Minireview

Molecular physiology and pathophysiology of stromal interaction molecules

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Impact statement

Intracellular Ca^{2+} signaling is a fundamentally important regulator of cell physiology. Recent studies have revealed that Ca^{2+}-binding stromal interaction molecules (Stim1 and Stim2) expressed in the membrane of the endoplasmic reticulum (ER) are essential components of eukaryote Ca^{2+} signal transduction that control the activity of ion channels and other signaling effectors present in the plasma membrane. This review summarizes the most recent information on the molecular physiology and pathophysiology of stromal interaction molecules. We anticipate that the work presented in our review will provide new insights into molecular interactions that participate in interorganelle signaling crosstalk, cell function, and the pathogenesis of human diseases.

Abstract

Ca^{2+} release from the endoplasmic reticulum is an important component of Ca^{2+} signal transduction that controls numerous physiological processes in eukaryotic cells. Release of Ca^{2+} from the endoplasmic reticulum is coupled to the activation of store-operated Ca^{2+} entry into cells. Store-operated Ca^{2+} entry provides Ca^{2+} for replenishing depleted endoplasmic reticulum Ca^{2+} stores and a Ca^{2+} signal that regulates Ca^{2+}-dependent intracellular biochemical events. Central to connecting discharge of endoplasmic reticulum Ca^{2+} stores following G protein-coupled receptor activation with the induction of store-operated Ca^{2+} entry are stromal interaction molecules (STIM1 and STIM2). These highly homologous endoplasmic reticulum transmembrane proteins function as sensors of the Ca^{2+} concentration within the endoplasmic reticulum lumen and activators of Ca^{2+} release-activated Ca^{2+} channels. Emerging evidence indicates that in addition to their role in Ca^{2+} release-activated Ca^{2+} channel gating and store-operated Ca^{2+} entry, STIM1 and STIM2 regulate other cellular signaling events. Recent studies have shown that disruption of STIM expression and function is associated with the pathogenesis of several diseases including autoimmune disorders, cancer, cardiovascular disease, and myopathies. Here, we provide an overview of the latest developments in the molecular physiology and pathophysiology of STIM1 and STIM2.

Keywords: STIM1, STIM2, store-operated calcium entry, calcium signaling, endoplasmic reticulum, signal transduction

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Introduction

Calcium (Ca^{2+}) is a universal intracellular signaling molecule that controls many cellular processes by acting over a wide spatio-temporal range within multiple subcellular compartments including the cytosol, mitochondria, endoplasmic reticulum (ER), and nucleus. Ca^{2+} signals consist of time-dependent changes in the concentration of Ca^{2+} in the cytosol ($[Ca^{2+}]_c$) and subcellular organelles that are generated by the mobilization of Ca^{2+} into the cytosol *via* entry through Ca^{2+}-permeable ion channels localized in the plasma membrane (PM) and the ER membrane. A major Ca^{2+} entry pathway in non-excitable and excitable cells is store-operated Ca^{2+} entry (SOCE) by which Ca^{2+} influx across the PM is activated by a decrease in the Ca^{2+} concentration within the lumen of the endoplasmic reticulum ($[Ca^{2+}]_{ER}$). Since Dr. James Putney first proposed in 1986 that lowering $[Ca^{2+}]_{ER}$ activated Ca^{2+} channels in the PM, investigators have focused on identifying the molecular basis of store-operated channels (SOCs), the signaling mechanisms involved in SOC activation and inactivation, and the cellular functions controlled by SOCE.

SOC current can be conducted by several types of ion channels. The most well-characterized SOC is the Ca^{2+} release-activated Ca^{2+} (CRAC) channel. Although the

biophysical properties of CRAC channels in a wide range of cell types were defined by numerous investigators in the 1990s, the molecular constituents controlling the activation and regulation of these channels were unknown for many years. In 2005 and 2006, results from studies in independent laboratories revealed two proteins necessary for SOCE: stromal interaction molecule 1 (STIM1) and Orai1.[1–6] STIM1, a type I single-pass ER transmembrane protein that is activated consequent to depletion of ER Ca^{2+} stores, was found to be essential for CRAC channel gating.[3–5] The Orai1 protein was found to form the ion-conducting pore subunit of CRAC channels.[4–6] The current consensus model of SOCE suggests that STIM1 functions as the main sensor of $[Ca^{2+}]_{ER}$ stores and activator of Orai1. Compared to STIM1 and Orai1, relatively little is known about the roles of STIM1 and Orai1 homologues, namely STIM2, Orai2 and Orai3, in SOCE and other cellular functions.[7]

In this review, we focus on the molecular physiology and pathophysiology of STIM1 and STIM2. After a brief review of cellular Ca^{2+} signal transduction, we will summarize recent advances in our understanding of STIM proteins with a particular emphasis on STIM2, the lesser studied of the two STIM proteins. Following a discussion of their structure-function properties, we will describe the role of STIM in regulating SOCE and other cellular functions. Finally, we will discuss the pathophysiological implications of disrupted STIM-dependent signaling in cancer, metabolic disease, immunological disorders, and other diseases.

An overview of intracellular Ca^{2+} homeostasis and signaling

Intracellular Ca^{2+} homeostasis is a fundamentally important property of all cells that is crucial for regulating a wide range of cell functions and cell viability, and is precisely regulated by Ca^{2+} entry into and out of the cytosol. In resting, unstimulated cells, $[Ca^{2+}]_c$ is maintained at a low level (50–200 nm) relative to the $[Ca^{2+}]$ in the extracellular space (1–2 mM) by the actions of Ca^{2+}-ATPases and counter-ion exchangers that remove Ca^{2+} from the cytosol. Subsequent to cellular stimulation, these same Ca^{2+} handling mechanisms participate in the regulation of dynamic changes in Ca^{2+} signals and rapidly restore $[Ca^{2+}]_c$ to pre-stimulus, basal levels, since prolonged elevation of $[Ca^{2+}]_c$ is detrimental to cell viability.

Spatial and temporal changes in $[Ca^{2+}]_c$ produced after exposure of cells to hormones, neurotransmitters, growth factors, and mechanostimulation are essential signals in eukaryotic cells that regulate cellular growth and proliferation, differentiation, gene expression, motility, secretion, and cell survival.[8,9] Following stimulation, $[Ca^{2+}]_c$ increases consequent to release of Ca^{2+} through PM and ER (or sarcoplasmic reticulum (SR) in myocytes) Ca^{2+}-permeable channels. Ca^{2+} signals also can be affected by Ca^{2+} released from mitochondria, Golgi apparatus, and acidic Ca^{2+} stores.[10–17] The increases in $[Ca^{2+}]_c$ exhibit temporally distinct patterns that can be sustained, transient, or oscillatory. Dynamic changes in $[Ca^{2+}]_c$ are shaped by proteins that transfer Ca^{2+} across the PM to the extracellular space and sequester Ca^{2+} into membrane-bound organelles such as the ER/SR, mitochondria, and Golgi apparatus. During the past four decades, the molecular biology, physiology and pathophysiology of Ca^{2+} transport mechanisms including plasma membrane Ca^{2+}-ATPases (PMCAs), sarcoendoplasmic reticulum Ca^{2+}-ATPases (SERCAs), secretory-pathway Ca^{2+}-transport ATPases (SPCAs), the mitochondrial voltage-dependent anion transporter (VDAC), and mitochondrial Ca^{2+} uniporter (MCU) have been well characterized. Detailed information about recent advances in our knowledge of these Ca^{2+} handling mechanisms and other aspects of intracellular Ca^{2+} signal transduction is available elsewhere.[9,18,19]

Store-operated Ca^{2+} entry—The nexus of PM and ER Ca^{2+} signaling

SOCE is a primary source for intracellular Ca^{2+} signaling in non-excitable cells such as fibroblasts, cells of the immune system, and hepatocytes. It is also present in excitable cells including endocrine and neuroendocrine cells, neuronal cells, cardiomyocytes, and vascular smooth muscle cells. A unique property of this Ca^{2+} influx mechanism is that it is controlled by the concentration of Ca^{2+} sequestered within the ER/SR.[20] Activation of SOCE is triggered by cellular stimuli that cause a decrease in $[Ca^{2+}]_{ER}$. This can occur, for example, in cells after the application of G protein-coupled or tyrosine kinase receptors agonists that stimulate phospholipase C activity, inositol 1,4,5-trisphosphate (IP_3) production, and IP_3 receptor (IP_3R)-mediated release of Ca^{2+} from the ER. After more than 30 years of study, we now know that SOCE is critically important in the maintenance of cytosolic and ER Ca^{2+} homeostasis, as well as the regulation of spatial and temporal changes in $[Ca^{2+}]_c$, protein biosynthesis, gene expression, and cell viability.[21–27]

The biophysical properties of SOCE were established during the early 1990s. Patch-clamp electrophysiology was used to first characterize CRAC currents in mast cells and T cells.[28,29] CRAC currents have been detected in a wide range of cell types from immune cells, to hepatocytes, fibroblasts, and intestinal epithelial cells, suggesting broad functional importance.[30] The Ca^{2+} current through these channels, I_{CRAC}, exhibits strong inward rectification, a positive reversal potential, low single-channel conductance and is voltage-independent.[28,29] Furthermore, I_{CRAC} is highly selective for Ca^{2+} over other divalent and monovalent cations.[29] The small pore size, ~3.9 Å, of the CRAC channels contributes to its high Ca^{2+} selectivity.[31] CRAC channels also exhibit Ca^{2+}-dependent fast inactivation. The channels are inactivated by local negative feedback from Ca^{2+} entering the channel, but is unaffected by Ca^{2+} release from intracellular stores.[32]

The molecular basis of SOCE was discovered in 2005 and 2006.[1–6] These studies revealed that STIM1 functioned as the sensor of ER/SR Ca^{2+} stores and an activator of SOCE,[1–3] while Orai1 formed the ion conducting, pore subunit of the CRAC channel.[4,33] STIM1 and Orai1 are both necessary and sufficient for SOCE.[34,35] Depletion of the ER Ca^{2+} stores causes a destabilizing conformational change of

STIM1 to reveal the Orai1-activating domain and initiate oligomerization and translocation of STIM1 molecules to ER-PM junctions.[36,37] This allows STIM1 interaction with PM Ca^{2+} channel Orai1 and activation of SOCE.[23]

