Chapter 1

Regulation of Calcium Signaling by STIM1 and ORAI1

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Abstract

STIM1 and ORAI1 proteins are regulators of intracellular Ca^{2+} mobilization. This Ca^{2+} mobilization is essential to shape Ca^{2+} signaling in eukaryotic cells. STIM1 is a transmembrane protein located at the endoplasmic reticulum, where it acts as an intraluminal Ca^{2+} sensor. The transient drop of intraluminal Ca^{2+} concentration triggers STIM1 activation, which relocates to plasma membrane-endoplasmic reticulum junctions to bind and activate ORAI1, a plasma membrane Ca^{2+} channel. Thus, the Ca^{2+} influx pathway mediated by STIM1/ORAI1 is termed store-operated Ca^{2+} entry (SOCE). STIM and ORAI proteins are also involved in non-SOCE Ca^{2+} influx pathways, as we discuss here. In this chapter, we review the current knowledge regarding the role of SOCE, STIM1, and ORAI1 in cell signaling, with special focus on the modulation of the activity of kinases, phosphatases, and transcription factors that are strongly influenced by the extracellular Ca^{2+} influx mediated by these regulators.

Keywords: calcium, signaling, SOCE, STIM, ORAI

1. Introduction

Cell signaling is the network of reactions and interaction of molecules that allow cells to react to a wide range of stimuli. In this response, many pathways are involved, so cells are able to adapt to changing conditions. One of the mechanisms to respond to external stimuli is mediated by receptors, that is, proteins located at the plasma membrane that communicate the extracellular and the intracellular medium. A significant strategy that cells acquired early in their evolution was the modification of the composition of the intracellular milieu, so the ionic

composition is different across the plasma membrane. This strategy is expensive in terms of the consumption of energy, since the ionic composition of the intracellular medium is modified by pumping out some ions from the cytosol. However, this is cost-efficient because it provided the possibility to proliferate and to gain cellular specialization. In this regard, free calcium (Ca^{2+}) concentration in the cytosol of cells is much lower than that observed in the external medium, so there are mechanisms to remove the excess of free Ca^{2+} from the cytosol, such as extruding Ca^{2+} to the extracellular medium or to intracellular Ca^{2+} stores. This pumping is carried out by plasma membrane Ca^{2+} pumps and by endoplasmic reticulum Ca^{2+} pumps, respectively. Also, buffering of Ca^{2+} with Ca^{2+}-binding proteins is another strategy to keep cytosolic free Ca^{2+} concentration ($[Ca^{2+}]i$) within the low nanomolar range (~100 nM). The reason why the $[Ca^{2+}]i$ is tightly controlled is because this level is a second messenger in cell signaling, that is, transient variations of $[Ca^{2+}]i$ communicate a signal to be transmitted. For instance, during fertilization of mammalian oocytes, a series of short-term cytosolic increases of $[Ca^{2+}]i$ occurs in the oocyte for ~20 h after the fusion with sperm. These transient and short spikes are required to release the arrest of the cell cycle and to stimulate the transition from the fertilized oocyte to 1-cell embryo (zygote). The level of $[Ca^{2+}]i$ is also involved in many other cellular events, like the control of gene expression, vesicular trafficking, neurotransmitter release, cytoskeletal dynamics, and so on.

Cytosolic Ca^{2+} spikes and Ca^{2+} waves are generated by the opening of Ca^{2+}-specific ion channels located at the plasma membrane and subcellular organelles. When they become activated, plasma membrane Ca^{2+} channels let the influx of extracellular Ca^{2+} so the $[Ca^{2+}]i$ rapidly increases, triggering the activation of Ca^{2+}-sensitive effectors. As the main intracellular Ca^{2+} store, the endoplasmic reticulum (ER) also contains Ca^{2+} channels that become activated upon certain stimuli to let the transient release of Ca^{2+} to the cytosol. Then, elevated $[Ca^{2+}]i$ activates Ca^{2+} pumps to reduce the level of free Ca^{2+} in the cytosol, making possible the temporal increase of $[Ca^{2+}]i$ which is essential for its role as a messenger. The speed of the Ca^{2+} rise, as well as the Ca^{2+} removal, together with the time that this elevation lasts, define the temporal Ca^{2+} signaling, or Ca^{2+} signature, a critical point in the activation of subsequent events. Similarly, the specific distribution of Ca^{2+} channels and pumps define the spatial Ca^{2+} signature. The spatiotemporal control of the Ca^{2+} signaling is relevant for determining the regulation of different signaling pathways that finally lead to diverse actions. In summary, it is not only important to know how Ca^{2+} levels are altered upon specific stimuli, but also their specific duration, shape, and subcellular localization.

In this chapter, we summarize the current knowledge regarding the role of the STIM and ORAI proteins family. Because of their role as ER intraluminal Ca^{2+} sensors, STIM proteins have been recently involved in the modulation of several Ca^{2+}-dependent signaling pathways. ORAI proteins are Ca^{2+} channels located at the plasma membrane that regulate the influx of Ca^{2+}, in some cases under the control of STIM proteins. Thus, cooperation of both proteins is critical for Ca^{2+} influx, Ca^{2+} signaling, and cell physiology.

