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Review

# Tuning store-operated calcium entry to modulate $Ca^{2+}$-dependent physiological processes

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A B S T R A C T

The intracellular calcium signaling processes are tightly regulated to ensure the generation of calcium signals with the specific spatiotemporal characteristics required for regulating various cell functions. Compartmentalization of the molecular components involved in the generation of these signals at discrete intracellular sites ensures the signaling specificity and transduction fidelity of the signal for regulating downstream effector processes. Store-operated calcium entry (SOCE) is ubiquitously present in cells and is critical for essential cell functions in a variety of tissues. SOCE is mediated via plasma membrane $Ca^{2+}$ channels that are activated when luminal $[Ca^{2+}]$ of the endoplasmic reticulum ($[Ca^{2+}]_{ER}$) is decreased. The ER-resident stromal interaction molecules, STIM1 and STIM2, respond to decreases in $[Ca^{2+}]_{ER}$ by undergoing conformational changes that cause them to aggregate at the cell periphery in ER-plasma membrane (ER-PM) junctions. At these sites, STIM proteins recruit Orai1 channels and trigger their activation. Importantly, the two STIM proteins concertedly modulate Orai1 function as well as the sensitivity of SOCE to ER-$Ca^{2+}$ store depletion. Another family of plasma membrane $Ca^{2+}$ channels, known as the Transient Receptor Potential Canonical (TRPC) channels (TRPC1-7) also contribute to sustained $[Ca^{2+}]_i$ elevation. Although $Ca^{2+}$ signals generated by these channels overlap with those of Orai1, they regulate distinct functions in the cells. Importantly, STIM1 is also required for plasma membrane localization and activation of some TRPCs. In this review, we will discuss various molecular components and factors that govern the activation, regulation and modulation of the $Ca^{2+}$ signal generated by $Ca^{2+}$ entry pathways in response to depletion of ER-$Ca^{2+}$ stores. This article is part of a Special Issue entitled: ECS Meeting edited by Claus Heizmann, Joachim Krebs and Jacques Haiech.

## 1. Introduction

The intracellular calcium signaling processes are tightly regulated to ensure the generation of spatiotemporally specific calcium signals. The magnitudes of calcium signals vary depending on the stimuli, with low intensity stimulus generating oscillatory responses and higher-intensity stimulus producing a more sustained response. Such responses may be transient in nature or persist for a long period of time. The pattern and characteristics of these cytosolic $Ca^{2+}$ signals critically determine the regulation of cell function. Compartmentalization of the molecular components involved in the generation of these signals at discrete intracellular sites ensures the signaling specificity and transduction of the signal to elicit responses in downstream effector processes [1]. $[Ca^{2+}]_i$ increases are initiated by intracellular release of $Ca^{2+}$ mediated by $Ca^{2+}$ channels, such as inositol 1,4,5-triphosphate receptor ($IP_3R$), and by $Ca^{2+}$ influx channels in the plasma membrane. Among the $Ca^{2+}$ entry mechanisms, store-operated calcium entry (SOCE) is ubiquitously present in cells and is required for regulation of essential cell functions in a variety of tissues. SOCE is mediated via plasma membrane $Ca^{2+}$ channels that are activated when luminal $[Ca^{2+}]$ in the endoplasmic reticulum ($[Ca^{2+}]_{ER}$) is decreased. Physiologically, ER-$Ca^{2+}$ stores are depleted by the action of $IP_3$, which is generated following hydrolysis of the plasma membrane phosphatidylinositol 4,5-bisphosphate ($PIP_2$), on its receptor $IP_3R$, a $Ca^{2+}$ channel located in the ER membrane. Experimentally, depletion of the ER-$Ca^{2+}$ stores can be induced by treating cells with inhibitors of the SERCA pump, such as thapsigargin and cyclopiazonic acid, which unmask a $Ca^{2+}$ leak pathway in the ER via an as yet unidentified pathway. Early work suggested the presence of a $Ca^{2+}$ sensing mechanism in the ER which conveys the change in status of $[Ca^{2+}]_{ER}$ to plasma membrane channels to cause activation of $Ca^{2+}$ entry. Extensive work over three decades culminated in the identification of the molecular components of SOCE as well as the underlying regulatory mechanism.

The ER-resident stromal interaction molecules, STIM1 and STIM2 were first identified as the $Ca^{2+}$ sensing proteins that respond to decreases in $[Ca^{2+}]_{ER}$ by undergoing conformational changes that cause them to aggregate near the plasma membrane [2–4]. Shortly after, Orai1 a four- transmembrane channel was discovered by two separate groups and established to be crucial for SOCE [5–8]. Discrete sites at the cell periphery where STIM1 and STIM2 aggregate in response to ER-$Ca^{2+}$ store depletion have been referred to as ER-plasma membrane (ER-PM) junctions, where the ER membrane and plasma membrane lie in close apposition to each other [9–11]. STIM clustering at these sites facilitates the recruitment, clustering and activation of Orai1 channels. Orai1 forms the pore of the Calcium Release Activated Calcium (CRAC) channel, which is highly selective for $Ca^{2+}$ and mediates the inwardly

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rectifying $Ca^{2+}$-release activated $Ca^{2+}$ current ($I_{CRAC}$). This was the first store-operated $Ca^{2+}$ current to be described in studies using T-lymphocytes and mast cells [12–18]. Since the discovery of STIM1 and Orai1, substantial evidence has been provided to establish that these two proteins are sufficient for the generation of $I_{CRAC}$ in many cell types [19–21]. Nonetheless, cell-specific differences in the currents activated by ER-$Ca^{2+}$ store depletion have been reported, suggesting that different channel components and/or regulatory proteins may underlie such diversity [22–24].