Orai1, a member of the Orai family of ion channel proteins, was initially identified through genome-wide RNAi screens in *Drosophila* S2 cells,[5,6,38] while human Orai1 was identified by linkage analysis of immune cells isolated from severe combined immunodeficient patients.[38] CRAC channels consist of six Orai1 subunits. Each subunit has four transmembrane domains and intracellular N- and C- termini.[4,6,39–41] Although not well studied, Orai2 and Orai3 also form SOCs and may form heteromultimeric channels.[42–44] The C-termini of each Orai homologue contains coiled-coil domains. The coiled-coil region of Orai1 is necessary for binding STIM1 following depletion of intracellular Ca^{2+} stores.[45,46] Additionally, a conserved region within the N-terminus of Orai1 is required for SOC channel opening.[46] More detailed information about the molecular biology, protein biochemistry, and biophysical properties of the Orai ion channels can be found in several recently published review articles.[47–50]

Although SOCE is mediated by STIM1 interaction with Orai1 at ER-PM junctions, other regulators of SOCE have been identified. Many of these regulators act directly on STIM1 and/or Orai1, while others regulate SOCE indirectly. Proteins that directly regulate SOCE by binding STIM1 and/or Orai1 include CRACR2A, SARAF, junctate, α-SNAP, STIMATE, and junctophilin-4 (Table 1).[51–57] Junctate and junctophilin-4 are components of the ER-PM junctions that facilitate STIM1 recruitment, clustering, and interaction with Orai1 at the ER-PM junctions.[52,56] STIMATE and CRACR2A are also positive regulators of SOCE. Through direct interactions with STIM1 and/or Orai1, these proteins facilitate STIM1 clustering in plasma membrane-associated ER membrane (PAM) nanodomains.[51,54] α-SNAP is also a positive regulator of SOCE, but it interacts with existing STIM1-Orai1 clusters to facilitate a molecular rearrangement necessary for the activation of SOCE.[57] In contrast, SARAF, a negative regulator of SOCE, directly interacts with STIM1 to facilitate slow Ca^{2+}-dependent inactivation of SOCE.[53]

Other proteins indirectly affect SOCE, including mitsugumin 29 (MG29), RAS association domain family 4 (RASSF4), and the ubiquitin-proteasome system (UPS).[90–92] MG29, a synaptophysin-related protein expressed in skeletal muscle is localized in SR-PM junctions and is required for maintenance of SR-PM junctions. The role of MG29 in SOCE was revealed in knockout mice studies. Decreased SOCE was observed in skeletal myocytes isolated from mice in which the expression of MG29 gene expression was silenced.[90,93] The MG29 null cells, however, did not exhibit any change in STIM1 and Orai1 expression levels.[90,93] Collectively, this suggests MG29 may play a role in facilitating SOCE in skeletal muscle by maintaining the integrity of the SR-PM junctions where STIM1 and Orai1 interact. RASSF4 was identified as a positive regulator of SOCE in a siRNA screen of HeLa cells.[91] Further experiments showed that RASSF4 regulates SOCE by affecting the translocation of STIM1 to ER-PM junctions. Rather than recruiting STIM1 *via* direct interactions, RASSF4 affects steady state PM phosphatidylinositol 4,5-bisphosphate (PIP_2) levels, thereby regulating targeting of STIM1 to the ER-PM junctions.[91] Finally, the UPS has also been shown to affect SOCE by regulating STIM1 surface expression.[92] In HEK 293 cells treated with proteosome inhibitors, STIM1 surface expression and SOCE were significantly increased. Additionally, overexpression of an E3 ubiquitin ligase resulted in decreased STIM1 surface expression levels. This suggests a novel role for the UPS in regulation of STIM1 localization and SOCE.

The process of SOCE whereby depletion of intracellular Ca^{2+} stores in the ER activates Ca^{2+} influx across the PM has been under investigation for more than 30 years. This has allowed for the identification of STIM proteins as Ca^{2+} sensors within the ER that activate PM channels of the Orai family. Since their discovery, recent experiments have allowed for the characterization of this process, as well as ways in which it is regulated as described above. We will continue this review with an in depth analysis of STIM1 and STIM2. We will discuss their roles in SOCE and other signaling pathways as well as the pathophysiology associated with these proteins and altered SOCE.

Molecular biology of STIM

STIM1 (initially named GOK) was identified in 1996 as a novel human gene cloned from chromosome region 11p15.5. This chromosomal region was known to be involved in a number of tumors and childhood malignancies.[94] Early work suggested STIM1 was a tumor suppressor gene as *in vitro* studies revealed a growth-inhibitory function of STIM1 in rhabdoid tumor and rhabdomyosarcoma cell lines.[95] The mouse homologue of STIM1, originally called SIM, was identified independently from a screen of stromal cell cDNA for proteins that recognized pre-B cells and promoted their proliferation.[96] Murine *Stim1* maps to a distal region of mouse chromosome 7 and its amino acid sequence shares 96% identity with human STIM1.[97]

A screen of gene databases in 2001 for STIM1-related sequences led to the discovery of STIM2, a STIM1 homologue present in vertebrates.[98] In the same study, a single STIM homologue, D-STIM, was identified in *Drosophila melanogaster* and *Caenorhabditis elegans*. Human *Stim2* was mapped to chromosome 4p15.1, mouse *Stim2* is located on chromosome 5, and *D-Stim* is on band 14A of the X chromosome.[98] cDNA and amino acid database alignment studies revealed that the genomic organization of STIM1 and STIM2 is highly conserved, suggesting that the two proteins evolved from a common ancestor.[98] Human and mouse STIM1 and STIM2 are each encoded by 12 exons spaced across more than 120 kb.[95] On the other hand, *D-Stim* is more compact; it is encoded by seven exons spaced over 4.2 kb.[98]

STIM1 and STIM2 are expressed at varying levels in a wide range of cell and tissue types.[98,99] While STIM1 is more abundant than STIM2 in platelets, macrophages, and T cells,[100–103] STIM1 and STIM2 are expressed at relatively equal levels in skeletal muscle, liver, and spleen.[100]

Table 1. STIM1 interacting proteins.

Binding Protein	Identity of binding protein	Cell type/*in vitro* method	Reference
Cytoskeletal/cell surface			
EB1, EB2, and EB3	Microtubule binding proteins	HEK 293, HeLa	58
PI(4,5)P2	Membrane phospholipid	Lipid-binding assay	59
GluA1 and GluA2	AMPA receptor subunit	Hippocampal neurons, rat cortical neurons	60,61
PMCA	PM ATPase that pumps Ca^{2+} out of the cell	HEK293	62
SOCE proteins			
STIM2	ER Ca^{2+} sensor	HEK 293, HEK 293T, Human platelets, HeLa, NIH 3T3	63–65
Orai1	CRAC channel	Human Platelets, rat cortical neurons, mouse dendritic cells, HEK 293, MEG-01, HeLa, parathyroid adenoma tissues, *Xenopus* oocytes	63,66–72
Orai2	Store-operated ion channel	Mouse dendritic cells, HEK293	44,66
Orai3	Store-operated ion channel	HEK293	44
TRPC1, TRPC4, TRPC5	Non-selective cation channels	Human platelets, parathyroid adenoma tissues, SY5Y neuroblastoma cells, HEK293	71,73–76
Nuclear transport			
Exportin1	Nuclear export	HEK 293T	62,77
Transportin1	Nuclear import	HEK 293T	77
ER proteins			
IP3R1 and IP3R3	ER Ca^{2+} channels	BAECs, MDCK, *Xenopus* oocytes	78,79,72
SERCA3, SERCA2b	ER Ca^{2+} ATPase	Human Platelets, *Xenopus* oocytes	62,63
Calnexin	ER chaperone/MAM protein	HEK293T	77
Calsequestrin1	Ca^{2+} buffering	HEK293	80
SARAF	Negative regulator of SOCE	MEG-01	81
STIMATE (TMEM110)	ER multi-transmembrane protein	HEK293	54
TMEM203	ER resident regulator of Ca^{2+} homeostasis	HEK293	82
SPPL3	ER membrane protein of the aspartyl protease family	HEK293T	83
Junctate	Ca^{2+} sensing ER protein	HeLa	52
Others			
SUR1	K_{ATP} channel subunit	HEK293T, mouse islets	84
$Ca_v1.2$ and $Ca_v1.3$	Voltage dependent Ca^{2+} channels	HEK293, HEK293T, SY5Y neuroblastoma cells, hippocampal neurons	74,85,86
Calmodulin	Ca^{2+} binding protein	CaM binding assay, isothermal titration calorimetry	59,87
AKAP	Scaffold protein	Hippocampal neurons	60
rPKA and cPKA	PKA subunits	Hippocampal neurons	60
PRKAA2	Energy sensor	HEK 293T	88
CRACR2A	Cytosolic Ca^{2+} sensing protein	HeLa	51
POST (TMEM110)	ER-resident membrane protein	HEK 293	62
Junctophilin-4	Contribute to the formation of junctional membrane complexes	HEK 293	56
α-SNAP	Cytosolic protein involved in intracellular trafficking and fusion of vesicles	HEK 293	57
Golli	Myelin basic protein	HeLa	89

In contrast, STIM2 is more highly expressed than STIM1 in dendritic cells, neurons, and the brain.[66,98,100,104] The STIM proteins also have varied expression levels during different stages of development in some tissues. For example, STIM1 is expressed in neonatal cardiomyocytes, but absent in adult cardiomyocytes.[105] Other cell types in which both STIM1 and STIM2 are expressed include beta cells, intestinal epithelial cells, endothelial progenitor cells, and pituitary gonadotrophs.[84,106,107]

The majority of STIM1 is localized in the ER membrane; however, STIM1 is also expressed in the PM[1,3,98,99,108–111] In contrast, STIM2 is located in the membranes of the ER and acidic organelles.[3,63,98,99,110] However, a small population of pre-STIM2 escapes ER targeting and is present in the cytosol proximal to the PM, where it constitutively interacts with Orai1.[112]

Splice variations of *Stim1* transcripts produce two isoforms of the protein: a long version (STIM1L) as well as the dominant shorter version (STIM1). STIM1L contains an extra 106-amino acid sequence in the cytosolic C-terminus of STIM1. While STIM1 is ubiquitously expressed in human tissues, STIM1L is expressed exclusively in skeletal muscle

Table 2. Function of STIM1 and STIM2 protein domains.