2. General overview of STIM and ORAI proteins

In humans, there are two different genes coding for STIM proteins: STIM1 and STIM2. STIM1 gene shows three known transcriptional variants that generate the proteins STIM1 (canonical),

Gene	Transcript(s)	Protein	Protein official name
ENSG00000167323	NM_001277961.1	NP_001264890.1	STIM1 isoform 1, or STIM1L
	NM_003156.3	NP_003147.2	STIM1 isoform 2 (canonical)
	NM_001277962.1	NP_001264891.1	STIM1 isoform 3, or STIMS
ENSG00000109689	NM_001169117.1	NP_001162588.1	STIM2 isoform 3
	NM_001169118.1	NP_001162589.1	STIM2.1, STIM2β
	NM_020860.3	NP_065911.3	STIM2.2, STIM2α
ENSG00000276045	NM_032790.3	NP_116179.2	ORAI1
ENSG00000160991	NM_001126340.2	NP_001119812.1	ORAI2 isoform a
	NM_001271818.1	NP_001258747.1	ORAI2 isoform a
	NM_001271819.1	NP_001258748.1	ORAI2 isoform b
	NM_032831.3	NP_116220.1	ORAI2 isoform a
ENSG00000175938	NM_152288.2	NP_689501.1	ORAI3

Table 1. Accession number for genes and reference sequences (RefSeq) of transcriptional variants and proteins.

STIM1L (the longest isoform), and STIM1S (the shortest isoform). For STIM2 gene, also three transcriptional variants have been described coding for proteins STIM2, STIM2.1 (or STIM2 beta), and STIM2.2 (or STIM2 alpha) (see **Table 1**).

Also in humans, three different genes code for ORAI proteins: ORAI1, ORAI2, and ORAI3. ORAI1 gene yields a single product (ORAI1 protein, also known as calcium release-activated calcium channel protein 1), whereas ORAI2 gene produces two variants (isoforms 1 and 2), and ORAI3 gene generates a single transcriptional variant and a single protein isoform (**Table 1**).

STIM1 protein is a positive regulator of store-operated Ca^{2+} entry (SOCE) [1, 2], a Ca^{2+} influx pathway regulated by the filling status of intracellular Ca^{2+} stores, mainly the ER. Although there is a significant pool of STIM1 at the plasma membrane, most STIM1 is ER-resident. When located at the ER, STIM1 shows a single transmembrane domain (TM) with the N-terminus toward the intraluminal space of this organelle. The Ca^{2+}-sensitive EF-hand domain, together with a sterile-α-motif (SAM), constitute an intraluminal Ca^{2+} sensor, with an apparent dissociation constant for Ca^{2+} of 250 μM [3]. When the intraluminal Ca^{2+} concentration drops below this Kd, the dissociation of Ca^{2+} from the EF-hand domain is transmitted to the SAM domain, and to the cytosolic domain of the protein leading to its activation [4]. The cytosolic domain shows a well-studied calcium release-activated calcium (CRAC) activation domain (CAD), with a series of short coiled-coil (CC) domains that bind to ORAI1 plasma membrane channels to activate Ca^{2+} influx [5]. STIM1 protein also shows a Ser/Pro rich domain, close to a short sequence of four amino acids that binds to the microtubule plus-end binding protein EB1 [6], and finally a terminal Lys-rich domain which is critical for the activation of non-ORAI1 Ca^{2+} channels, such as TRPCs [7].

STIM2 and STIM1 share >60% sequence identity, and STIM2 also senses intraluminal Ca^{2+} concentration although with different sensitivity, since the dissociation constant for Ca^{2+}

(~500 μM) is twofold higher than that of STIM1 [8], suggesting that STIM2 becomes activated with smaller changes in intraluminal Ca^{2+} levels, whereas STIM1 activates Ca^{2+} entry upon more severe conditions [9].

ORAI1 is a plasma membrane protein with four transmembrane domains with the N- and C-termini oriented to the cytosol. The Ca^{2+} channel is formed by a hexamer of ORAI1 monomers, with the Ca^{2+} pore in the center of the hexamer [10, 11]. Both the N- and C-terminal domains are involved in the binding to STIM1 [12]. The paralogues ORAI2 and ORAI3 share 63% and 58% sequence identity with ORAI1, being the extracellular loop 3 that connects TM domain 3 and 4, significantly larger in ORAI3.

3. STIM1-ORAI1-mediated Ca^{2+} influx

The mechanism of activation has been well documented for the complex STIM1-ORAI1. At resting state, STIM1 is distributed on the surface of the ER, where its cytosolic domain is folded in a tight state due to an intramolecular clamp between domains CC1a1 and CC3 [13]. The inactive and resting STIM1 is a dimer [14], and the activation of Ca^{2+}-release from intracellular stores due to the activation of the phosphoinositide pathway, leads to the transient depletion of Ca^{2+} levels within the ER. The consequent Ca^{2+} dissociation from the intraluminal domain of STIM1 triggers the activation of the protein in a more extended state that lets STIM1 to form oligomers [15]. In contrast to what it has been observed for inactive STIM1, which shows a high mobility on the ER surface while it is bound to EB1 and microtubules, STIM1 oligomers are quite immobile when they reach ER-PM junctions. This oligomerization has been extensively documented when STIM1 is targeted with fluorescent tags (**Figure 1**).