Another family of plasma membrane $Ca^{2+}$ channels, known as the Transient Receptor Potential Canonical (TRPC) channels, have also been shown to contribute to $[Ca^{2+}]_i$ elevation detected following store depletion induced by SERCA pump inhibitors and/or agonist stimulation. TRPC1 is the first member of the TRPC family to be cloned, with six additional members (TRPCs 2-7) subsequently identified. TRPC channels were discovered prior to Orai1 and STIM1 and all TRPC members have been proposed as channel components of SOCE in several studies. The TRPC channels exhibit diverse channel properties and can be divided into subgroups based on their biochemical and functional similarities: TRPC1/4/5, TRPC2 and TRPC3/6/7 [25,26]. Among these channels, strongest evidence has been provided for the involvement of TRPC1 in SOCE in several cell types [27]. However, TRPC-mediated currents, unlike $I_{CRAC}$, display a lower $Ca^{2+}$ selectivity, or are non-selective, with a larger conductance [23]. Collectively, currents associated with activation of TRPC channels in response to receptor stimulation and/or internal $Ca^{2+}$ store depletion have been referred to as $I_{SOC}$ [28]. TRPC1, which contributes to currents activated by ER-$Ca^{2+}$ store depletion, was shown to cluster in plasma membrane domains with STIM1 following stimulation of the cells. Importantly, STIM1 was shown to activate the channels (further described below). TRPC1/STIM1 channels have distinct characteristics and physiological functions, when compared to Orai1/STIM1 channels. In this review, we will discuss various molecular components and factors that activate, regulate and modify the $Ca^{2+}$ signal generated by $Ca^{2+}$ entry that is triggered in response to depletion of ER-$Ca^{2+}$.

## 2. Regulation of SOCE by STIM proteins

STIM1 and STIM2 are primary regulators of $Ca^{2+}$ entry mediated by Orai1 and TRPC channels. Importantly, signaling via these proteins spans from within the ER to the plasma membrane. Thus, critical molecular domains are localized within the N-terminus in the ER lumen for $Ca^{2+}$ sensing and in the cytosolic C-terminus for protein clustering as well as for interaction and activation of Orai1 and TRPCs (Fig. 1). Precise control of intramolecular interactions governs the requisite conformational changes involved in STIM interaction with and activation of Orai1. The STIM proteins share considerable amino acid (aa) sequence homology as well as molecular domain homology, including the EF hand, sterile α motif (SAM), coiled-coil regions (CC1-3) and the lysine-rich region (also known as the polybasic domain) (Fig. 1). Nonetheless, there are a few, highly significant differences between them that account for their distinct properties and functions. These same distinctions enable STIM1 and STIM2 to tune SOCE-generated $[Ca^{2+}]_i$ signals for modulating downstream $Ca^{2+}$-dependent physiological functions.

### 2.1. Physiological relevance of ER-$Ca^{2+}$ sensing

STIM1 and STIM2 sense changes in $[Ca^{2+}]_{ER}$ via the N-terminal EF hand domain located within the ER lumen. There are two $Ca^{2+}$-binding motifs within the EF hand domain: canonical and non-canonical. Both motifs and the SAM domain form what is known as the EF-SAM core, which functions as the $Ca^{2+}$ sensing machinery of both STIMs. It has been suggested that a loop in the canonical motif binds to a single $Ca^{2+}$ ion, while a second loop in the non-canonical motif stabilizes the first loop by forming backbone hydrogen bonds [29–31]. The $Ca^{2+}$ binding affinities of the EF hand domains in the STIM proteins are significantly distinct. The canonical motif within the STIM1-EF hand domain has a stronger affinity for $Ca^{2+}$ when compared to that of STIM2. This necessitates a substantial depletion of the ER-$Ca^{2+}$ stores to induce a response in STIM1. In contrast, the STIM2 EF hand domain has a lower affinity that has been associated with a 3 aa difference in the sequence, which allows it to respond to smaller reductions in $[Ca^{2+}]_{ER}$ [3,32–34]. As a result, STIM2 has been proposed to play a role in maintaining resting $[Ca^{2+}]_i$ in cells, to gate Orai1 in cells stimulated with low [agonist], and to promote the clustering and function of STIM1/Orai1 in the ER-PM junctions when $[Ca^{2+}]_{ER}$ is high [3,35–37]. The latter role is physiologically relevant as cells are often stimulated with low [agonist] that induce small amounts of ER-$Ca^{2+}$ store depletion. As shown in Fig. 2, the pattern of $[Ca^{2+}]_i$ signals vary depending on the level of $[Ca^{2+}]_{ER}$. A more sustained and elevated response is seen when ER-$Ca^{2+}$ depletion is substantial, such as following stimulation with high [agonist]. On the other hand, more oscillatory responses are observed when cells are stimulated with low [agonist], which generates smaller depletion of the ER-$Ca^{2+}$. When STIM2 is expressed in cells, it forms puncta in the absence of stimulation and following low [agonist] stimulation, whereas STIM1 clusters only at relatively higher [agonist]. When co-expressed with STIM1, STIM2 recruits and facilitates STIM1 clustering in the ER-PM junctions. This results in an enhancement of SOCE due to activation of Orai1 by the recruited STIM1 [37,38]. STIM1 and STIM2 have distinct contributions to SOCE. Loss of either STIM1 or Orai1 eliminates SOCE, while knockdown of STIM2 alters the pattern of $[Ca^{2+}]_i$ responses [37,39]. Loss of STIM2 decreases $[Ca^{2+}]_i$ oscillations normally seen at low [CCh], while at high [CCh] the typical sustained elevation in $[Ca^{2+}]_i$ is converted to a more oscillatory response, more like what is detected with relatively lower [CCh] [37]. These findings suggest that STIM2 enhances the sensitivity of SOCE to low intensity stimuli.

The EF hand domains of the STIM proteins provide essential control of their activation in response to ER-$Ca^{2+}$ depletion. Mutations in the $Ca^{2+}$ binding domain of STIM1 EF hand to resemble those in STIM2 caused constitutive puncta formation of the protein and activation of Orai1 in absence of stimulation [34]. A key step in STIM-mediated activation of SOCE is oligomerization of the protein. The SAM domain has a critical role in oligomerization of STIM proteins and regulates the obligatory first step that triggers the conformational remodeling of the C-terminal domains. Deletion of the SAM domain impairs the ability of STIM proteins to form puncta [40]. Resolution of the structure of STIM1

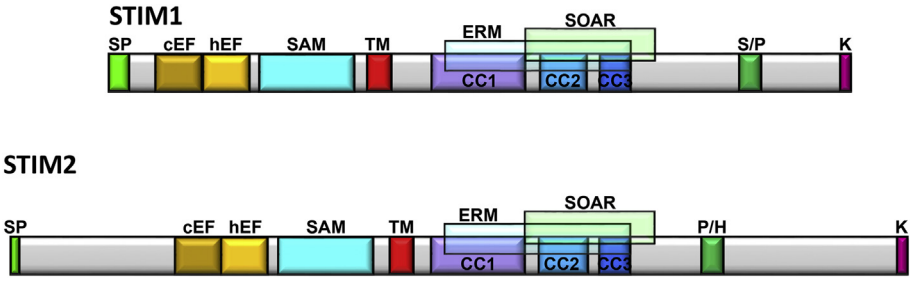


**Fig. 1.** Schematic diagram showing various domains of STIM1 and STIM2 proteins. SP: signal peptide; cEF: canonical EF hand; hEF: hidden EF hand; SAM: sterile-α-motif; TM: transmembrane region; CC1 to CC3: coiled-coil domains 1 to 3; ERM: ezrin/moesin/radixin domain; SOAR: STIM Orai1 Activating Region; S/P: serine/proline-rich region; P/H: proline/histidine-rich region; K: polybasic or lysine-rich domain.

