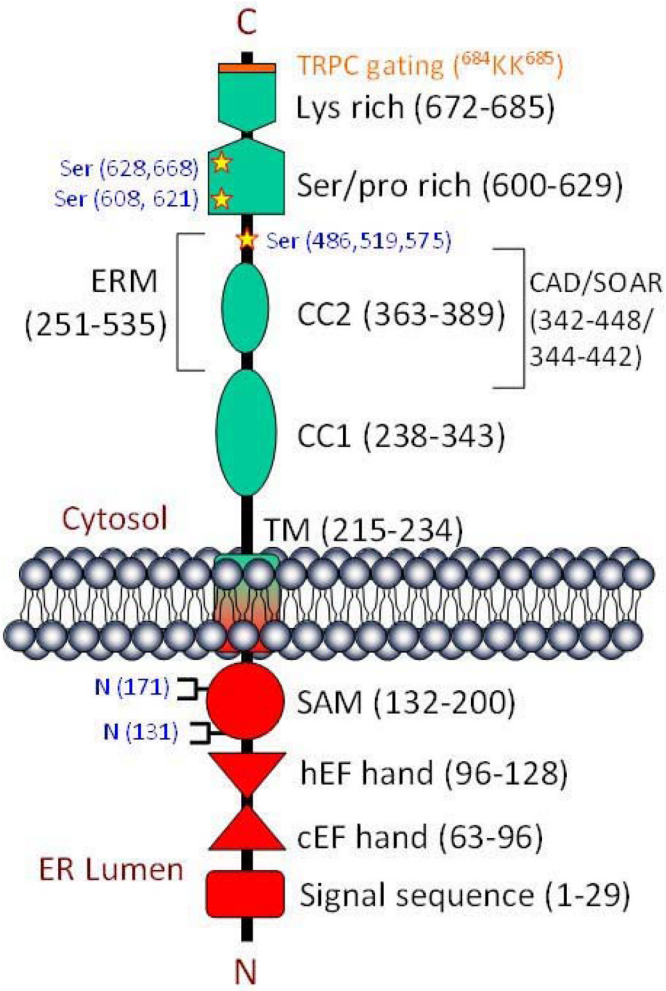

Figure 2.

STIM1 domains. This model (not drawn to scale) depicts the critical domains of STIM1 with its N-terminal in the ER lumen and the C-terminal in the cytosol. The amino acids that span individual domain are shown within parentheses. The lysine (K) residues, critical for TRPC channel gating (shown in orange), lie within the poly-K region in the cytoplasmic domain on STIM1. The cytoplasmic domain of STIM1 also contains the overlapping Orai1 activating regions (CAD/SOAR). Shown in blue are the residues that are glycosylated (N-terminal) or phosphorylated on serine (C-terminal). Abbreviations - c/hEF hand (canonical/hidden), SAM (sterile alpha motif), TM (trans membrane), CC1/2 (coiled-coil), ERM (Ezrin-Radixin-Moesin), CAD (CRAC activation domain), SOAR (STIM1 Orai activating region)