The binding of STIM1 to the microtubule plus-end tracking protein EB1, ensures the targeting of STIM1 to ER-PM junctions [16]. However, this binding to EB1 is not required for the activation of ORAI1. STIM1 dissociates from EB1 by a mechanism regulated by the phosphorylation of a set of serine residues (Ser575, Ser608, and Ser621) adjacent to the EB1-binding site [17]. This STIM1 phosphorylation is mediated by the kinases ERK1/2, which become activated in the absence of Ca^{2+}-influx by the activation of tyrosine kinase receptors at the plasma membrane [18–20]. Thus, STIM1 bound to EB1 travels to ER-PM junctions [6], but it dissociates from EB1 to bind to ORAI1 [17]. The physical interaction between STIM1 and ORAI1 is fully required for gating the Ca^{2+} channel. This interaction is mediated by the aforementioned CRAC activation domain (CAD) of STIM1 and both the cytosolic C-terminus and the N-terminus of ORAI1 [21], although the binding to the N-terminus is slightly weaker [22]. The stoichiometry of the complex STIM1-ORAI1 is also in the center of debate, but the current accepted proposal supports a 1:1 to 2:1 ratio in order to activate the channel [23, 24].

The high selectivity of ORAI1 for Ca^{2+} is due to the pore design, with a selectivity filter mediated by the acidic glutamate residue E106 at the first transmembrane domain [26]. Once Ca^{2+} influx is activated, a negative feedback controls the excessive Ca^{2+} entry, and Ca^{2+}/calmodulin mediates this mechanism of inactivation. A short domain (residues 470–491) with seven acidic amino acids, close to the CAD binding domain, is directly involved in the Ca^{2+}-dependent inactivation [27]. Also in this report, Mullins et al. identified a membrane-proximal N-terminal domain of ORAI1 (residues 68–91) that binds calmodulin (CaM) in a Ca^{2+}-dependent manner

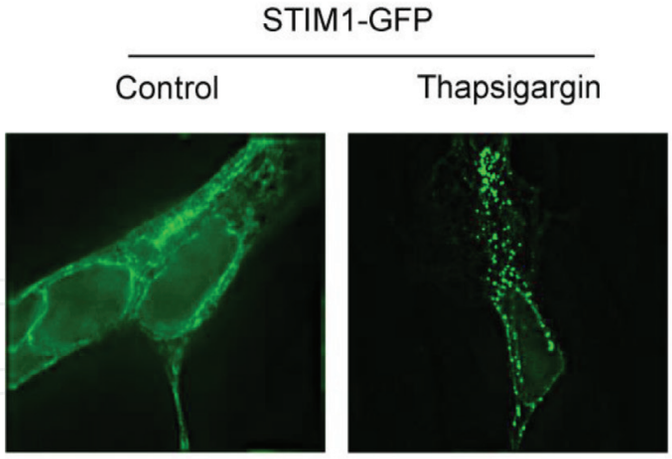

Figure 1. HEK293 cells stably expressing STIM1-GFP were incubated in Hank's balanced salt solution (HBSS) (left) or in Ca^{2+}-free HBSS with 1 μM thapsigargin (right) to trigger Ca^{2+} store depletion. After 10-min incubation cells were fixed and visualized under wide-field fluorescence microscopy. In control cells (left panel), STIM1-GFP showed a localization that matched with endoplasmic reticulum. Thapsigargin induced aggregation of STIM1-GFP revealed by the clustering of GFP fluorescence (right panel), a result that demonstrated that the recombinant protein STIM1-GFP was sensitive to store depletion (reprinted from reference [25]).

[27], supporting a model in which Ca^{2+}/CaM binds to the N-terminus of ORAI1 to trigger channel inactivation.

The large Rab GTPase CRACR2A mediates another mechanism that controls and prevents excessive Ca^{2+} entry. At low intracellular Ca^{2+} levels, CRACR2A enhances the binding of STIM1 to ORAI1, but at higher $[Ca^{2+}]i$, that is, after ORAI1 activation, CRACR2A dissociates from ORAI1, inhibiting SOCE [28]. ORAI1 residues involved in the binding to CRACR2A are the same as those that bind Ca^{2+}/CaM, so Ca^{2+}/CaM and Ca^{2+}-free CRACR2A are competitors for ORAI1.