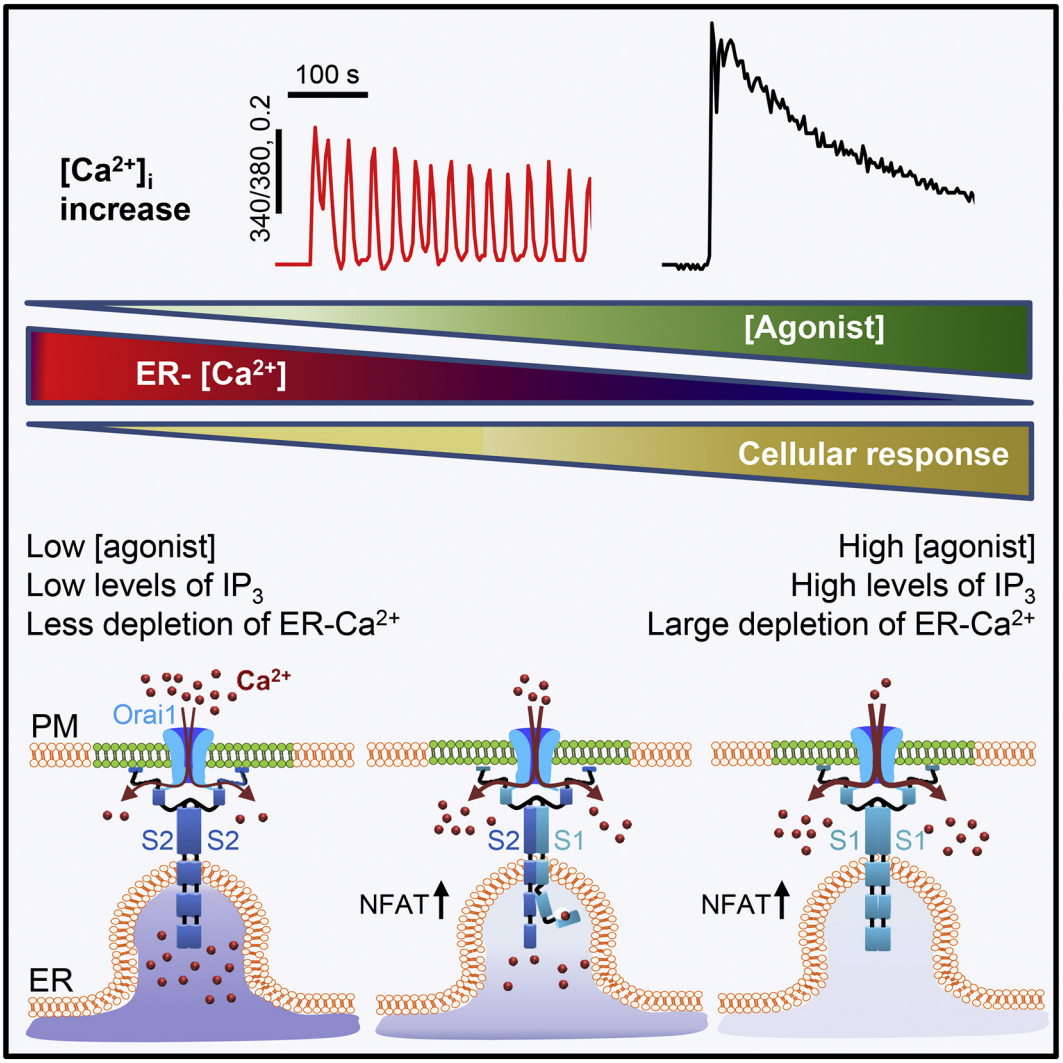


**Fig. 2.** Relationship between $[Ca^{2+}]_{ER}$ and regulation of SOCE by STIM proteins. Reproduced with permission from [38]. This model shows the pattern of $Ca^{2+}$ signals generated by increasing stimulus intensities. At ambient ER-$[Ca^{2+}]$ STIM2 is the primary regulator of Orai1 function, causing a low-activity state of the channel. Stimulation of cells with low [agonist] triggers small reduction in ER-$[Ca^{2+}]$ and generates an oscillatory $Ca^{2+}$ signaling pattern. Under these conditions, activation of Orai1 is dependent on the recruitment and activation of STIM1 in ER-PM junctions by STIM2, resulting in a relatively higher level of Orai1 activity. At relatively high [agonist] stimulation induces substantial depletion of ER-$Ca^{2+}$, causing sustained (non-oscillatory) $Ca^{2+}$ elevation. Under these conditions, Orai1 activity is determined by STIM1, which can respond robustly at low ER-$[Ca^{2+}]$. Activation of NFAT is observed when Orai1 is gated by STIM1.

EF-SAM domain revealed that destabilizing the intramolecular interactions within the core is central to the oligomerization of this portion of STIM1 [41,42]. Studies reported by Ikura and coworkers have shown fundamental differences in the oligomerization dynamics of the EF-SAM portions of STIM1 and STIM2 in response to decreases in $[Ca^{2+}]_{ER}$ [33,34,41]. The EF-SAM core in STIM1 displays rapid partial unfolding coupled with oligomerization, relative to the domain in STIM2. The dampened aggregation kinetics and increased stability of STIM2 EF-SAM core, relative to STIM1, is a possible contributing factor for the differential effects of the two proteins on Orai1 function and $Ca^{2+}$ signaling.