Domain	Function
EF-hand	ER Ca^{2+} sensor
SAM	Protein interaction domain. When ER Ca^{2+} stores are full, maintains STIM in folded conformation Upon Ca^{2+} store-depletion, facilitates STIM oligomerization
CC1-CC3	ER retention and stability Under-store replete conditions, inhibitory helix in CC1 keeps STIM in inactive conformation Under store-deplete conditions, elongates to facilitate interaction with Orai1
SOAR (within CC2-CC3)	Minimal region required for Orai1 activation
K-rich	Initial recruitment of STIM to ER-PM junction STIM1 gating of TRPC channels Electrostatic interactions with PM phospholipids

and neonatal heart.[105,113] STIM1L plays an important functional role in myocytes as a fast activator of SOCE. Actin binding localizes STIM1L in clusters with Orai1 at the PM for rapid activation of SOCE following depletion of SR Ca^{2+}.[113]

Unlike STIM1, three distinct forms of STIM2 generated by post-translational modifications have been identified.[112] In HEK 293 cells, the majority of STIM2 exists as an ER membrane protein produced after cleavage of a 101-amino acid signal peptide at the N-terminus. Another smaller population, referred to as preSTIM2, escapes ER targeting and remains in the cytosol with the signal peptide intact. A third variant of STIM2 results from cleavage of the signal peptide into a smaller signal peptide fragment (SPF). These isoforms play distinct functional roles in cells. STIM2 senses and responds to changes in $[Ca^{2+}]_{ER}$ to activate SOCE, while pre-STIM2 associates with the PM and regulates store-independent Ca^{2+} entry. In contrast, the SPF of STIM2 functions as a transcriptional regulator.[112] Since the initial discovery and description of STIM2 isoforms, we still do not fully understand their physiological roles. Further investigation into the expression and function of these variants in other types of cells could yield insightful information that will further define the multiple roles of STIM2 in cellular physiology.

More recently, two independent studies have reported expression of a splice variant of STIM2, termed STIM2β, in $CD8^+$ and $CD4^+$ T-cells, Jurkat cells, human monocytes, and C2C12 cells .[114,115] STIM2β is highly conserved and expressed in many tissue types. STIM2β contains an additional eight amino acids in the channel-activating domain encoded by exon 9. Unlike STIM2, STIM2β inhibits Orai1-mediated SOCE. Although STIM2β localization and puncta formation resembles STIM2, Orai1 recruitment into puncta formed by STIM2β is significantly reduced. STIM2β interaction with Orai1 is mediated by heterodimerization with STIM1 or STIM2. Although the underlying mechanism of STIM2β inhibition of Orai1 is unclear, it may be mediated by a sequence-specific inhibitory allosteric and Ca^{2+}-dependent interaction with Orai1.[114]

STIM: Structure/function considerations

STIM proteins are type 1A single-pass transmembrane proteins located in the ER (Figure 1). STIM1 is 685 amino acids long. The nucleotide sequence of STIM2 encodes a 746-amino acid protein that is post-translationally processed into a 732-residue protein. D-STIM, the smallest of the STIM proteins, contains 570 amino acids.[98] The STIM proteins are largely conserved and share high homology through most of their length, but differ considerably in the distal C-terminal end. At the N-terminus, STIM proteins contain a pair of conserved cysteine residues, a Ca^{2+}-binding helix-loop-helix EF-hand domain, and a sterile α-motif (SAM) (Figure 2). Also conserved in the three STIM proteins is an N-linked glycosylation site at the N-terminus of the SAM domain. STIM1 contains a second N-linked glycosylation site within the SAM domain that is absent in STIM2 and D-STIM. The transmembrane region is highly conserved and contains a single cysteine residue in each STIM protein. STIM proteins have a series of α-helices, forming coiled-coil domains that mediate protein–protein interactions. Distal to the coiled-coil domains, STIM1 has a serine- and proline-rich region, whereas STIM2 contains a histidine- and proline-rich domain (Figure 2). Other than a similar lysine-rich tail at the C-terminus, the amino acid sequences of STIM1 and STIM2 diverge beyond the coiled-coil domains.

The molecular basis of STIM1-mediated SOCE activation following ER Ca^{2+} depletion was discovered in HeLa cells using site-directed mutagenesis, nuclear magnetic resonance, and Ca^{2+} imaging.[116] STIM1 is maintained in an inactive conformation through hydrophobic interactions between the Ca^{2+}-bound EF-hand regions and the SAM domain. After ER Ca^{2+} levels decrease, Ca^{2+} dissociates from the EF-hand. The EF-hand motif is essential for STIM function as an ER Ca^{2+} sensor (Table 2). The Ca^{2+} binding domain of the EF-hand enables STIM proteins to sense changes in $[Ca^{2+}]_{ER}$, which, under resting conditions, ranges between 0.2 and 0.6 mM.[117] The Ca^{2+}-binding domain comprised a canonical Ca^{2+}-binding EF-hand (cEF), a non-canonical EF-hand (ncEF), and a SAM domain. The cEF binds a single Ca^{2+} ion; the ncEF hand does not bind Ca^{2+}.[116] The SAM domains in STIM1 and STIM2 are highly conserved, exhibiting more than 85% sequence similarity. The SAM functions as a protein interaction domain and is composed of five α-helices that closely associate with the Ca^{2+}-bound EF-hand to stabilize the STIM proteins as monomers (Table 2). As $[Ca^{2+}]_{ER}$ decreases, Ca^{2+} dissociates from the EF-hand. This results in a partial unfolding of the EF-SAM complex, which leads to oligiomerization of the STIM proteins.[36,64,116]

Although binding assays have shown that the STIM1 and STIM2 EF-SAM domains exhibit similar Ca^{2+} binding affinities (K_d ~0.2–0.6 mM and ~0.5 mM, respectively), STIM2 has a greater sensitivity to smaller decreases in $[Ca^{2+}]_{ER}$ than STIM1.[118,119] The STIM2 EF-SAM domain is more stable than that of STIM1 in the absence of Ca^{2+}

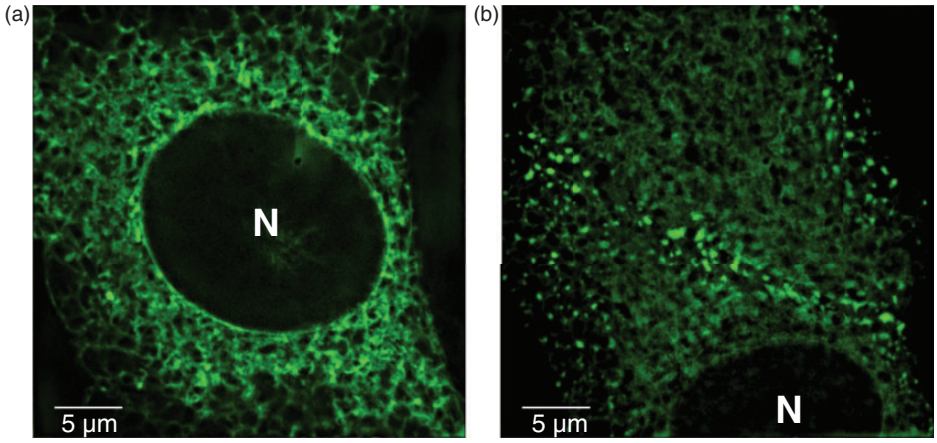

Figure 1. STIM1 and STIM2 have a reticular ER distribution. NIH 3T3 cells transiently expressing STIM1-YFP (a) or STIM2-YFP (b). Images shown are a z-projection of a z-stack acquired on a Nikon's N-SIM super resolution microscope. N: nucleus. (A color version of this figure is available in the online journal.)

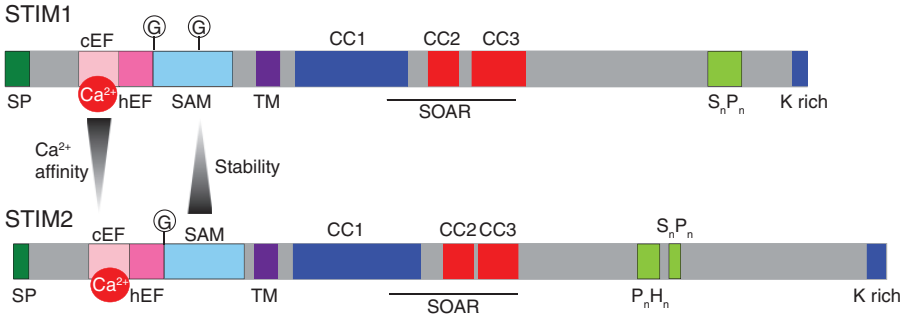

Figure 2. Comparison of the structure of human STIM1 and STIM2, highlighting each functional domain. SP: signal peptide; cEF: classical EF hand; hEF: hidden EF hand; SAM: sterile α motif; G: N-glycosylation site; TM: transmembrane domain; CC: coiled-coil domain; SOAR: STIM-Orai activating region; S_nP_n: serine proline rich domain; P_nH_n: proline histidine rich domain; K rich: lysine rich domain. As indicated by the gradient arrows, the STIM2 EF-hand has a lower Ca^{2+} affinity but its EF-SAM domain is more stable than that of STIM1. (A color version of this figure is available in the online journal.)

(Figure 2). While STIM1 EF-SAM partially unfolds upon dissociation of Ca^{2+}, STIM2 EF-SAM maintains a highly folded secondary structure.[119] How the EF-SAM domain of STIM2 participates in regulation of SOCE is unclear. Although the STIM2 EF-SAM regions share 88% sequence similarity to that of STIM1, detailed structural information about the STIM2 EF-SAM domain is limited. Biophysical studies of recombinant human STIM proteins indicate that the STIM2 SAM domain is more structurally stable than the STIM1 SAM and does not readily aggregate.[119] This suggests that loss of the EF-SAM interaction does not lead to oligomerization of STIM2 molecules to the same extent as STIM1.