STIMATE, a protein encoded by TMEM110 gene, is an ER-resident protein and a modulator for the activity of the STIM1-ORAI1 complex [29]. When Ca^{2+} dissociates from STIM1, the conformational change to a more extended structure of STIM1 facilitates the binding between STIMATE and STIM1-CC1 domain, avoiding the inhibition of CAD domain. This is the reason why STIMATE promotes the full extended conformation and the formation of STIM1 clustering at ER-PM junctions. In addition, the protein SARAF has been described as a negative regulator of SOCE [30]. As an ER membrane-resident protein, SARAF associates with STIM1 to promote Ca^{2+}-dependent inactivation of SOCE. In this regard, a conserved STIM1 (448–530) C-terminal inhibitory domain (CTID) has been reported to regulate Ca^{2+}-dependent inhibition [31]. CTID shows the capability to promote access of SARAF to the STIM1-ORAI1 activation region (SOAR or CAD), thus promoting inactivation of SOCE.

Additional regulators of the complex STIM1-ORAI1 have been reported, including septins [32] and RASSF4 [33]. Septin filaments and phosphatidylinositol-4,5-bisphosphate (PIP_2) polarize in ER-PM junctions before store-depletion and facilitate STIM1 targeting to these junctions, where STIM1 recruits ORAI1. On the other hand, RASSF4 (RAS association domain family 4) also regulates SOCE by affecting the translocation of STIM1 to ER-PM junctions. Finally, a recent report has shown that ORAI1, as well as STIM1 phosphorylated at ERK1/2-target sites, are recruited at the leading edge of migrating cells, where ORAI1 binds cortactin, a regulator

of plasma membrane ruffling [34]. This membrane ruffling is the reorganization of the cortical cytoskeleton required for the formation of filopodia and lamellipodia, and STIM1-KO (knockout) and ORAI1-KO cells, engineered by CRISPR/Cas9 genome editing, showed defective membrane ruffling and largely diminished cell migration [34], demonstrating that Ca^{2+} influx through STIM1-activated ORAI1 is essential for these events.

4. The role of STIM1 and ORAI1 on cell signaling

Given the importance of Ca^{2+} in many signaling pathways, the impact of STIM1, ORAI, and SOCE on cell signaling is also remarkable. Increasing evidence prove the significant role of this Ca^{2+} entry pathway in cell physiology and tissue homeostasis, and we focus here on the role of STIM and ORAI proteins on modulators of signaling pathways, such as kinases, phosphatases, and transcription factors. We also describe recent findings that unravel how STIM1 and ORAI1 are modulated by posttranslational modifications.

4.1. MAPK pathway

The close relationship between SOCE and mitogen-activated protein kinases (MAPKs) was revealed by Machaca and Haun [35], when they investigated the inactivation of SOCE in Xenopus oocyte maturation. SOCE is an active pathway in almost all eukaryotic cells, but during M-phase of cell cycle it becomes inactivated [36–38]. Machaca and Haun demonstrated that SOCE inactivation at germinal vesicle breakdown of Xenopus oocytes coincided with an increase in levels of MAPK and maturation-promoting factor (MPF), but they also demonstrated that MPF triggered SOCE inactivation by inhibiting the coupling between store depletion and SOCE activation, and not by blocking Ca^{2+} influx through SOCE channels [35].

In cells at interphase, some evidence supports a role for SOCE on ERK1/2 activation. In this regard, it has been proposed that SOCE activates extracellular signal-regulated kinases 1/2 (ERK1/2) in parotid acinar cells [39] and melanoma cells [40]. This proposal fits well with SOCE as an upstream regulator of ERK1/2. However, this proposal does not seem to be applicable to all cell lines, since ERK1/2 can be activated in the absence of extracellular Ca^{2+} and therefore in the absence of Ca^{2+} influx in HEK293 cells [20], the Ishikawa adenocarcinoma cell line [18], osteosarcoma U2OS cells [34], and prostate PC3 cells [19]. In addition, STIM1 knockdown did not modify phosphorylation of MEK1/2-ERK1/2 in gastric cancer cells [41], and STIM1 and ORAI1 knockdown did not inhibit the activation of ERK1/2 in response to EGF [42]. Moreover, ERK1/2 is fully activatable in STIM1-KO PC3 cells [19], with no active SOCE, demonstrating that SOCE is dispensable for ERK1/2 activation.

On the contrary, increasing evidence demonstrates that SOCE is a target for ERK1/2 activity, and that ERK1/2 is an upstream regulator of STIM1 and SOCE (reviewed in [43]). Pozo-Guisado et al. reported that STIM1 is phosphorylated by ERK1/2 at residues Ser575, Ser608, and Ser621 [25]. This phosphorylation is required for the full activation of STIM1 and for triggering the dissociation of STIM1 from microtubules [17]. Accordingly, phospho-STIM1 is enriched at the leading edge of migrating cells, that is, in the vicinity of receptor tyrosine

kinases [34], where phospho-STIM1 acts in cooperation with ORAI1 to regulate the Ca^{2+} influx that rules cell migration. Consequently, phospho-STIM1 is an effector of ERK1/2 and an essential mediator for the activation of Ca^{2+} influx upon stimulation of cells with IGF-1 [20], or EGF [18, 19, 34].