### 2.2. STIM Orai1 Activating Regions

A small fragment within the cytoplasmic region of STIM proteins, known as SOAR (STIM Orai1 Activating Region) or CAD (CRAC Activation Domain), is the minimal sequence required for interaction with and activation of Orai1 [43,44]. SOAR of STIM2 (SOAR2) shares > 80% amino acid identity with that of STIM1 (SOAR1) and this was further confirmed by homology modeling of SOAR2, which showed almost exact superimposition of its backbone with that of SOAR1 [45,46]. However, coupling of the two SOARS with Orai1 as well as activation of the channel are remarkably different. STIM2 (also referred to as STIM2α or STIM2.2) is a weaker activator of Orai1 than STIM1, inducing less channel currents and $Ca^{2+}$ entry when it is expressed with Orai1 [3,37,45,47]. The stronger Orai1 binding and gating exhibited by STIM1 is due to a single aa ($Phe^{394}$) in SOAR1. Substitution of the corresponding aa in SOAR2 with leucine ($Leu^{485}$) accounts for the reduced binding of STIM2 with Orai1, as well as weaker activation of the channel [45]. Thus, a second level in the tuning of Orai1 channel function is provided by the SOAR domains in the two proteins. Recently, an alternatively spliced variant of STIM2 (STIM2.1 or STIM2β) was identified by two groups [48,49]. STIM2.1 is suggested to have an inhibitory effect on SOCE due to the insertion of 8 additional aa (VAASYLIQ) within its SOAR domain. According to the presently available data, this STIM2 variant cannot bind to Orai1 efficiently or gate the channel. However, it can still bind and recruit STIM1 to ER-PM junctions and thus could serve as a scaffold for STIM1-Orai1 interaction under certain conditions [48,49]. Hence, recruitment and activation of Orai1 by different STIM proteins can result in variable magnitudes of

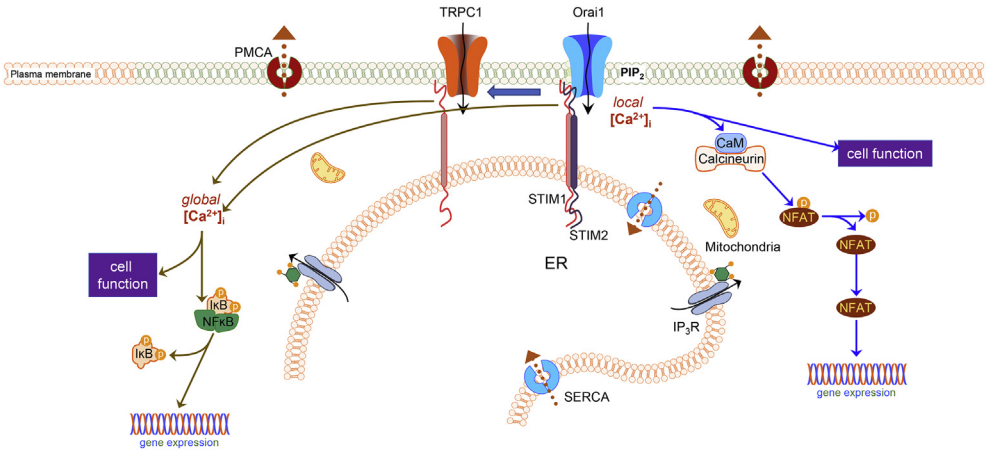


**Fig. 3.** Tuning of SOCE-generated $Ca^{2+}$ signals for regulation of cell function. Agonist stimulation of the cell stimulates $PIP_2$ hydrolysis to generate $IP_3$, which binds to $IP_3R$ causing $Ca^{2+}$ release from the ER lumen. In response to the decrease in ER-$[Ca^{2+}]$, STIM proteins aggregate and translocate to ER-PM junctions (denoted in green in the plasma membrane) where they recruit and activate Orai1. Plasma membrane $PIP_2$ has been suggested to play an important role in assembly of Orai1/STIM complexes. The resulting increase in $[Ca^{2+}]$ near the channel pore, termed local $[Ca^{2+}]_i$, drives activation of NFAT. In some cell types, the increase in local $[Ca^{2+}]_i$ triggers recruitment of TRPC1 to the plasma membrane which is also activated by STIM1 and amplifies the $Ca^{2+}$ signal generated by Orai. TRPC1-dependent increase in $[Ca^{2+}]_i$ is utilized by cells to regulate distinct functions such as activation of NFκB and exocrine gland function. Activities of PMCA, SERCA, and mitochondria contribute to modulation of SOCE-generated $[Ca^{2+}]_i$ signal. CaM: calmodulin; ER: endoplasmic reticulum; $IP_3R$: inositol 1,4,5-triphosphate; NFAT: nuclear factor of activated T cells; NFκB: nuclear factor kappa-light-chain-enhancer of activated B cells; $PIP_2$: phosphatidyl 4,5-bisphosphate; PMCA: plasma membrane $Ca^{2+}$-ATPase pump; SERCA: sarco-endoplasmic reticulum $Ca^{2+}$-ATPase pump.

SOCE to generate distinct patterns of $[Ca^{2+}]_i$ signals. How the pattern of $[Ca^{2+}]_i$ signals is coupled to the regulation of downstream effector functions has not yet been fully characterized in different cell types. Furthermore, there is a significant gap in our knowledge regarding the status and function of endogenous STIM proteins as most of the function and localization studies, excepting those with knockdown or knockout of the proteins, have been carried out with heterologously expressed proteins.

### 2.3. The polybasic domain

The C-terminus of both STIMs contain a positively charged polybasic domain that binds electrostatically to the plasma membrane $PIP_2$. The basic function of this domain is to anchor activated STIM proteins within ER-PM junctions such that the SOAR domain is available for recruitment and activation of Orai1. The polybasic domain of STIM2 shows a stronger affinity for $PIP_2$ than STIM1 [50,51]. The combined effect of a lower $Ca^{2+}$-binding affinity of its EF hand and a stronger plasma membrane $PIP_2$ binding of its polybasic domain likely enables STIM2 to form stable clusters within the ER-PM junctions when cells are stimulated with low [agonist] or, in some cases, in the absence of stimulation. STIM proteins lacking this domain are not able to form puncta following ER-$Ca^{2+}$ store depletion. However, Orai1 can bind to the SOAR domain of the STIM1 mutant lacking its polybasic domain (STIM1ΔK) and induce recovery of its clustering in ER-PM junctions. In contrast, Orai1 does not bind to, or induce, recovery of STIM2ΔK5 [37], most likely due to the weaker binding of STIM2-SOAR to Orai1. Notably, STIM2ΔK5 also displays poor interaction with STIM1 [38]. This suggests that in STIM2, the polybasic domain is critical for targeting the protein to ER-PM junctions and the SOAR domain is involved in interactions with other STIM proteins and Orai1. As noted above, STIM1 and STIM2 can co-cluster within ER-PM junctions and recruit Orai1 to cause assembly of Orai1/STIM complexes. Thus, the relative amounts of STIM1 and STIM2 within the ER-PM domain can contribute to the stability of channel complex via interactions of their polybasic domains with the plasma membrane. Current models support the suggestion that at low [agonist] there will be relatively more STIM2 than STIM1 in the complex (Fig. 2). Under these conditions, the stronger STIM2-polybasic domain will be critical for this stability.