The cytoplasmic region of STIM proteins is composed of three conserved coiled-coil (CC) domains located within a larger ezrin/radixin/moesin (ERM) homology domain. ERM proteins are a family of proteins that mediate attachment of cytoskeletal proteins to the PM.[120] Structurally, ERM proteins exist in a conformationally inactive form. Intramolecular interactions within the ERM complex block PM and cytoskeletal protein binding sites. A conformational change that displaces the electrostatic intramolecular interactions opens up the protein binding sites, yielding an active form of the ERM protein.[121–123] ERM domains have been identified in several proteins located at the PM, including focal adhesion kinase, talin, and protein tyrosine phosphatases. They play an important role in mediating protein–protein and protein–phospholipid interactions that facilitate the anchorage of protein complexes at specific membrane sites.[124] We currently do not understand how the ERM complex affects STIM localization and function.

The CC domains of STIM1 contain the CRAC activation domain (CAD)[125] or STIM1-Orai1-activating region (SOAR) that is required for Orai1 activation (Table 2).[126] The SOAR region is highly conserved and spans amino acids 344–442 in human STIM1. Direct binding of the SOAR domain to an α-helical region of the Orai1 C-terminus is required for full channel activation and SOCE. When expressed on its own, SOAR binding to the C-terminus of Orai1 is sufficient for activation[126]; however, when the full-length protein is transiently expressed in cells, binding of SOAR to the N-terminus of Orai1 is also required for channel activation.[125] Co-immunoprecipitation assays have shown that the SOAR domain directly binds to both the C- and N-termini of Orai1. Deletion of the Orai1 C-terminus

prevents STIM1 clustering and SOCE, whereas deletion of the N-terminus of Orai1 did not prevent clustering but abolished SOCE.[125] Dimerization of STIM1 molecules following Ca^{2+} depletion and EF-SAM destabilization is also facilitated by interactions of the STIM SOAR domain.[127–129] The cytosolic coiled-coil domains (CC1, CC2, and CC3) of STIM1 have been implicated as important facilitators of STIM1 oligomerization in response to ER Ca^{2+} store depletion. Under store-replete conditions, the SOAR domain is inactive due to electrostatic interactions between an inhibitory helix in CC1 and the SOAR domain spanning CC2 and CC3.[130,131] Consequent to a decrease in $[Ca^{2+}]_{ER}$, unfolding and dimerization of EF-SAM domain cause a conformational change in the cytosolic terminal, removing inhibition of CC1, allowing unfolding and elongation of the C-terminus. STIM1 unfolding reveals a polybasic domain in SOAR that interacts with Orai channels and the PM.[37]

Although human STIM2 SOAR (SOAR2) shares 76% sequence identity and 91% similarity with that of STIM1 (SOAR1),[132] variations in amino acid sequence result in discrete structural and functional differences between STIM1 and STIM2. X-ray crystallography and single-wavelength anomalous diffraction of human SOAR1 indicate that each SOAR1 monomer is made up of a group of four α-helices (Sα1-Sα4), with two long α-helices (Sα1 and Sα4) at the N- and C- terminal regions flanking two short α-helices (Sα2 and Sα3).[128] A cluster of positively charged residues in Sα1 of SOAR1 is important for binding of STIM1 to Orai1.[133] Additionally, a phenylalanine (F394) in Sα2 of SOAR1 is critical for STIM1 interaction with and activation of Orai. The positively charged residues are conserved in SOAR2. However, the replacement of F394 in SOAR1 with a leucine residue in SOAR2 may contribute to the decreased ability of STIM2 to interact with and activate Orai1.

The CC domains have also been shown to be important for ER retention and stability of STIM1 (Table 2).[77] Mutation analysis of STIM1 showed that mutants lacking the CC domains were either expressed on the cell surface or secreted, while mutants with an intact CC domain remained localized to the ER. STIM1 lacks the KKXX ER-retention sequence present in STIM2, so it is uncertain if the CC domain plays a similar localizing function in STIM2.[134] Mutational analysis also showed the CC domains is important for stabilizing the STIM proteins. The half-life of mutants lacking the CC domain is shorter than that of the full protein or mutant containing the CC domain.[77]

STIM proteins also have a polybasic lysine-rich (K-rich) domain (Table 2). Although not sufficient on its own for activation of Orai, the K-rich domain facilitates the initial recruitment of STIM1 to ER-PM junctions (Figure 3).[125,126] The K-rich domain of STIM1 also participates in the regulation of TRPC channel gating [135] and may be important for mediating the electrostatic interactions that enables the binding of STIM proteins to PIP_2.[59] STIM1 and STIM2 exhibit different affinities for PIP_2, with STIM2 having a greater affinity for the plasma membrane phospholipid

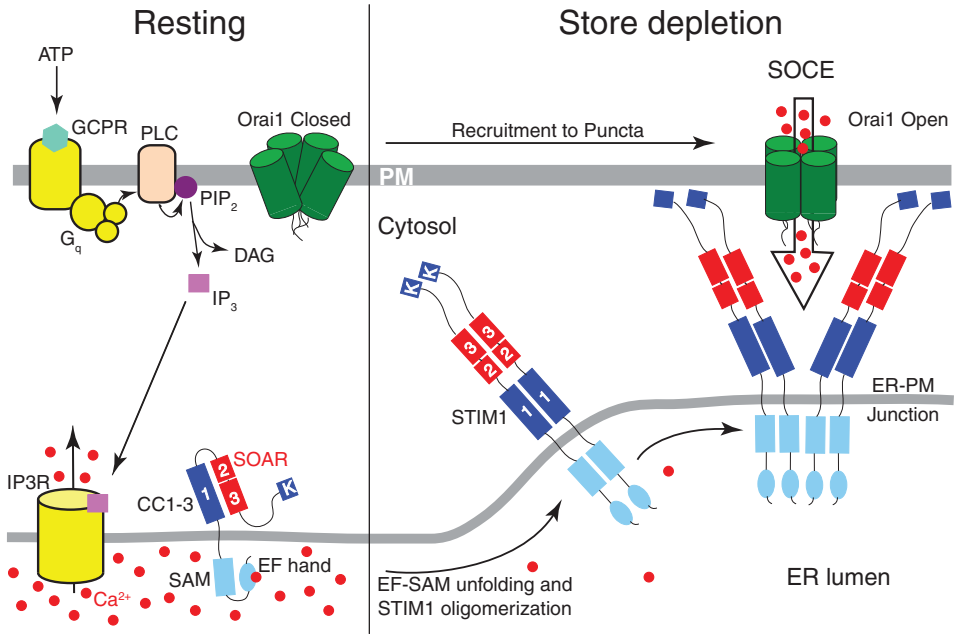

Figure 3. Consensus model of SOCE. When the ER Ca stores are full at rest, Ca^{2+} is bound to the STIM1 EF-hand. In the Ca^{2+}-bound mode, the EF-hand has a stabilizing interaction with the SAM domain, maintaining monomeric distribution of the STIM1 molecule. When an agonist, such as ATP, binds to and activates a G-coupled protein receptor (GCPR), phospholipase C (PLC) cleaves phosphatidylinositol 4,5-bisphosphate (PIP_2), generating inositol trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 diffuses through the cytosol, then binds to and opens IP3Rs in the ER membrane, resulting in discharge of the ER Ca^{2+} stores. Following ER Ca^{2+} store-depletion, Ca^{2+} dissociates from the EF-hand, initiating a destabilization-coupled STIM1 oligomerization process. Conformational changes in the EF-hand cause EF-SAM unfolding, destabilization, and subsequent aggregation of multiple STIM molecule EF-SAM domains. This oligomerization in the luminal domains also triggers conformational changes throughout the C-terminal domain of STIM1. At rest the coiled-coil (CC) domains are folded, but oligomerization results in an unfolded, extended conformation of the CC domains. Finally, STIM1 multimerizes and moves to ER-PM junctions, where it recruits and opens Orai1 channels to facilitate SOCE. Note: STIM2 is not pictured in this model as the role of STIM2 in SOCE is still unclear. Likewise, variants of the STIM proteins are not illustrated as they appear to play cell-specific roles as discussed in the text. (A color version of this figure is available in the online journal.)

than STIM1. Tetramers of STIM1 bind PIP_2, whereas dimers of STIM2 are sufficient for PIP_2 binding. A conserved cysteine residue located immediately upstream of the K-rich domain in STIM2 may contribute to the difference in lipid binding between the STIM isoforms. This cysteine residue is essential for dimerization of STIM2. Furthermore, STIM1 has two conserved proline residues within its K-rich domain that are not present in STIM2. The absence of proline in STIM2 allows the formation of a short α-helix; a feature of a similar lipid-binding signal sequence in yeast. When the proline residues in STIM1 K-rich domains are mutated, there is a two-fold increase in lipid affinity, suggesting that the absence of proline in STIM2 could account for its increased lipid affinity.[59] The functional consequence of increased affinity of STIM2 over STIM1 for PM lipids remains to be determined.

In summary, STIM1 and STIM2 are conserved and share high homology throughout the length of the molecule. Common to both isoforms are protein domains required for STIM function, namely a Ca^{2+}-binding EF-hand motif, a SAM domain that stabilizes STIM proteins and regulates protein–protein interactions, three coiled-coil domains containing an Orai activating region, and the K-rich domain that facilitates protein anchoring to the PM during STIM activation (Table 2). Although each of domains exists in both STIM1 and STIM2, there are a few amino acid changes that contribute to different binding affinities and activation kinetics between STIM1 and STIM2. These differences may partially explain the diversity of the molecular functions regulated by STIM1 and STIM2 that we describe below.

Posttranslational regulation of STIM

STIM proteins undergo posttranslational modifications and interact with other regulatory proteins. Studies using Calyculin A, a serine/threonine phosphatase inhibitor, have revealed that STIM1 is phosphorylated in K562 cells.[99] Phosphorylation of STIM1 at Ser575, Ser608, and Ser621 by extracellular signal-regulated kinases 1/2 (ERK1/2) regulates SOCE in HEK293 cells.[136] ERK1/2 phosphorylation of STIM1 is inversely related to $[Ca^{2+}]_{ER}$: STIM1 phosphorylation increases after ER Ca^{2+} store depletion, but decreases upon store refilling.[137] Phosphorylation of STIM1 at tyrosine residues has also been shown to be important for activation of SOCE in human platelets.[138] These studies show that inhibition of phosphorylation decreases the formation of STIM1 puncta and STIM1-Orai1 interactions, and reduces SOCE.