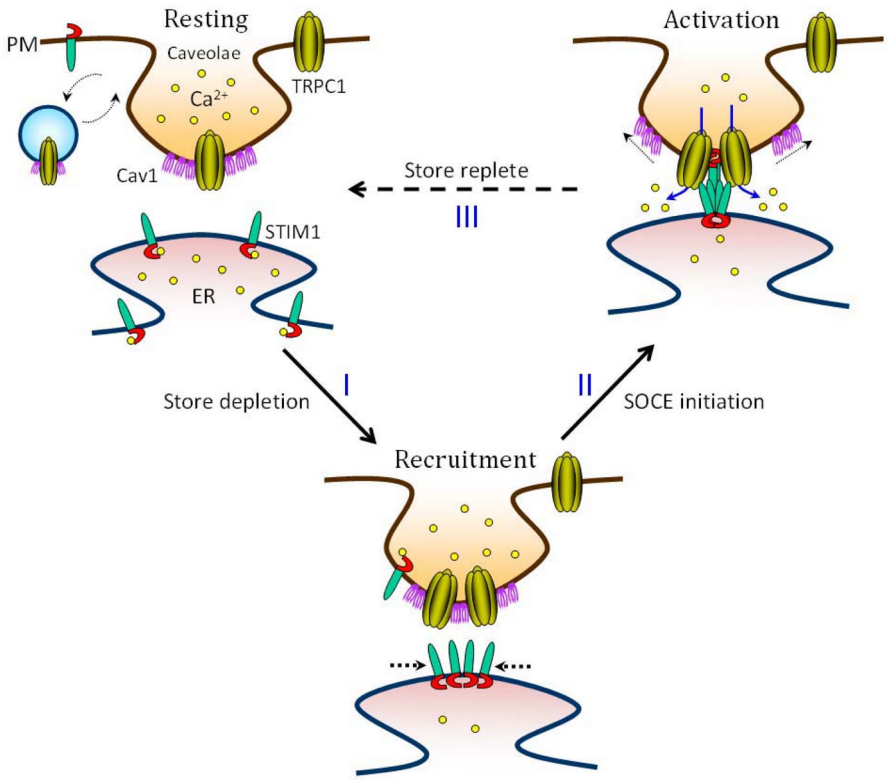

Figure 3.

Steps in TRPC1 channel activation. This model shows the compartmentalization TRPC1-mediated Ca^{2+} influx at ER-caveolar/membrane raft juxtaposed microdomains. In *'Resting'* state (ER-stores filled)- (a) STIM1, predominantly in the ER, is bound to Ca^{2+} and displays a diffused localization pattern, (b) PM-associated STIM1 is also shown, however, its contextual relevance is currently unknown, (c) PM-TRPC1 differentially associates with caveolin1 (cav1) enriched - caveolar and non-caveolar microdomains and (d) a steady-state PM trafficking of TRPC1 is achieved by vesicular activity. Between resting and activation, we propose an intermediate step of channel *'Recruitment'*, wherein, following ER Ca^{2+} *store-depletion* (step-I), (a) STIM1 unbinds Ca^{2+} and engages in oligomeric cluster (puncta) formation, (b) TRPC1 is recruited to caveolar raft domains wherein Cav1 scaffolds PM-TRPC1 thus retaining the channel at discrete ER apposed PM microdomains. The channel *'Activation'* is marked by *SOCE initiation* (step II) where, (a) STIM1 interacts with TRPC1 and (b) activates the channel resulting in the increase of $(Ca^{2+})_{cyt}$ with the subsequent dissociation of Cav1. $(Ca^{2+})_{cyt}$ is then sequestered back to the ER to refill the ER-stores as indicated by *store replete* (step III), following which (a) STIM1 binds Ca^{2+} and dissociates from TRPC1 getting back to the resting state. As the filled status of ER controls the STIM1-puncta kinetics it also determines the dynamic and reversible STIM1-TRPC1 associations. The molecular events ensuing each step (step I through step III) outlined in this model may not necessarily reflect their exact physiological sequence and further investigation in this aspect is necessary to delineate the exact