## 3. STIM2-STIM1 interactions in the tuning of SOCE

Most recently, it has been reported that STIM2 and STIM1 have a concerted role in the regulation of SOCE. Several lines of data suggest that a small population of STIM2 likely resides within the ER-PM junctions of unstimulated cells where it recruits and constitutively activates Orai1. This is supported by data showing that knockdown of STIM2 decreases resting $[Ca^{2+}]_i$ in some cell lines while overexpression leads to constitutively clustered STIM2 and increased activity of endogenous Orai1 channel [37]. The latter observation suggests that STIM2 can acquire an activated conformation without depletion of ER-$Ca^{2+}$, i.e. with access of SOAR2 domain to Orai1. This has recently been confirmed using a STIM2-derived conformational sensor (YFP-OASF2-CFP) that displayed low FRET, which is indicative of a disruption in CC1-CC3 domain interaction. Similar measurements with the STIM1-derived conformational sensor (YFP-OASF1-CFP) demonstrated high resting FRET, unless Orai1 was co-expressed or mutations were introduced to disrupt CC1-CC3 interaction [38]. The relaxed conformation of STIM2 C-terminus can be a major determining factor in the constitutive clustering of STIM2 in ER-PM junction and activation of Orai1 in unstimulated cells.

It is well established that STIM1-STIM1 interactions are necessary for activation of the protein and its translocation to ER-PM junctions and regulation of Orai1. We have shown previously that STIM2 facilitates recruitment of STIM1 to ER-PM junctions at low [agonist] [37]. Consistent with this, knockdown of STIM2 decreased SOCE function at low, but not high, stimulus intensities. Notably, STIM1 is critically required for SOCE even at these low [agonist] suggesting that STIM1 can activate Orai1 if STIM2 is present but cannot do so on its own [37]. These findings led to the proposal that STIM2-STIM1 interaction triggers activation of STIM1 under conditions where $[Ca^{2+}]_{ER}$ is high and STIM1 cannot respond by itself (Fig. 3). The critical role of STIM2 in regulating STIM1 function was demonstrated using the conformational sensor for STIM1, YFP-OASF1-CFP [38]. As noted above, the OASF domain of STIM1 is in an inactive conformation due to CC1-CC3 interactions, which causes a high FRET signal. When STIM2 is expressed together with YFP-OASF1-CFP, it reduces the FRET signal. This confirms that binding of STIM2 to STIM1 causes remodeling of the STIM1 C-terminus triggering an activated conformation. This results in Orai1/STIM1 coupling and gain of Orai1 function in cells with minimal ER-$Ca^{2+}$ store depletion [38]. The SOAR of STIM2 plays an important role in mediating its binding with and causing the conformational remodeling of STIM1. STIM2 protein lacking SOAR (STIM2ΔSOAR) showed very weak interaction with STIM1 as less STIM2ΔSOAR (*c.f.* STIM2) co-immunoprecipitated with STIM1. Furthermore, STIM2ΔSOAR was unable to change the FRET signal of the YFP-OASF1-CFP

when co-expressed in the same cell [38]. There is increasing data to show that STIM2-STIM1 interactions can modulate Orai1 function. Studies in αT3 and NIH3T3 cells have suggested that STIM2 recruits and stabilizes STIM1 in plasma membrane-associated ER membrane junctional nanodomains, thus facilitating its interaction with Orai1 and activation of SOCE [52]. STIM2.1 (or STIM2β) a highly conserved alternatively spliced isoform of STIM2, inhibits of SOCE [48,49]. Interestingly, although STIM2.1 does not by itself strongly bind Orai1, it is recruited to Orai1 channels by forming heterodimers with other STIM isoforms. Analysis of STIM2.1 mutants and Orai1-STIM2.1 chimeras suggested that it actively inhibits SOCE through a sequence-specific allosteric interaction with Orai1. Additionally, it was recently reported that STIM2.1 can also interact with STIM1 and be recruited to ER-PM junctions [53]. Contrary to earlier predictions, the SOAR1–SOAR2.1 heterodimer activated Orai1 channels but prevented clustering of the channels. Similar results were obtained with the concatenated SOAR1-SOAR1F394H heterodimer. Interestingly, expression of full-length STIM1 with STIM2.1 suppressed the sustained agonist-induced $Ca^{2+}$ oscillations, suggesting a possible dominant negative effect being exerted by STIM2.1 [53]. Thus, STIM proteins appear to have an inherent flexibility in their ability to regulate SOCE as a means to shape the amplitude and dynamics of cytosolic $Ca^{2+}$ signals. SOCE function can be predicted to increase in the following order: Orai1/STIM2.1-STIM2.1 < Orai1/STIM2.1-STIM2 < Orai1/STIM2-STIM2 < Orai1/STIM2-STIM1 < Orai1/STIM1-STIM1 [38,53]. Having the ability to tune SOCE as required for normal regulation of physiological functions or in response to exigencies and stress is of immense value to the cell.

## 4. Regulators of ER-PM junctions and SOCE-generated $[Ca^{2+}]_i$ signals

Several proteins promote the stability and remodeling of the ER-PM junctions required for regulating and maintaining Orai1/STIM1 function. Recent studies have identified a STIM1 interacting protein, STIMATE/TMEM110, which promotes its active conformation and stabilizes STIM1-dependent ER-PM junctions [54,55]. Another protein called SARAF (SOCE-associated regulatory factor) regulates STIM1 to control the slow $Ca^{2+}$-dependent inactivation of Orai1. Both these regulatory proteins are recruited to ER-PM junctions after stimulation when Orai1/STIM1 cluster. Another group of ER proteins known as extended synaptotagmins (E-Syt1, E-Syt2, and E-Syt3) have $PIP_2$-binding domains through which they interact with the plasma membrane $PIP_2$. In resting cells, E-Syt1 displays diffuse ER localization, while E-Syt2 and E-Syt3 appear to predominantly localize in ER near the PM [56,57]. It is suggested that E-Syt2 and E-Syt3 mediate ER-PM tethering and remodeling of the junctions (e.g. expansion), while E-Syt1 is recruited to the junctions in response to $Ca^{2+}$ entry. The exact function of E-Syts in modulating SOCE in different cell types has not yet been elucidated.