N-linked glycosylation has been implicated in the regulation STIM1. A STIM1 mutant in which two consensus glycosylation site arginine residues (N) were mutated to glutamine (Q) N131Q/N171Q (QQ) failed to rescue SOCE in neuroblastoma × glioma hybrid cells.[139] Alternatively, when this mutant was expressed in HEK293 cells, SOC currents were unaffected; however, arachidonic-acid-regulated Ca^{2+}-selective (ARC) channel currents were significantly reduced due to the inability of the STIM1 QQ mutant to be expressed in the PM.[109] Interestingly, other specific glycosylation site mutant combinations have differing effects. N131D/N171Q (DQ) resulted in a gain of function mutant as opposed to loss of function seen in the QQ mutant.[140] The DQ mutant increased the rate of STIM1 translocation to the ER-PM interface and reduced current latency. Furthermore, when co-expressed with Orai1, STIM1 DQ decreases Orai1 expression, alters STIM1:Orai1 stoichiometry and affects Ca^{2+} homeostasis.[140] Although STIM2 contains one of two glycosylation sites found in STIM1, the role of glycosylation in STIM2 function has not yet been defined.

Direct interactions between STIM and other proteins can influence STIM function. STIM1 and STIM2 both contain a calmodulin (CaM) binding site in the K-rich domain that may serve as an additional mechanism by which Ca^{2+} regulates STIM proteins. STIM binds CaM in a Ca^{2+}-dependent manner. In the presence of Ca^{2+}, STIM1 and STIM2 bind CaM with high affinity ($K_d = 0.8$ and 0.9 μM, respectively). When Ca^{2+} is not bound, however, STIM1 and STIM2 bind to CaM with much lower affinity (K_d of 55 and 150 μM, respectively).[87] Although the functional significance of CaM-binding remains uncertain, recent evidence suggests that binding of Ca^{2+}/CaM to STIM2 competitively inhibits interactions of the STIM2 K-rich domain with liposomes and PIP_2 in the PM.[59] This interaction may prevent Ca^{2+} overload by reducing STIM2-mediated ER-PM contacts when $[Ca^{2+}]_c$ is elevated, and consequently diminish the store-operated Ca^{2+} current.

Interactions between STIM1 and other proteins also can contribute to reducing SOCE. Two Ca^{2+}-dependent modes of SOCE inactivation have been reported: fast inactivation and slow inactivation. Fast inactivation is Ca^{2+}-dependent and mediated through a CRAC modulatory domain in the C-terminus of STIM1, glutamate residues in the Orai C-terminus, and CaM. [141–144] Slow Ca^{2+}-dependent inactivation is mediated by SOCE-associated regulatory factor (SARAF), an ER resident protein.[53] Like STIM proteins, SARAF is a single-pass ER membrane protein. The ER lumenal domain of SARAF regulates its inhibitory action mediated by the cytosolic domain that inactivates SOCE through interactions with the C-terminal domain of STIM1.[53]

Another mechanism of STIM regulation by protein-protein interactions involves STIM-activating enhancer encoded by TMEM110 (STIMATE), an ER-resident protein. Proteomic analysis and genetic manipulation identified STIMATE as a positive regulator of Ca^{2+} entry *via* a direct interaction with STIM1.[54,55] The C-terminus of STIMATE binds to the coiled coil 1 (CC1) domain in STIM1, causing disruption of the auto-inhibitory STIM1 CC1-SOAR domain interaction and promotes translocation of STIM1 to ER-PM junctions. The interaction of STIMATE with STIM2 has not yet been investigated.

STIM 2 in SOCE and Ca^{2+} homeostasis

It has been well documented that STIM1 is essential for SOCE (Figure 3).[1–3] Under resting conditions, when the ER Ca^{2+} stores are full, Ca^{2+} is bound to the STIM EF-hand and STIM1 is distributed evenly in the ER membrane. Consequent to store depletion, Ca^{2+} dissociation from STIM1 results in partial unfolding, destabilization, and

oligomerization of STIM1 molecules. This is followed by translocation of STIM1 to ER-PM junctions where the STIM oligomers bind to Orai1, forming distinct puncta.[2,127,145] Interaction of the SOAR domain of STIM1 with Orai1 opens the channels and activates SOCE.[67,125,126] In contrast, the role STIM2 in SOCE remains unclear. Studies have reported that STIM2 activates SOCE in mast cells, Jurkat T cells, and HEK 293 cells.[1,68,146,147] However, stable overexpression of STIM2 was found to inhibit SOCE when stably overexpressed in HEK293, PC12, A7r5, and Jurkat T cells.[110,148] The underlying mechanisms that account for the different roles of STIM2 in the regulation of SOCE have not been defined. One possible explanation may be differences in tissue culture conditions used to grow the cells. In the experiments that found stable overexpression of STIM2 inhibited SOCE, the cells were maintained in a selective media containing geneticin (G418).[110] G418 is an aminoglycoside antibiotic that inhibits STIM2-mediated SOCE.[68] This suggests that the inhibition of SOCE observed in these experiments could be a result of direct effects of G418 on SOCE. In support of this idea, experiments in which STIM2 was transiently overexpressed in the absence of G418 showed a modest increase in SOCE.[64] Another possibility is an adaptive mechanism in which extended STIM2 overexpression gradually downregulates SOCE. This is consistent with a study that has shown prolonged overexpression of STIM2 significantly decreased maximal SOCE.[64]

Knockdown studies have also provided contradictory information about the role of STIM2 in SOCE. Silencing of STIM2 in murine T cells, cortical neurons, and mast cells significantly reduced SOCE.[100,146,149] Knockdown of STIM2 in HeLa cells, B cells, MDA-MB-231 cells, and mouse embryonic fibroblasts (MEFs) resulted in a reduction of SOCE, but much smaller than that observed after STIM1 was knocked down.[3,103,150,151] In contrast, in *Xenopus* oocytes, STIM2, but not STIM1, played a predominant role in SOCE.[152] Alternatively, silencing of STIM2 expression in HEK293, neutrophil-like HL-60 cells, rat pulmonary arterial smooth muscle, airway myocytes, and CD4 T^+ cells did not affect SOCE.[1,103,148,153–155] In mouse neurons, where STIM2 is the dominant isoform, there was a significant reduction of SOCE following knockout of STIM2.[100] However, in arterial smooth muscle cells where STIM2 is expressed at much lower levels than STIM1, there was no effect of STIM2 knockdown on SOCE.[154] The differences presented for a role of STIM2 in SOCE may reflect cell type, experimental approach, or the methodologies used for genomic editing. Additionally, it may be difficult to accurately assess STIM2 function using siRNA due to variable degrees of *Stim2* knockdown.

Although the role of STIM2 may be cell type-specific, it may also depend on the ratio of STIM1 and STIM2 protein expression levels (STIM1:STIM2) within cells. An investigation of HEK cells transiently overexpressing STIM2 found that transfection with 2 μg *Stim2* cDNA inhibited SOCE, but no effect was observed in cells transfected with 0.5 μg *Stim2* cDNA.[148] In the same study, it was discovered that STIM2 activated SOCE when co-overexpressed with Orai1. These findings suggest that the STIM1:STIM2 ratio may affect signaling. This phenomenon may be in part caused by a signal-trap mechanism found in Chinese hamster ovary (CHO) and HEK293 cells.[156] In these cell types, Orai1 undergoes constant recycling with only about 40% localized in the PM at any given point in time. After ER Ca^{2+} store depletion, STIM1 traps Orai1 and prevents its recycling away from the PM, and results in an enrichment of Orai1 at the PM. However, in cells in which STIM1 is highly overexpressed, Orai1 becomes trapped within STIM1 clusters intracellularly and does not translocate to the PM after discharge of ER Ca^{2+} stores. The trapping of Orai1 leads to a reduction in SOCE.

Basal Ca^{2+} homeostasis is critical to maintain, as deviations in baseline Ca^{2+} can interfere with ER and mitochondrial function as well as receptor-mediated Ca^{2+} signaling. Aside from a role in SOCE, some studies have shown STIM2 regulates Ca^{2+} homeostasis. siRNA screening of the human signaling proteome identified STIM2 as a strong positive regulator of basal $[Ca^{2+}]$.[95] While STIM2 knockdown in HeLa, HUVEC, and HEK293T cells resulted in decreased resting $[Ca^{2+}]$ in the ER and cytosol, cells in which human STIM2 was over-expressed exhibited increased basal $[Ca^{2+}]_c$.[64] This is in contrast to knockdown of STIM1 which did not significantly lower $[Ca^{2+}]_c$ and had a much smaller effect on $[Ca^{2+}]_{ER}$ than STIM2. This observation was later confirmed in HEK293 cells, human myoblasts, human breast cancer cells, and in murine neuronal cells.[100,113,148,151] STIM2 is partially active in unstimulated cells under resting conditions, which may account for its role as a regulator of Ca^{2+} homeostasis. Unlike STIM2 which is fully activated by low agonist concentrations and low levels of ER Ca^{2+} store depletion, STIM1 is activated by high agonist concentrations and full store-depletion in mast cells, Jurkat T cells, and HEK293 cells.[146] Thus, STIM2 would respond to passive leakage of Ca^{2+} from the ER to activate SOCE and allow for Ca^{2+} entry and refilling of the ER stores, while STIM1 would remain inactive until the cell was exposed to a higher level of stimulus-induced decrease in $[Ca^{2+}]_{ER}$.