Other MAPKs, such as p38 MAPK, have been shown to regulate SOCE, although by an indirect mechanism. Transforming growth factor beta (TGFβ) regulates megakaryocyte maturation and platelet formation by upregulating the expression of the serum-glucocorticoid inducible kinase SGK1 [44, 45], which is p38 MAPK-dependent [46]. SGK1 stimulates nuclear translocation of transcription factor NF-κB, which upregulates ORAI1 expression, increasing SOCE. This increase was demonstrated to be sensitive to p38 MAPK inhibition, SGK1 inhibition, and NF-κB inhibition, demonstrating the role of p38 MAPK in the upregulation of SOCE [47]. Another proposal was reported by Sundivakkam et al. [48], who reported that p38 MAPK directly phosphorylates STIM1. In this report, it was shown that pharmacological inhibition of p38 MAPK increased SOCE and that p38β knockdown prevented STIM1 phosphorylation and potentiated SOCE. However, this report did not identify the phosphorylated Ser/Thr residue(s), since the findings were based on the use of a phospho-Ser antibody [48], but not a site-specific phospho-specific antibody.

4.2. cAMP and PKA

The crosstalk between SOCE and cAMP-activated pathways has been investigated thoroughly since the molecular description of STIM1 and ORAI1. For instance, the Ca^{2+}/CaM-stimulated adenylyl cyclase 8 (AC8) was found to be activated by SOCE and co-localized with STIM1 and ORAI1 in lipid rafts [49]. Interestingly, other authors found that lowering the concentration of free Ca^{2+} within the ER led to recruitment of adenylyl cyclases, enhancing the production of cAMP with the subsequent PKA activation, being this action independent of the $[Ca^{2+}]i$ [50]. Because activation of STIM1 and translocation to ER-PM junctions were required for coupling ER-Ca^{2+} depletion and adenylyl cyclase activity, without altering $[Ca^{2+}]i$, those authors proposed the occurrence of a pathway termed store-operated cAMP signaling (SOcAMPS), a pathway that was later confirmed for other AC isoforms, such as AC3 [51]. More recently, it was confirmed that other Ca^{2+} channels, including TRPC1, were also involved in the activation of ACs [52]. It has been reported that STIM1 interacts with the plasma membrane adenylyl cyclase 6 to regulate melanogenesis [53], and this interaction is mediated by the Ser/Pro-rich C-terminal region of STIM1. These reports, together with the finding that ORAI1- and SOCE-deficient fibroblasts showed impaired cAMP production and cAMP-dependent signaling [54], strongly support the direct relationship between Ca^{2+} depletion at ER stores, STIM1 activation, and enhanced production of cAMP.

As for other pathways, the regulation between STIM1 and PKA seems to be reciprocal. In addition to the CRAC channel ORAI1, the plasma membrane-resident STIM1 activates store-independent arachidonic acid regulated Ca^{2+} (ARC) channels, and this activation depends on the phosphorylation of STIM1 at Thr389 by PKA, which requires the scaffold protein AKAP79 [55]. This phosphorylation triggers a structural change in the SOAR region of STIM1 (also known as CAD) being essential for the selective activation of ARC channels [56].

4.3. Other kinases and pathways

It is known that Ca^{2+}-influx is upregulated by phosphoinositide 3-kinase (PI3K) signaling in platelets [57, 58]. Because PI3K signaling involves activation of SGK1, and this kinase has been shown to be a stimulator of ORAI1 expression [59], it is accepted that PI3K modulates SOCE by upregulation of the CRAC channel. In B16B6 melanoma cells, constitutive activation of Src and PKB/Akt was revealed to be due to the activation of SOCE in lipid rafts, which promoted Ca^{2+}-dependence of the Src activity to trigger tumor signaling events [60], as it reported for lung metastasis of melanoma cells in a xenograft mouse model [61]. However, this is not shared by other cancer cells, as in prostate PC3 cells, with low levels of active ERK1/2 due to constitutive activation of PKB/Akt, Src is fully activatable in a Ca^{2+}-independent manner by epidermal growth factor (EGF) [19].

Protein kinase C (PKC) phosphorylates ORAI1 at residues Ser27 and Ser30 [62]. More precisely, Kawasaki et al. demonstrated that the knockdown of the isoform PKCβ led to an increase of Ca^{2+} influx, and that recombinant PKC phosphorylated ORAI1 in vitro and in vivo at these two amino acids, an effect that inhibited the Ca^{2+} transport through ORAI1 [62]. No other phospho-residues have been characterized in detail in ORAI1. In airway smooth muscle cells, rottlerin, a PKCδ-selective inhibitor, reduced phorbol esters-triggered SOCE, without affecting total levels of STIM1 and ORAI1 [63]. However, the mechanism of this inhibition remains to be elucidated. PKC also inhibited SOCE in hepatocytes treated with amiodarone, an experimental design to mimic the accumulation of lipids during steatosis [64]. Because selective inhibition of PKC reversed SOCE to normal values, it was concluded that lipid accumulation triggers PKC-dependent SOCE impairment. Also, accumulation of palmitate is cytotoxic in kidney cells, and high levels of palmitate triggered Ca^{2+} depletion in the ER, in addition to mitochondrial stress. This depletion is antagonized by the inhibition of fatty acid transporters, inhibition of phospholipase C (PLC), and inhibition of PKC [65]. Once again, the mechanism that links PKC and the regulation of STIM1/ORAI1 remains elusive.