Remodeling of plasma membrane $PIP_2$ as well as the actin cytoskeleton locally at the sites of Orai1/STIM1 assembly can also potentially impact the assembly and function of Orai1/STIM1. Septins are suggested to be involved in plasma membrane $PIP_2$ regulation and affect the assembly of Orai1/STIM1 in the ER-PM junctions. Septins assemble as filaments following binding with plasma membrane $PIP_2$ and their major proposed role is to function as diffusion barriers [58]. Knockdown of septin altered the assembly and function of Orai1/STIM1 likely due to disruptions of essential plasma membrane domains and the architecture of ER-PM junctions. Since $PIP_2$ remodeling in the plasma membrane can affect the status of cortical actin, altering $PIP_2$ levels can induce cytoskeleton remodeling. Conversely, cytoskeletal remodeling can modify the plasma membrane $PIP_2$ microdomains. Hartzell et al. reported that $Ca^{2+}$-dependent retrograde flow of actin corrals ER with Orai1/STIM1 complexes at T cell synapses which governs CRAC channel localization. In this case, $Ca^{2+}$ influx via Orai1 acts as a trigger for the actin remodeling [59]. Another study has also described $PIP_2$-dependent changes in Orai1/STIM1 function and regulation. Maleth et al. reported that initial assembly of the Orai1/STIM1 complex takes place within $PIP_2$-poor microdomains. Subsequently, Orai1/STIM1 complex is associated with $PIP_2$-rich regions, either due to remodeling of plasma membrane $PIP_2$ within ER-PM junctions or relocation of the channel complex. While the exact mechanism is not well understood, this study showed that the feedback regulation of Orai1 channel activity occurs within the $PIP_2$-rich microdomain [60]. Proteins involved in lipid transfer have also been mapped to the ER-PM junctions and these can also be dynamically modulated (recently reviewed in [61]) and contribute to the stability of the Orai1/STIM1 complex.

Activities of molecular components (e.g., $IP_3$Rs, SERCA PMCA, or mitochondria) that are involved in regulating the $Ca^{2+}$ homeostasis also contribute to shaping the spatiotemporal characteristics of the Orai1/STIM1-generated $Ca^{2+}$ signal. Interestingly, PMCA, mitochondria and $IP_3$R have been shown to colocalize with STIM1 [62–64]. PMCA function as well as mitochondrial $Ca^{2+}$ uptake have been reported to alter the pattern of $[Ca^{2+}]_i$ increases and impact cellular function [63,65–68]. $Ca^{2+}$ entering the cell via SOCE is taken up into the mitochondria via MCU-mediated $Ca^{2+}$ uptake resulting in energization of the organelle. Conversely, mitochondrial $Ca^{2+}$ accumulation (1) reduces $Ca^{2+}$-dependent inactivation of SOCE, (2) modifies store depletion and thus affects SOCE indirectly, and (3) delays store refilling, prolonging sustained SOCE. In addition, mitochondria supplies ATP and/or other intermediate metabolites which impact SOCE. $Ca^{2+}$ uptake into mitochondria can lead to increase in ROS which is reported to modify SOCE and have significant consequences on cellular $Ca^{2+}$ handling. Impairment of SOCE due to mitochondrial malfunction adversely affects T and mast cell activation and decreases cell proliferation. Excessive $Ca^{2+}$ uptake into the mitochondria may lead to apoptosis or cell death, e.g. traumatic brain injury [69].

Mitochondrial regulation of SOCE is reported to sustain $Ca^{2+}$ entry that is critical for clonal expansion of activated T cells. Mitochondrial uncouplers that collapse mitochondrial potential ($\Delta\Psi$) prevent sustained SOCE in T cells. Importantly, inhibition of $Ca^{2+}$ uptake by mitochondria, prevents Orai1-mediated activation of NFAT-dependent gene expression. An important factor in the regulation of SOCE by mitochondria are their relative proximity to the CRAC channels. For example, mitochondria translocate to plasma membrane in response to SOCE in T cells which determines uptake of $Ca^{2+}$ into mitochondria near the site $Ca^{2+}$ entry, thus contributing to $Ca^{2+}$-dependent inactivation of CRAC channels. An increasing array of studies demonstrate that SOCE is remodeled in cancer and might control critical disease processes such as cell proliferation, cell migration and resistance to apoptosis Metabolic reprogramming, required for tumor cell proliferation and survival, is characterized by the high glycolytic rate and defective ATP synthesis by mitochondria. This altered metabolism also determines the response of the cells to certain chemotherapeutic drugs [70].

Low cytosolic $Ca^{2+}$ concentration is maintained by function of $Ca^{2+}$-ATPases, SERCA and PMCA in the ER and plasma membrane, respectively. Following stimulation, the function of these calcium pumps are modulated and significantly impact the complex and dynamic regulation of $[Ca^{2+}]_i$ signals. SERCA is responsible for replenishing ER $Ca^{2+}$ stores and protein folding/maturation with aberrant function causing depletion or overloading of ER-$Ca^{2+}$ store, ER stress, misfolding of proteins and dysregulation of chaperones. Mutations and altered expression levels of SERCA isoforms have been identified in various cancers, such as cancers of colon, gastric, lung, myeloid leukemia and choroid plexus tumors which cause changes in proliferation, migration and cellular signaling mechanisms. Altered PMCA function has also been associated with cancer such as breast cancer. Enhanced expression of _PMCA maintains low $[Ca^{2+}]_i$ which attenuates apoptosis by suppressing uptake of $Ca^{2+}$ into mitochondria. On the contrary, down-regulation of PMCA in colon or oral squamous cell carcinoma, increases $[Ca^{2+}]_i$ which enhances cell proliferation

[71].