Functional versatility of STIM proteins

Studies of the interactions between STIM1, STIM2, and other proteins have provided strong support for the hypothesis that STIM1 and STIM2 play multifunctional roles in cells (Tables 1 and 3). STIM1 regulation of Ca^{2+} signaling is neither limited to its actions on SOCs nor is it dependent on ER Ca^{2+} store depletion. In addition to Orai and TRPC channels, STIM1 regulates ARC channels independent of $[Ca^{2+}]_{ER}$.[109] Regulation of ARC channels by STIM1 is thought to be mediated through interactions with STIM1 expressed in the PM and not in the ER membrane. Control of ARC channels appears unique to a subpopulation of STIM1 protein that is expressed in the PM, and does not involve STIM2 since biotinylation assays indicate that STIM2 is not localized within the PM.[110] STIM1 has also been shown to inhibit voltage-dependent Ca^{2+} channels (VDCCs) in rat neurons, A7r5 cells, mouse HL-1 cardiomyocytes, and HEK293 cells.[85,86,157] The inhibitory effect of STIM1 on VDCC, L-type, alpha 1c subunit ($Ca_v1.2$) depends on binding of the SOAR domain with

Table 3. STIM2 interacting proteins.

Binding Protein	Identity of binding protein	Cell type/*in vitro* method	Reference
Cytoskeletal/cell surface			
EB1 and EB3	Microtubule binding proteins	HEK293, HeLa	58
PI(4,5)P2	Membrane phospholipid	Lipid-binding assay	59
GluA1 and GluA2	AMPA receptor subunit	Hippocampal neurons, rat cortical neurons	60,61
Spectrin α and β chains	Scaffold protein	MIN6, NIH 3T3	***
SOCE proteins			
STIM1	ER Ca^{2+} sensor	HEK293, HEK293T, Human platelets, HeLa, NIH 3T3	64,65,98,112
Orai1	CRAC channel	Human Platelets, rat cortical neurons, mouse dendritic cells, HEK293	63,66,68,69
Orai2	Store-operated Ion Channel	Mouse dendritic cells	66
TRPC3	Non-selective cation channel	HUVEC	
Nuclear transport			
Exportin1	Nuclear export	HEK293T	77
Transportin1	Nuclear import	HEK293T	77
ER Proteins			
IP3R1	ER Ca^{2+} channel	BAECs	79
SERCA3	ER Ca^{2+} ATPase	Human Platelets	63
Calnexin	ER chaperone/MAM protein	HEK293T	77
GRP78/BiP	ER chaperone	NIH 3T3	***
Others			
Calmodulin	Ca^{2+} binding protein	CaM binding assay, isothermal titration calorimetry	59,87
AKAP	Scaffold protein	Hippocampal neurons	60
rPKA and cPKA	PKA subunits	Hippocampal neurons	60
PRKAA2	Energy sensor	HEK293T	88
Liprin-alpha-1, 2, and 3	Focal adhesion regulator	MIN6, NIH 3T3	***
Heat Shock Cognate 71 kDa protein	Molecular chaperone	NIH 3T3	***
Rab11 family-interacting protein	Endosomal recycling	NIH 3T3	***

***Nelson HA and Roe MW: unpublished observations.

the C-terminus of $Ca_v1.2$. STIM1 interaction also has been shown to stimulate long-term internalization of the VDCCs from the PM of rat hippocampal neurons.[86]

More recently, we found that STIM1 plays a role in regulating ATP-sensitive potassium (K_{ATP}) channels and SOC channels in MIN6 cells, an insulin-secreting β-cell line derived from mouse insulinoma.[84] Co-immunoprecipitation studies demonstrated that STIM1 directly interacts with the nucleotide binding fold-1 (NBF1) of the sulfonylurea receptor 1 (SUR1) subunit of the K_{ATP} channels expressed in MIN6 cells and mouse islets of Langerhans. Since K_{ATP} channels are critically involved in the control of pancreatic β-cell membrane potential and stimulus-secretion coupling, our findings suggest that STIM1 couples PM ion channel activity and $[Ca^{2+}]_{ER}$ to regulate membrane excitability and β-cell function.[84]

In addition to regulating several different Ca^{2+} entry pathways, STIM1 has also been shown to play a role in the efflux of cytosolic Ca^{2+} through interactions with PMCA and SERCA2b.[21,158] In Jurkat T cells, immunoprecipitation of endogenous PMCA pulled down endogenous STIM1, demonstrating a direct physical interaction.[21] Interaction of STIM1 with PMCA decreased PMCA mediated Ca^{2+} efflux. In Jurkat cells, when extracellular Ca^{2+} is removed, basal $[Ca^{2+}]_c$ is restored following a two-phase (fast then slow) exponential decay pattern. While STIM1 overexpression significantly reduced the slow phase of Ca^{2+} clearance, STIM2 overexpression had no effect on Ca^{2+} clearance rates. The STIM1 cytosolic C-terminus encompassing the proline-rich domain appears to be responsible for the interaction with PMCA and the attenuation of Ca^{2+} efflux mediated by PMCA. Although STIM2 contains an analogous proline-rich domain, whether STIM2 also binds PMCA remains unknown. While the STIM1-PMCA interaction reduces Ca^{2+} efflux, an interaction of STIM1 with SERCA2b potentiates Ca^{2+} clearance from the cytosol into the ER in HeLa cells during SOCE activation.[158] Taken together, these studies suggest STIM1 shapes dynamic changes in $[Ca^{2+}]_c$ through its interactions with Ca^{2+}-ATPases and by regulation of SOCE.[21,158]

STIM1 has also been implicated in the regulation of other second messenger signaling pathways in cells. In HEK 293 cells, Orai1, STIM1, and adenylyl cyclase type 8 (AC8) were found to be co-localized in lipid raft domains of the PM.[159] When STIM1 and Orai1 are co-expressed in HEK293 cells, AC8 activity increases proportionally to the increase in SOCE. Inhibitors of SOCE reduced the effects on AC8 activity in HEK293 cells overexpressing AC8 and in non-manipulated MIN6 cells.[159] This work suggests SOCE mediated by STIM1 regulates Ca^{2+}-dependent activation of AC8. Alternatively, in NCM460 cells, ER Ca^{2+} depletion, independent of any changes in $[Ca^{2+}]_c$, leads to the

recruitment of ACs to PAM domains and therefore increased cAMP accumulation and protein kinase A (PKA) activation.[160] Furthermore, STIM1 translocation was required for linking reduced ER Ca^{2+} to increased AC activity. These findings suggest the existence of a store-operated cAMP signaling pathway regulated by STIM1. Interestingly, recent work has shown that cAMP can induce STIM1 translocation, but not Orai1 clustering or SOCE in pancreatic islet cells.[161] This suggests a bi-directional control of signaling pathways between STIM1 and cAMP.

STIM1 may also function as a sensor of cellular stressors. Studies of DT40 cells indicate that STIM1 functions as a sensor of oxidative stress.[162] Oxidant (buthionine sulfoximine)-induced S-glutathionylation at cysteine 56 near the EF-hand decreases Ca^{2+} binding, activating STIM1 and SOCE independent of ER Ca^{2+} content. Consequently, $[Ca^{2+}]_c$ is increased at rest and after cellular activation. This elevation of cytosolic Ca^{2+} leads to impaired mitochondrial function and induction of apoptotic cell death.[162] Recent studies of HEK293 and HeLa cells indicate that STIM1 participates in cellular responses to heat stress.[163] Using fluorescence confocal imaging, investigators found that heating HeLa cells from 23.5°C to 50°C induced GFP-STIM1 puncta formation without ER Ca^{2+} store depletion. Upon cooling, the number of STIM1 puncta was reduced by 80%, but did not return to baseline. During the cooling period, Orai1-mediated Ca^{2+} influx was initiated, resulting in a large rise in $[Ca^{2+}]_c$. Physiologically, relevant temperature changes have also been shown to modulate STIM1-dependent nuclear factor of activated T-cells (NFAT) activation and gene expression in Jurkat cells.[163]

STIM proteins also have important functional roles beyond Ca^{2+} signaling. Glutathione S-transferase (GST)-pull down and immunoprecipitation assays revealed an interaction of both STIM1 and STIM2 with microtubule plus end tracking proteins EB1 and EB3.[58,164] Some investigators have proposed that microtubules facilitate SOCE by organizing STIM1 localization and movement.[165] Other studies have shown that the interaction of STIM1 with EB1 was dispensable for SOCE, but important in the formation of contacts between the ER and microtubules that participate in dynamic changes in ER spatial organization and remodelling.[58] This may have important implications in regulating the transition from interphase to mitosis or meiosis. During interphase, STIM1 binds to EB1 via a T-R-I-P sequence located in the distal end of its C-terminus, maintaining proper ER spatial arrangement.[164,166] Phosphorylation of STIM1 near the T-R-I-P domain during mitosis inhibits the STIM1-EB1 interaction, causing dissociation of the ER from the microtubules. This allows exclusion of the ER from the mitotic spindle and proper separation of organelles during cell division. Interestingly, phosphorylation of STIM1 in this region was also shown to inhibit SOCE during mitosis, suggesting phosporylation may play a role in differential regulation of STIM1 function.[167] Furthermore, the effects of phosphorylation on STIM1-EB1 interactions and SOCE are independent. This was demonstrated as loss of the STIM1-EB1 interaction failed to block SOCE,[58] while a phospho-deficient STIM1 mutant restored SOCE independent of EB1.[166] It is uncertain whether STIM2 has similar functional interactions with microtubule plus end tracking proteins. STIM2 does associate with EB1 and EB3 in B16F1mouse melanoma cells, albeit to a lesser extent than STIM1.[58] The consequences of such interaction have not been studied.

A broader range of STIM1 functions were suggested following the identification of Partner of STIM1 (POST).[62] POST is a 10-transmembrane–spanning segment adaptor protein located in the ER and PM. Although POST-Orai1 binding is independent of ER Ca^{2+} store depletion, lowering $[Ca^{2+}]_{ER}$ triggers binding of POST to STIM1 and POST movement within the ER towards the cell membrane where it inhibits PMCA activity, and may thereby sustain elevated $[Ca^{2+}]_c$. POST also has been shown to facilitate interactions between STIM1 and PMCA, SERCA2, Na^+/K^+-ATPase, and the nuclear transporters, importin-β, and exportin. PMCA, SERCA, and Na^+/K^+-ATPase are all regulators of intracellular Ca^{2+}. STIM1 interaction with these transporters may serve to modulate their activity and coordinate $[Ca^{2+}]_c$. The proposed interaction between STIM1 and the nuclear transport proteins was further confirmed in an independent study that used co-immunoprecipitation in combination with mass spectrometry to identify exportin1, transportin1, and calnexin as both STIM1- and STIM2-associated molecules in HEK293T and HeLa cells.[77] An interaction with importin, exportin, and transportin following reduction in $[Ca^{2+}]_{ER}$ suggests the possibility that nuclear import/export can be directly modulated by store depletion. The significance of STIM interactions with calnexin and nuclear transport factors remains unclear, but future studies may reveal more complex interactions and functional consequences of these associations.