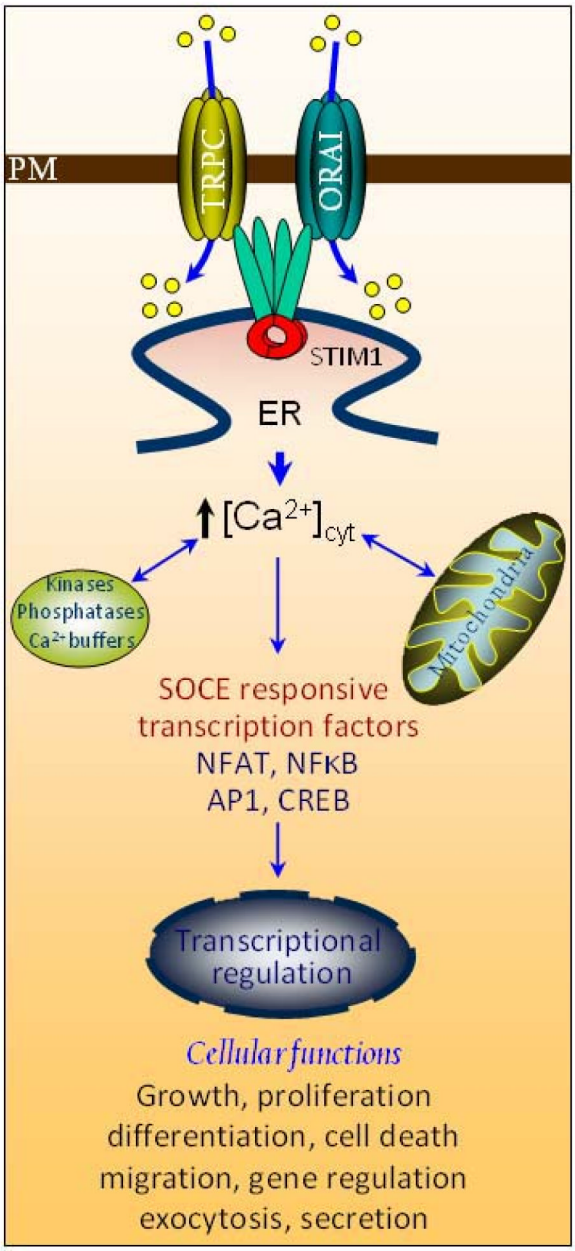

Figure 4.

STIM1 activated SOCE and cell function. This model illustrates downstream signaling following STIM1-activated SOCE. Activation of PM-SOCC (TRPC/Orai channels) by STIM1 accounts for store-operated Ca^{2+} signaling following increase in $(Ca^{2+})_{cyt}$. To impact on cell signaling, the SOCE-induced $(Ca^{2+})_{cyt}$ reciprocates with organelle such as mitochondria and signaling intermediates including kinases (such as PKC, CaMKs, ERK), phosphatases (such as calcineurin) and Ca^{2+} binding proteins like calmodulin. On a long term, SOCE influences a variety of cellular functions and brings about observable physiological changes by gene regulation. As STIM1 has the potential to influence many signaling pathways, it might be pivotal to studying pathological mysteries such as cancer and neurodegeneration.

Table 1

TRPC-STIM1 association and physiological response

Interaction	Cell line/tissue	Method	Physiology
TRPC1-STIM1	HEK293 (40, 82, 87, 121, 123, 143) HSG (68, 79, 124, 144) HL-7702 (normal human liver cell line) (145) Human platelets (125, 146) Human Parathyroid (147) Human VSMCs (130, 132) Intestinal epithelial cells (133) Pulmonary artery cells (141–142) Human glomerular mesangial cells (148) Mouse pancreatic acinar cells (128)	Co-IP, GST-pull down RNAi, TIRF, FRET Ca^{2+} measurements Confocal imaging Co-fractionation Co-expression	SOCE activation Cell proliferation Cell migration Wound healing Transcriptional regulation (NFκB, CREB) Ternary complex with Orai1
TRPC2-STIM1	HEK293 (40, 123)	Co-IP, GST-pull down	n.d
TRPC3-STIM1	HEK293 (40, 87, 123, 143, 149)	Co-IP (indirect)	Agonist-induced Ca^{2+} entry
TRPC4-STIM1	HEK293 (40, 87, 123) Human glomerular mesangial cells (148)	Co-IP, GST-pull down, RNAi, Ca^{2+} measurements	SOCE activation
TRPC5-STIM1	HEK293 (87)	Co-IP	SOCE activation
TRPC6-STIM1	HEK293 (40, 87, 143)	Co-IP (indirect)	Agonist-induced Ca^{2+} entry
TRPC7-STIM1	HEK293 (150)	Co-IP (no interaction)	n.d