## 5. Modulation of SOCE-generated $Ca^{2+}$ signals by TRPC channel regulation

All TRPC channels are activated in response to stimulation of the cells with various agonists. Thus, $Ca^{2+}$ entry via these channels could potentially be activated coincidentally with Orai1 following agonist stimulation and $IP_3$-mediated decrease in $[Ca^{2+}]_{ER}$. Consistent with this, $I_{CRAC}$ is not the only current activated by store depletion in some cell types. These cells display currents which have a lower $Ca^{2+}$ selectivity, or are non-selective, with a larger conductance [23]. Collectively, these have been referred to as $I_{SOC}$ and have been associated with activation of TRPC channels in response to receptor stimulation and/or internal $Ca^{2+}$ store depletion [72–74]. Some TRPC channels are activated by passive or active (i.e. mediated by agonist) depletion of ER-$Ca^{2+}$ stores, while others are only activated following receptor stimulation [72–74]. The latter channels are considered store-independent or receptor-operated. For example, activation of TRPC6 and TRPC7 in most cases is achieved via store-independent mechanisms where a role for channel regulation by $PIP_2$ or DAG has been proposed. Of all TRPCs, the available data on TRPC1 are the most consistent and strongest in support of its role in SOCE. Nonetheless, in a relatively small number of studies TRPC3, TRPC4, and TRPC5 have also been shown to be activated by store depletion. It is important to recognize that the presence of TRPC channel activity in cells does not preclude the essential role of Orai1/STIM1 channels in SOCE. Loss of either of these components eliminates $Ca^{2+}$ entry and CRAC channel activity in cells displaying SOCE. In contrast, knockdown of TRPC channels only causes partial reduction of $Ca^{2+}$ entry and in some cases causes a change of $I_{SOC}$ to $I_{CRAC}$, which is eliminated by knockdown of Orai1 or STIM1. Most notably, the component of $Ca^{2+}$ entry associated with TRPC channel function is also abrogated by knockdown of either STIM1 or Orai1, indicating that Orai1 and STIM1 govern TRPC channel activity [75–80]. The additional $Ca^{2+}$ entry associated with TRPC channels are physiologically relevant and utilized by cells to regulate functions that are distinct from those governed by Orai1.

The polybasic domain of STIM1 plays an important role in activating the TRPC channels [81,82]. TRPC1 and TRPC4 have been proposed to be the main channels that interact with and are activated by STIM1, conferring ER-$Ca^{2+}$ store dependency/sensitivity on these channels. The negatively charged aspartate residues in TRPC1 ($^{639}$DD$^{640}$) and TRPC4 ($^{647}$EE$^{648}$) electrostatically interact with the positively charged $^{684}$KK$^{685}$ of STIM1. Swapping the charges on either the STIM1 polybasic domain (KK to EE) or on TRPC1 or TRPC4 (DD to EE) abrogates STIM1-TRPC interactions and SOCE [82,83]. When other TRPCs form a heteromeric channel complex with either TRPC1 or TRPC4, they gain store dependency due to STIM1 gating of TRPC1 or TRPC4. For example, TRPC3 and TRPC6 are not activated by store depletion and do not interact with STIM1. However, heteromeric complexes of TRPC1/TRPC3 and TRPC4/TRPC6 can interact with and be gated by STIM1. Studies that either knockdown endogenous STIM1 or overexpress STIM1 (wild type or various mutants) established the importance of STIM1-TRPC interactions for channel activity. Loss of endogenous STIM1 impaired TRPC1-mediated SOCE and $I_{SOC}$, whereas co-expression of STIM1 with TRPC1 boosted SOCE [75,80,81]. Notably, the STIM1-TRPC1 interaction is driven by ER-$Ca^{2+}$ store depletion since no such interaction was evident in unstimulated cells, as shown by studies utilizing co-immunoprecipitation, FRET and TIRFM techniques [81,82,84–87].

Several studies have addressed the critical functional interaction between Orai1 and TRPC1. It has been proposed that Orai1 physically modulates TRPC channels and confers STIM1-mediated activation in response to store depletion [78]. This study hypothesized that native SOCE is mediated by TRPC channels with Orai1 acting as a regulatory subunit, based on the findings that Orai1 physically interacts with the N- and C-termini of TRPC3 and TRPC6 in coimmunoprecipitation experiments [78]. Another prediction was that Orai1 and TRPC1 might form heteromeric channels, with properties distinct from that mediated by Orai1 or TRPC alone [77]. However, neither of these proposals has been further supported by other studies. Cheng et al. demonstrated that $Ca^{2+}$ entry via Orai1 triggers recruitment of TRPC1 to the plasma membrane. Increased TRPC1 expression in the surface membrane is prevented by blocking SOCE with 1 μM $Gd^{3+}$, removal of extracellular $Ca^{2+}$, knockdown of Orai1, or expression of dominant-negative mutant Orai1 lacking a functional pore (E106Q). This study also revealed that while Orai1-mediated $Ca^{2+}$ entry can trigger recruitment of TRPC1, gating of the channel is achieved by electrostatic interaction with STIM1 [75]. Thus, coordinated regulation of the surface expression of TRPC1 by Orai1 and gating by STIM1 provides a mechanism for rapidly modulating and amplifying SOCE-generated $Ca^{2+}$ signals. By recruiting ion channels and other signaling pathways, Orai1 and STIM1 concertedly impact a variety of critical cell functions that are dependent on SOCE, but not Orai1-mediated $Ca^{2+}$ entry per se. This is further demonstrated in acinar cells isolated from salivary glands as well as pancreas of TRPC1$^{-/-}$ mice, in which TRPC1 has been genetically ablated but Orai1 expression and localization is still maintained. Despite this, $Ca^{2+}$-dependent $K^+$ as well as $Ca^{2+}$-activated $Cl^-$ channel activities are substantially reduced in cells from salivary glands and exocrine pancreas of TRPC1$^{-/-}$ mice [88,89]. In the same cells, blocking Orai1 function or SOCE with 2-aminoethoxydiphenyl borate also eliminates the channel activities. These findings indicate that in exocrine gland acinar cells, TRPC1-mediated $Ca^{2+}$ entry preferentially regulates these ion channel activities and is primarily associated with fluid secretion. However, TRPC1 function is still dependent on Orai1-mediated $Ca^{2+}$ entry. Thus, it can be suggested that TRPC1-mediated $Ca^{2+}$ entry can amplify and/or modify the pattern of Orai1-generated $Ca^{2+}$ signal as required for regulation of $K^+$ and $Cl^-$ fluxes in these cells. Modification of SOCE-generated $[Ca^{2+}]_i$ signals by TRPC1 has been further established in studies showing that knockdown of TRPC1 changes the pattern of $[Ca^{2+}]_i$ elevation from sustained to oscillatory in a salivary gland cell line [90]. The residual $Ca^{2+}$ influx via Orai1 is sufficient for activation of NFAT but not the $Ca^{2+}$-activated $K^+$ channel. Together these studies provided strong evidence that TRPC channel function can overlap with Orai1 channels both spatially and temporally. Yet, the channels impact distinct downstream physiological functions. The specific effector proteins that are involved in sensing $Ca^{2+}$ for regulation of cell function are not yet known for TRPC1. In the case of Orai1, calmodulin and calcineurin relay channel function to activate NFAT1, a key regulator of $Ca^{2+}$-dependent gene expression.