STIM1 has also been shown to interact with type 3 IP_3 receptor (IP3R3) in mouse pancreatic acini and Madin-Darby Canine Kidney Epithelial (MDCK) cells, and IP3R1 in bovine aortic endothelial cells (BAECs).[78,79,168] STIM2 was found to bind with IP3R1 in BAECs.[79] The functional consequences of these interactions are not well characterized. In murine pancreatic acini and *Drosophila melanogaster* neurons, the association of IP3R3 and STIM1 forms part of a signaling complex at the ER-PM junctions that stabilizes the STIM1-Orai1 interaction to facilitate SOCE.[79,169] It is likely that the interaction of STIM1 and STIM2 with IP3R contributes to the regulation of IP3R function. In BAECs, STIM1 positively regulated Ca^{2+} release stimulated by ATP or bradykinin, whereas an increased interaction between STIM1 and IP3R3 mediated by polycistin1 and the PI3K/AKT pathway was shown to decrease Ca^{2+} release in MDCK cells.[79,169]

While most studies looking to identify STIM associated proteins have focused on STIM1, a study in adult rat brains and hippocampal neurons investigated STIM2 specifically.[60] This study showed STIM2, but not STIM1, is important for regulation of dendritic spine formation in rat neurons.[60] In addition to regulating spine morphogenesis, STIM2 also was found to participate in the regulation of synaptic transmission as well as synaptic delivery and removal of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs).[60,170] AMPARs are non-selective transmembrane cation channels allowing the

passage of Na^+ and K^+, and are the primary mediators of excitatory neurotransmission in the brain. AMPAR is composed of four subunits (GluA1–4). GluA1 has two well-defined phosphorylation sites in the cytoplasmic tail that regulate activity of the AMPAR; Ser-831 and Ser-845. In nervous tissues, co-immunoprecipitation experiments revealed STIM2 interacts with GluA1, AKAP, and both the regulatory and catalytic subunits of PKA.[60] STIM2 facilitates phosphorylation of GluA1 by recruiting PKA to the AMPAR independent of SOCE. More interestingly, it was shown that cAMP initiates STIM2 translocation to ER-PM junctions where it acts in a signaling complex with PKA to phosphorylate GluA1 and enhance cell surface delivery of the AMPAR.[60] Although this function was thought to be a unique property of STIM2, other co-immunoprecipitation studies have demonstrated that both STIM1 and STIM2 interact with GluA1 and GluA2 in rat cortical neurons.[61] The STIM-AMPAR subunit interaction was important for amplifying the SOCE response *via* Ca^{2+} entry through AMPARs. Although STIM1 interacted with AMPARs in a SOCE-dependent context, STIM1 was not important for dendritic spine morphogenesis, suggesting a novel role as a central organizer of excitatory synapses unique to STIM2 in neural cells.

We have used co-immunoprecipitation to pull down STIM2 and mass spectrophotometry to identify novel STIM2 binding proteins from mouse insulinoma (MIN6) and NIH 3T3 fibroblast cell lysates (Table 3). Alpha and beta chain spectrin were both pulled down with STIM2 in MIN6 and NIH 3T3 cells. Spectrin is a cytoskeletal protein that forms a scaffold important for maintaining PM and cytoskeletal integrity. The STIM2-spectrin interaction has not been explored, but could be important for anchoring STIM2 at ER-PM junctions. Liprins 1, 2, and 3 were also pulled down with STIM2 in both the NIH 3T3 and MIN6 cells. Liprins interact with the LAR family of transmembrane protein tyrosine phosphatases and may facilitate their localization to focal adhesions. The functional significance of a STIM2-Liprin interaction remains to be determined. Inducible heat shock cognate 71 kDa protein (also termed heat shock 70 kDa protein 8, Hsc70 or Hsp73) and Rab11 family-interacting protein also were pulled down by STIM2 in NIH 3T3 cell lysates. An interesting and potentially important STIM2 binding partner we identified in NIH 3T3 cells was GRP78, another member of the heat shock protein 70 family. This protein, also known as binding immunoglobulin protein (BiP) is located in the ER lumen where it regulates folding and assembly of proteins in the ER as well as participates in ER stress signaling through the unfolded protein response signaling pathways. Future investigations of the STIM2-GRP78 interaction could provide exciting new insights into the function of STIM2 in ER stress and cell death.

In summary, recent studies have demonstrated that STIM1 and STIM2 are multifunctional signaling effectors that participate in a wide array cellular signaling pathways. STIM1 has been implicated in Ca^{2+} clearance and various transport mechanisms to coordinate $[Ca^{2+}]_c$.[21,62,77,158] Not only does STIM1 activate Orai1, but STIM1 also regulates K_{ATP} channels and ARC channels.[84,110] STIM1 may play a role in cAMP and IP3R signaling pathways.[78,79,159,160] STIM1 also functions as a sensor of various kinds of cellular stress, interacts with the microtubule cytoskeleton and is involved in remodeling of the ER.[58,162,163,166] Finally, although not as thoroughly studied as STIM1, emerging evidence indicates that STIM2-mediated signaling plays an important role as a central organizer of excitatory synapses in neural cells.[60]

Pathophysiology of STIM

Ca^{2+} entry through SOCs controls a diverse range of cellular functions, such as exocytosis, cell proliferation, gene transcription, and cell death. It is not surprising therefore that several human diseases have been associated with disruption of *Stim1* and *Stim2* expression. A growing body of evidence indicates that aberrant SOCE contributes to the pathogenesis of immunodeficiency disorders, cancer, neurodegeneration, cardiovascular disease, musculoskeletal disorders, and diabetes.

Immune system diseases associated with disruption of STIM1 and STIM2

Although abnormal SOC activity may result from absence or mutation in the channel protein, Orai1, studies have shown mutations in STIM1 contribute to abnormal SOCE and the pathogenesis of disease. For example, a nonsense mutation in *Stim1* was discovered in a patient with complex immunodeficiency disorder (CID).[171] The mutation introduced a premature stop codon in *Stim1*, resulting in a truncated STIM1 lacking the SAM domain. Examination of fibroblasts isolated from the individual with CID showed loss of STIM1 expression and decreased SOCE. Expression of wild type (WT) human STIM1 restored normal patterns of Ca^{2+} entry following ER Ca^{2+} store discharge. Expression of WT STIM2 also increased SOCE, although to a smaller extent than with that of WT STIM1. It is interesting to note that mRNA levels of *Stim2* were not altered in the CID patient fibroblasts. This raises the likelihood that the level of endogenous STIM2 is not sufficient to compensate for the loss of STIM1; however, when overexpressed, STIM2 can partially compensate for the loss of STIM1 function. Severe immunodeficiency was also observed in another cohort of patients possessing a mutation that caused reduced *Stim1* mRNA expression and absence of STIM1 protein expression.[172,173] The association between STIM mutations and immunodeficiency can be explained by the resulting defects in SOCE as this pathway of Ca^{2+} entry controls T cell proliferation, cytokine production and expression, regulatory B cell and mast cell function, and neutrophil phagocytosis.

SOCE and the STIM molecules are critical components of Ca^{2+} signals that regulate cellular processes in many types of immune cells. Mast cells from mice lacking STIM1 exhibit impaired Ca^{2+} influx and reduced activation of transcription factors NF-κB and NFAT. Furthermore, following IgE stimulation, STIM1-deficient mast cells had significantly less degranulation and cytokine production, demonstrating an important role for STIM1 mediated SOCE in mast cell activation and anaphylaxis.[175] T cells

isolated from mice lacking STIM1 or STIM2 had reduced SOCE, decreased NFAT translocation to the nucleus, and lower cytokine production.[103] Stim1/Stim2 double-knockout mice displayed a lymphoproliferative phenotype and a reduced number of regulatory T cells. Other studies have found impaired T cell receptor mediated activation of T cells in the absence of SOCE in patients lacking Orai1 or STIM1.[171,176] SOCE has also been shown to play a key role in the function of natural killer (NK) cells; cytotoxic lymphocytes important for viral and antitumor immunity. NK cells deficient for Orai1 and STIM1 exhibited reduced cytokine production, defects in the exocytosis of cytotoxic granules, and an inability to lyse tumor targets *in vitro*.[173,177] In addition to NK cell deficits, NKT cell development is impaired in STIM1- STIM2-deficient mice.[178]

While absent or reduced SOCE can cause immunodeficiency, overactive SOCs are also linked to the pathogenesis of human disease. One such example is the contribution of SOCE to airway inflammation and bronchoconstriction. A 3,5-bistrifluoromethylpyrazole derivative, BTP2, blocks SOCs and interleukin (IL)-2 production in T cells. When BTP2 was orally administered in sensitized guinea pigs, it reduced asthmatic bronchoconstriction.[179] Additionally, in sensitized Brown Norway rats, BTP2 reduced antigen-induced eosinophil infiltration and IL-4 and leukotriene production in airways. These studies raise the possibility that SOCE contributes to the development of bronchial asthma via Ca^{2+} influx activation of T cell cytokine production and release of inflammatory mediators.[179–181] Increases in SOCE are also thought to enhance proliferation of T cells. This may account for the elevated cytokine production in intestinal mucosa associated with inflammatory bowel disease.[182–184] A more detailed review of the roles of STIM1 and STIM2 in immune diseases is available elsewhere.[174]

Abnormal SOCE and its role in the pathogenesis of cancer

STIM1, Orai1, and SOCE have also been implicated in carcinogenesis. Studies have indicated that cancer cell proliferation, apoptosis, metastasis, and tumor neovascularization are affected by disruption of SOCE and other components of intracellular Ca2+ signal transduction.[185] Several forms of cancer are associated with altered Ca^{2+} homeostasis and abnormal expression of Ca^{2+} channels and other Ca^{2+} regulatory proteins.[186]

Initially, STIM1 was identified as a potential tumor suppressor in rhabdomyosarcomas and rhabdoid tumors.[95] While STIM1 is highly expressed in skeletal myocytes, *Stim1* mRNA has not been detected in rhabdomyosarcoma or rhaboid tumor cell lines. Furthermore, when STIM1 is overexpressed in cells derived from rhaboid tumors or rhabdomyosarcomas lacking STIM1 expression, the cells die. Collectively, these data suggest loss of STIM1 expression is critical for the establishment or progression of these tumors.