In 2011, Mungai et al. reported that hypoxia augmented cellular reactive oxygen species (ROS), without a significant alteration of energy charge values [66]. Hypoxia triggered an increase in $[Ca^{2+}]i$, relocalization of STIM1 to ER-PM junctions, and phosphorylation of AMP-activated protein kinase (AMPK), in the absence of its upstream regulator LKB1 (liver kinase B1). These events were due to the ROS-dependent activation of ORAI1, which led to an increase in $[Ca^{2+}]i$ and activation of CaMKK2 (or CaMKKβ), an upstream activator of AMPK [66, 67]. Finally, a recent report from Yang et al. described how STIM1-ORAI1 mediated autophagy in endothelial progenitor cells exposed to oxidized low-density lipoprotein to mimic hypercholesterolemia. This treatment caused stimulation of Ca^{2+} influx mediated by STIM1-ORAI1, activation of CAMKK2 and decrease of mTOR activity with the subsequent activation of autophagy [68]. Similarly, in hepatocarcinoma cells, mitochondrial fission increased cytosolic Ca^{2+} levels that activated the NF-κB pathway, upregulating STIM1 expression and the subsequent SOCE [69]. The relative increase of $[Ca^{2+}]i$ also activated NFAT-dependent upregulation of Drp1, promoting a positive loop to rise levels of mitochondrial fission.

4.4. NF-κB, NFAT, CREB, and other transcription factors

As we mentioned above, SOCE is essential for platelet activation, and it is known the key role of ORAI1 in response to thrombin [70, 71]. ORAI1 transcripts were found significantly reduced

in platelets and megakaryocytes from SGK1-KO mice, and transfection of megakaryocyte with constitutively active SGK1 increased phosphorylation of the IκB kinase (IKKα/β), which phosphorylates the inhibitor protein IκBα, promoting nuclear translocation of NF-κB subunit p65 [59]. In addition, Eylenstein et al. defined, by chromatin immunoprecipitation (ChIP) experiments, the promoter regions accounting for NF-κB-sensitive genomic regulation of STIM1 and ORAI1 [72], supporting further the conclusion that upregulation of ORAI1 and STIM1 by SGK1-dependent NF-κB signaling leads to the upregulation of SOCE, which in turn upregulates expression of other transcription factors like fibroblast growth factor 23 (FGF23) [73]. Other transcription factors are known to regulate STIM1 expression, such as Wilms tumor suppressor 1 (WT1) and early growth response 1 (EGR1), which were found by analyzing the STIM1 promoter with the TESS search system (University of Pennsylvania) [74]. Finally, NEUROD2, a neurogenic transcription factor, has been described as a negatively regulator of STIM1 expression, an activity that limits the level of STIM1 in cortical neurons [75].

Regarding NF-κB, reciprocal regulation seems to link this transcription factor and SOCE. In addition to the activation of SOCE by NF-κB described above, Liu et al. reported that T-cell activation triggered by the binding of antigen to T-cell receptor stimulated SOCE and that this Ca^{2+} entry activated a PKCα-mediated phosphorylation of p65 NF-κB at Ser536, an event that controls nuclear localization and transcriptional activity of NF-κB [76].

STIM1 and ORAI1 are also well-known activators of the protein phosphatase calcineurin, which activates the nuclear factor of activated T cells (NFAT) [77]. Once NFAT becomes dephosphorylated by calcineurin the transcription factor is internalized into the nucleus. Indeed, the activation and nuclear translocation of NFAT was the reporter used by Feske et al. when they searched for regulators of SOCE using a Drosophila RNA interference screening, a report that led to the description of ORAI1 as the channel that mediates the Ca^{2+} release-activated Ca^{2+} current, or CRAC [78]. Because NFAT modulates the expression of a wide range of genes, it is involved in many pathways, and also in the regulation of the expression of other regulators of transcription, including IRF4, BATF, and Bcl-6 [79]. NFAT is not the only transcription factor activated by the axis STIM1-ORAI1-Ca^{2+}/CaM-calcineurin because Ca^{2+} influx through ORAI1 stimulates the transcription factor EB (TFEB), promoting the activation of chemokines genes [80]. SOCE also activates the Ca^{2+}/cAMP response element binding protein (CREB), a transcription factor that regulates expression of many genes, at least in cultured smooth muscle cells and intact arteries [81]. In this regard, it was observed that mitochondrial Ca^{2+} uptake was reduced in lymphocytes lacking STIM1 or ORAI1, an effect that was due to reduced mitochondrial Ca^{2+} uniporter (MCU) expression [82]. ChIP and promoter analyses revealed that CREB directly binds the MCU promoter, revealing that SOCE regulates the Ca^{2+} uptake capability of mitochondria by regulating Ca^{2+}-dependent activation of CREB [82].