In addition to TRPC1, other TRPC subtypes also appear to be regulated by Orai1, such as TRPC4 and TRPC5. TRPC5 forms an outwardly rectifying nonselective cation channel that can be directly activated by increases in $[Ca^{2+}]_i$ induced by Orai1–mediated $Ca^{2+}$ entry [91]. TRPC4 channels can also be similarly modulated by $Ca^{2+}$ [92], although exactly how Orai1 determines TRPC4 channel activity is not yet known. Furthermore, TRPC channels (e.g. TRPC3) that interact with TRPC1 to form heteromers can in principle be activated by STIM1 and also recruited to the plasma membrane. It is interesting to note that the trafficking of TRPC1/TRPV4 channel in smooth muscle cells, where both channels contribute to SOCE, have been shown to be dependent on Orai1 channel function [93,94].

Unlike STIM1, there has been far fewer studies that looked at the role of STIM2 in TRPC channel function. In mouse salivary glands, a targeted knockout of STIM2 markedly decrease pilocarpine-induced fluid secretion but only at low [pilocarpine] [37]. During wound healing, migration of intestinal epithelial cells is dependent on TRPC1-medicated SOCE. Rapid restitution of the epithelial layer is regulated by the intracellular levels of polyamines. An increase in [polyamines] stimulated STIM1 to promote the TRPC1-STIM1 interaction to enhance SOCE. In contrast, STIM2 expression and interaction with TRPC1 was inhibited. Exogenous expression of STIM2 in these epithelial cells

inhibits STIM1 translocation to the plasma membrane, adversely affecting TRPC1-STIM1 interaction [95]. Two different store-dependent currents in HEK293 cells have been reported to require STIM2. While the TRPC1-mediated $I_{max}$ current requires both STIMs, the $I_{min}$ current is solely dependent on STIM2 [96]. $I_{min}$ channels are reported to be store-independent but are relatively selective for divalent cations with single channel conductance of 1.2 pS. In contrast $I_{max}$ channels have higher conductance (17 pS) but are less selective for divalent cations. However, the latter can be activated by $InsP_3$, $Ca^{2+}$, or store depletion.

A reciprocal shift in the expression of TRPC1, Orai1, STIM1 and STIM2 in human colon carcinoma cells has been proposed to contribute to the $Ca^{2+}$ remodeling processes that drive tumorigenesis. Orai1, STIM1 and TRPC1 expression, SOCE, $I_{CRAC}$ and $I_{SOC}$ display enhancement while STIM2 expression is downregulated. This is suggested to favor the interactions of STIM1 with Orai1 and TRPC1 [97]. Upregulation of STIM2 expression contributes to the phenotypic switch of pulmonary arterial smooth muscle cells from a contractile to proliferative mode [98] while STIM2-mediated SOCE is crucial for the stability of mushroom dendritic spine structures [99]. In both the muscle and neuronal cells, STIM2 is proposed to mediate SOCE via its interaction with TRPC6 and Orai2. Further studies are required to elucidate the molecular interactions between STIM2 and TRPC channels, and how such interactions affect their store depletion-dependent functions.

## 6. Conclusion

Multiple molecular components and processes coordinately regulate SOCE to facilitate and dynamically modulate the amplitude and spatiotemporal characteristics of $Ca^{2+}$ signals generated due to $Ca^{2+}$ entry. The model in Fig. 3 summarizes the major mechanisms and components discussed above in this review. Both the Orai1/STIM1 and TRPC1/STIM1 complexes mediate $Ca^{2+}$ entry. Distinct effector proteins localized near the channel and in the cytosol detect local and global changes in $[Ca^{2+}]_i$ resulting in regulation of distinct cellular functions. Orai1 function is dependent on its regulatory proteins, STIM1 and STIM2, as well as the assembly of the channel with these proteins in ER-PM junctions. STIM2 and STIM1 act in a concerted fashion to tune the function of Orai1 channels at different levels of ER-$Ca^{2+}$ store depletion to efficiently regulate cell function. Several proteins as well as plasma membrane lipids, e.g. $PIP_2$, are involved in formation and stabilization of the ER-PM junctions where they support the assembly of Orai1/STIM1 as well as the regulation of the channel. Knowledge of these are slowly emerging and further details are required to understand their physiological relevance in different cell types. While Orai1/STIM1 form the primary SOCE channel and generate the initial $Ca^{2+}$ signal, other channels such as TRPCs, can be recruited to ER-PM junctions via interaction with STIM1 and inserted into the plasma membrane by a mechanism that is dependent on Orai1-mediated $Ca^{2+}$ entry. Alternately, some TRPCs can be directly activated by initial $[Ca^{2+}]_i$ elevation due to SOCE or by changes in $PIP_2$/DAG in the plasma membrane. Importantly, activation of these additional channels modifies the initial $Ca^{2+}$ signal, amplitude and pattern, which leads to the regulation of cell functions that cannot be accomplished by Orai1 alone. For example, TRPC1-mediated SOCE leads to a global increase in $[Ca^{2+}]_i$ which regulates a distinct set of downstream events, e.g. activation of NFκB-dependent gene expression and $Ca^{2+}$-dependent $K^+$ channels (Fig. 3) [90]. Since assembly of Orai1 and STIM proteins in ER-PM junctions is critical for SOCE, future studies should be directed to determine the exact functions of the various molecular components that contribute to these junctions and how they impact function in different cells. Finally, as most studies reported until now have utilized overexpression systems, knowledge is lacking regarding the localization, status and regulation of endogenous Orai1, STIM1, and STIM2. Such studies will provide further understanding and insights into the regulation and physiological impact of $Ca^{2+}$ signals generated by SOCE.

## Conflict of interest

The authors declare no conflict of interest.

## Transparency document

The Transparency document associated with this article can be found, in online version.

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