Ca^{2+} influx regulates myoblasts differentiation, and shortly after the molecular description of STIM and ORAI proteins, it was reported that silencing STIM1, Orai1, or Orai3 reduced SOCE and myoblast differentiation [83]. This positive effect on myoblasts correlated with the expression of MEF2 and myogenin, two transcription factors involved in skeletal muscle development, although it is still unclear the molecular pathway that links STIM1/ORAI1 and the activation of the transcription factors. In cerebellar granule neurons cultured in low concentration of extracellular potassium, mimicking resting conditions, SOCE promoted the degradation of transcription factor Sp4, a regulator of neuronal morphogenesis and function [84].

Another important molecular interactor of STIM1 is the hypoxia-inducible factor-1 alpha (HIF-1α), which is upregulated during hepatocarcinoma growth [85]. Li et al. found that HIF-1α directly controls STIM1 transcription, but also that STIM1-mediated SOCE is required for HIF-1α accumulation in hepatocarcinoma cells via activation of Ca^{2+}/CaM-dependent protein kinase II, revealing a mutual dependence of STIM1 and HIF-1α in the regulation of Ca^{2+} transport and tumor growth [85].

High levels of ORAI1 and STIM1 are found in many types of cancer cells. In gastric cancer tumor progression, this higher expression is associated with a negative impact on survival rates of patients, an effect that was partially due to targeting expression of metastasis-associated in colon cancer-1 (MACC1) [86], an essential regulator of the transcription for the gene coding for the hepatocyte growth factor receptor, MET. Similarly, a recent report described that STIM1 promotes cell migration and the epithelial-to-mesenchymal transition (EMT) by activating TGF-β, Snail and Wnt/β-catenin pathways in prostate cancer cells [87].

Finally, an excellent report from Stephan Feske laboratory [54], described how SOCE is crucial for mitochondrial fatty acid oxidation, and that Ca^{2+} entry through ORAI1 was essential to activate adenylyl cyclase, cyclic AMP production, the transcriptional regulator peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α) and peroxisome proliferator-activated receptor alpha (PPARα), which is mediated by the activation of CREB.

5. ORAI3 and calcium signaling

Shortly after the molecular description of ORAI1 as the CRAC channel, it was revealed the essential role of other ORAI proteins in cell signaling. The involvement of ORAI3, together with ORAI1, in arachidonic acid-regulated Ca^{2+} (ARC) channels was early proposed [88, 89]. In contrast to SOC channels, the activation of ARC channels depends on the pool of STIM1 resident in the plasma membrane [90]. More interestingly, ORAI1 and ORAI3 show a differential sensitivity to reactive oxygen species, due to the extracellularly located Cys195 residue which is found in ORAI1, but not in ORAI3. The differential redox sensitivity underlies the differential responses between naïve and T helper lymphocytes, an event that lets T(H) cells proliferate and secrete cytokines in oxidative environments [91].

ORAI3, but not ORAI1, was also involved in the activation of PLCδ in response to arachidonic acid, an activation that controls oscillation frequency of Ca^{2+} spikes triggered by carbachol [92]. ORAI3 channels are overexpressed in estrogen receptor-positive breast cancer cells [93], and it was later demonstrated, using the MCF-7 cancer cell line, that silencing ORAI3 slows down cell cycle and triggers arrest at G1 phase [94]. EGF triggers Ca^{2+} entry through ORAI3, and the channel is transcriptionally upregulated by the estrogen receptor alpha (ERα) [95]. It is now accepted that cancer cells show a remodeling of ORAI proteins, with an enhanced participation of ORAI3 compared to noncancerous cells, suggesting that heteromerization of ORAI3 and ORAI1 is a common feature in malignant transformation [96].

6. Future directions

During the past decade, a significant progress was made regarding the molecular description of the proteins involved in store-operated Ca^{2+} entry. Although some details remain unclear,

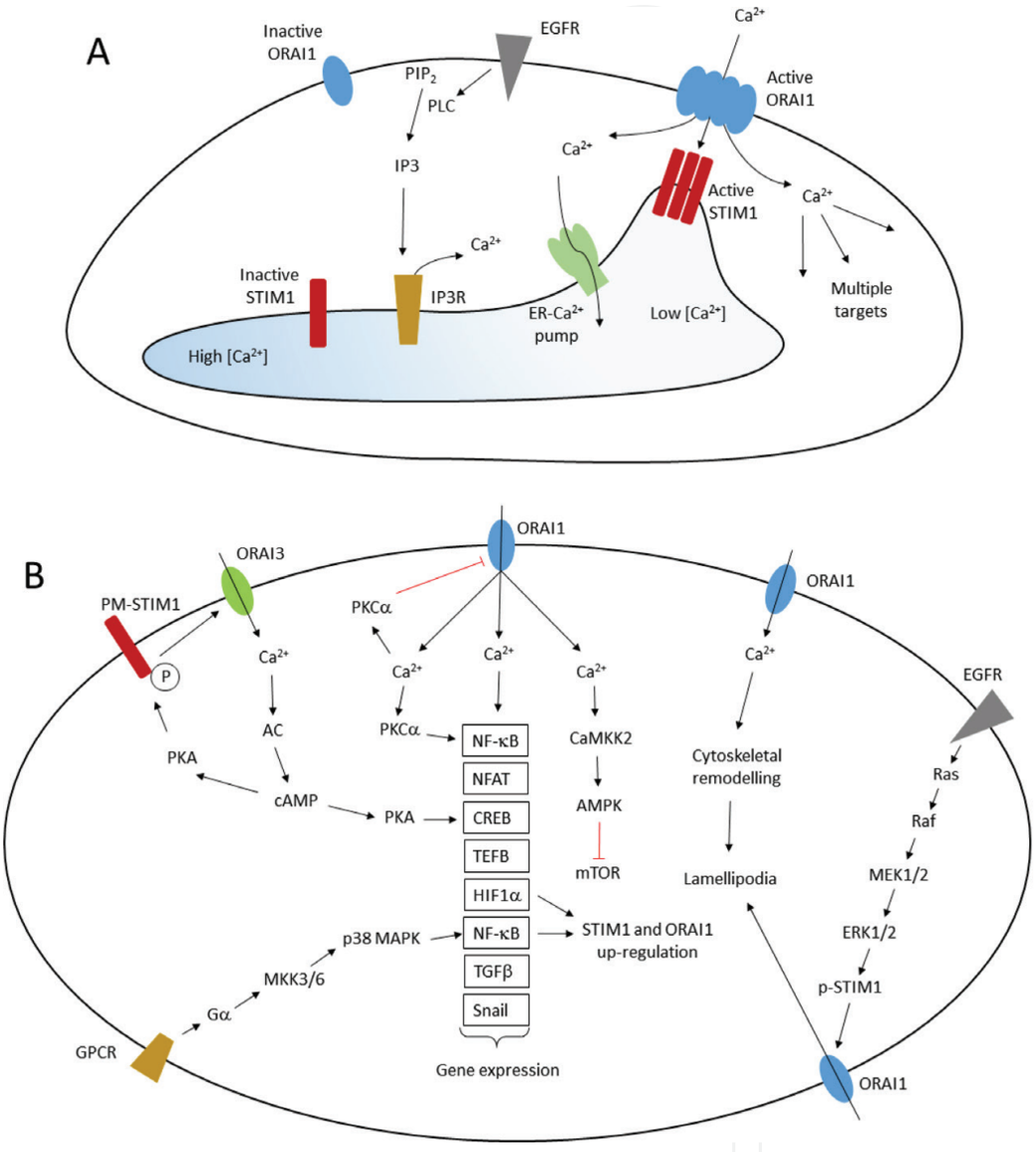

Figure 2. Activation of SOCE by STIM1/ORAI1 and pathways involved in SOCE-dependent signaling. Panel A: diverse stimuli that triggers the activation of the phospholipase C pathway, such as activation of EGF receptor (EGFR), stimulate the production of inositol 1,4,5-trisphosphate (IP3) which binds and activates IP3 receptor (IP3R) at the endoplasmic reticulum (ER). This activation leads to the release of Ca^{2+} from the ER, with the subsequent transient depletion of intraluminal [Ca^{2+}] and the activation of STIM1. Ca^{2+}-unbound STIM1 aggregates in oligomers and translocates to plasma membrane (PM)-ER junctions where it binds and activates ORAI1. Extracellular Ca^{2+} entry through ORAI1 activates multiple Ca^{2+}-dependent targets, as shown in panel B, but also provides a Ca^{2+} source to replenish intraluminal Ca^{2+} levels. This replenishment is accomplished by the ER-Ca^{2+}-ATPase which pumps Ca^{2+} into the ER lumen. Panel B: schematic illustration of the most important pathways regulated by STIM1/ORAI1. AC, adenylyl cyclase; GPCR, G protein-coupled receptor; PM-STIM1, plasma membrane-resident STIM1; p-STIM1, phosphorylated STIM1.

a topology of STIM1-ORAI1 contact sites, selectivity filters in ORAI1, and the role of post-translational modifications have been reported for both proteins. The involvement of STIM and ORAI proteins in different pathways is now much clearer, and they are now considered master regulators of Ca^{2+}-dependent signaling pathways. However, in many cases pathways were studied in cancer cell lines in vitro, so physiological models are required to evaluate the importance of STIM1 and ORAI1 in the pathophysiology of cells in vivo. Nevertheless, primary cell cultures and established cell lines constitute a widely accepted experimental approach for basic studies in cell signaling and understanding the role of STIM1/ORAI1 in cell biology and cell signaling. With these tools, we have reached the conclusion that STIM1 and ORAI1 are involved in the control of Ca^{2+} refilling within the ER. More important, STIM1 and ORAI1 directly modulate Ca^{2+} signaling in a wide variety of pathways, with a significant role in gene expression, cell migration, and tumor cell metastasis (**Figure 2**). Because the expression of STIM1/ORAI1 is deregulated in cancer cells, it is required to evaluate the relative importance of STIM1/ORAI1 as pharmacological targets for the treatment of disease, not only with the use of in vitro cell cultures, but also in animal models for the study of human disease.

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Conflict of interest

The authors declare no conflict of interests.

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