In contrast, a tumor growth promoting role for STIM1 was found in patients with cervical cancer and breast cancer.[151,187] In a study of 24 cervical cancer patients, expression of STIM1 directly correlated with tumor size and clinical outcome.[187] Increased expression of STIM1 correlated with larger primary tumors, greater metastasis, and a decreased five-year survival rate. Similarly, there was greater tumor growth and decreased patient survival in breast cancer patients with high *Stim1* mRNA expression levels compared to controls.[151] In rat and human glioblastoma cells, knockdown of STIM1 or Orai1 inhibited tumor cell proliferation and promoted apoptosis, suggesting a role for SOCE in glioblastoma formation.[26] S*tim2* expression was also found to be increased in a genome-wide gene expression analysis of 20 patients with primary glioblastoma.[188]

Others studies have provided additional support for the hypothesis that STIM1 and STIM2 participate in tumorogenesis by showing that SOCE is important for the vascularization in tumors by controlling the production of vascular endothelial growth factor (VEGF).[187] Knockdown of STIM1 in human cervical cancer cells impairs VEGF secretion and in SCID mice injected with SiHA tumor cells, SOCE inhibitors 2-aminoethoxydiphenyl borate (2-APB), and SKF 96365 reduced neovascularization.[187] In human umbilical vein endothelial cells (HUVEC), RNAi silencing of *Stim1*, *Stim2*, or *Orai1* reduced both VEGF-mediated Ca^{2+} influx and cell proliferation.[189] Furthermore, *in vitro* treatment of human endothelial progenitor cells with BTP2, an inhibitor of SOCE, decreased VEGF-induced cytoplasmic Ca^{2+} oscillations, cell proliferation, and tubologenesis.[190]

A role for STIM1 and Orai1 in tumor metastasis has also been proposed. Migration of tumors from the primary site to secondary locations is the leading cause of death of cancer patients. It has been shown that Orai1 and STIM1 play a critical role in tumor cell migration *in vitro* and tumor cell metastasis in mice.[191] Application of SKF96365, an inhibitor of SOCE, as well as lowering the expression level of Orai1 and Stim1 by RNA interference, reduced cancer cell migration and tumor metastasis. This suggests the loss of SOCE caused by reduction of STIM1 or Orai1 expression is an important molecular signaling mechanism that could be exploited to develop new therapeutic approaches that reduce tumor migration and metastasis. However, caution must be taken when using such an approach since SOCE is also critical for antitumor immunity.[192] In mice with a T cell specific deletion of both *Stim1* and *Stim2*, cytotoxic lymphocytes were unable to prevent tumor cell engraftment. Specifically, STIM1 and STIM2 were required for production of interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α), degranulation, and Fas ligand (FasL)-mediated tumor cell killing. This indicates that SOCE is important for antitumor immune responses and raises the possibility that inhibition of CRAC channels in cancer therapy has the potential to decrease the body's own ability to inhibit tumorgenesis.

While the STIM1-Orai1 pathway has been extensively implicated in the pathogenesis of cancer as described above, some studies have suggested that Orai3 plays a key regulatory role in cell proliferation and cancer progression. Orai3 has been found to be overexpressed in breast, prostate, and lung cancers.[193–196] Interestingly, Orai3 is implicated in estrogen receptor (ER)(+) breast tumors specifically, while the canonical STIM1/Orai1 pathway

appears to predominate in ER(−) breast cancers cells.[195] Orai3-mediated SOCE is required for cell cycle progression, particularly through G1 and G1/S transition phases. [194,197,198] Knockdown of Orai3 expression in an ER (+) breast cancer cell line (MCF7) inhibited cell cycle progression, induced apoptosis, and inhibited tumorigenesis.[194,198] Taken together, these studies suggest Orai3 is a specific ER-regulated Ca^{2+} channel associated with uncontrolled cell growth and tumorigenesis. Similarly, in lung cancer tissue, Orai3 expression was elevated, correlating with increased cell proliferation and high tumor grade.[196] In human prostate cancer cell lines, Orai3, but not Orai1 is significantly overexpressed.[193] In *in vitro* models used to mimick this change in Orai expression, Orai3 overexpression favored heteromerization with Orai1 to form an ER Ca^{2+} store-independent Ca^{2+} channel. The Orai3-Orai1 channels promote cell proliferation and reduce the number of Orai1-based SOCs, which are implicated in supporting vulnerability to apoptosis. *In vivo*, Orai3 knockdown in prostate cancer tumors reduced tumor growth, while overexpression of Orai3 increased tumor volume.[193] This suggests there may be a critical switch in cancer progression, shifting the Orai channels from Orai1 homomultimers to Orai3-Orai1 heteromultimers that promote cell growth and migration while preventing apoptosis.

Neurodegenerative diseases associated with disruption of STIM1 and STIM2

There is a growing body of evidence supporting a role of STIM1, STIM2, and SOCE in nervous system pathology, such as Alzheimer's disease (AD), Huntington's disease (HD), and ischemic damage of the brain.[100,199–201] For instance, it is known that mushroom dendritic spine structures are essential for memory storage and the loss of mushroom spines may explain memory defects in Alzheimer's disease (AD). Interestingly, it was shown that STIM2 is important for stabilization of neuronal mushroom spines via a STIM2-mediated neuronal store-operated Ca^{2+} entry (nSOC) and Ca^{2+}/calmodulin kinase II pathway.[199] This suggests STIM2 downregulation is associated with spine loss and memory decline. Consistent with this, overexpression of STIM2 in two murine models of AD rescued nSOC and prevented mushroom spine loss.[199,202] Furthermore, downregulation of STIM2 in familial Alzheimer's disease (FAD) is thought to be linked to presenilins, endoprotease complexes that catalyze the intramembrane cleavage of integral membrane proteins.[203] Mutations in presenilin 1 (PS1) are a major cause of FAD. Mutations in PS1 reduce STIM2 expression and diminish SOCE in human lymphocytes. In human neuroblastoma cells, expression of a mutant PS1 linked to AD was also shown to reduce SOCE.[204] Surprisingly, STIM2 knockdown rescued SOCE in these cells, which markedly contrasts with the results from STIM2 overexpression in the murine models. In this case, it appears STIM2 may be acting as an inhibitor of SOCE. Taken together, this data suggest that the STIM2-mediated SOCE pathway in neurons may be a therapeutic target for the development of novel treatments of memory loss and AD.

STIM1 and STIM2 are also important in the regulation of synaptic plasticity.[170,200,205] Long-term potentiation (LTP) and depression (LTD) are two widely studied models of synaptic plasticity implicated in information storage. Using a conditional knockout mouse models, deletion of both *Stim1* and *Stim2* enhanced LTP at CA3-CA1 hippocampal synapses.[200] This enhancement was associated with increased PKA mediated phosphorylation of the AMPAR subunit GluA1, cAMP response element binding protein (CREB), and $Ca_v1.2$. Physiologically, this resulted in impaired spatial learning and memory. In a *Stim2* conditional knockout mouse model, synaptic delivery and removal of AMPARs were impaired.[170] Similarly, STIM1 was shown to regulate mGluR1/TRPC3-dependent slow excitatory synaptic potentials.[205] Deletion of *Stim1* in mouse cerebellar Purkinje neurons has also been shown to cause impairments in cerebellar motor behavior. Taken together, these studies suggest STIM1 and STIM2 are key regulators of synaptic plasticity, mediated through their regulation of AMPAR cycling and Ca^{2+} signaling.

STIM2 has also been show to play a critical role in hypoxic neuronal cell death.[100] In neurons, STIM2 is the most abundant STIM isoform. SOCE was reduced in neurons from *Stim2* deficient mice ($Stim2^{-/-}$), but not in neurons isolated from *Stim1* knockout mice. Under anoxic conditions in the brain, excess $[Ca^{2+}]_c$ is thought to contribute to neuronal cell death. In $Stim2^{-/-}$ neurons, ischemia-induced Ca^{2+} accumulation was markedly reduced, suggesting decreased STIM2 may contribute to neuron survival. Indeed, when mice were subjected to ischemic conditions, $Stim2^{-/-}$ mice showed significantly less neuronal damage compared with wild type mice.[100] This data suggest that in cells where STIM2 is the predominant isoform, it may play a critical role in SOCE and pathophysiological conditions associated with SOCE and Ca^{2+} homeostasis.

Involvement of STIM1 and STIM2 in disorders of the cardiovascular system

Cardiac hypertrophy is an abnormal enlargement or thickening of the heart muscle that can result from increase in cardiomyocyte size or other changes in heart muscle machinery. Hypertrophic growth often accompanies various forms of heart disease that can lead to death. Orai1, STIM1, TRPC1, and therefore, SOCE play an important role in cardiac hypertrophy in mouse and rat cardiomyocytes.[105,206–208] Despite this data, the role of SOCE in adult cardiomyocytes is unclear. STIM1 expression and SOCE are absent in adult mouse cardiomyocytes, present in neonatal cardiomyocytes, suggesting a developmental regulation.[105] Interestingly, STIM1 expression was induced in adult cardiomyocytes by pathological stress to trigger SOCE and hypertrophy.

Hemostasis following blood vessel damage is the first stage of wound healing and depends on the coordinated activation of platelets. Ca^{2+} is a critical second messenger, controlling a variety of platelet responses, including integrin activation, secretion, shape change, and procoagulant activity.[209,210] SOCE mediated by Orai1 and STIM1 appears to be the main mechanism for Ca^{2+}-dependent platelet

activation and thrombosis.[210–216] A gain-of-function (GoF) STIM1 mutation in mice resulting in constitutively active STIM1 was lethal *in utero* in mice homozygous for the mutation due to uncontrolled bleeding.[214] Mice heterozygous for the mutation was viable, but had extremely low platelet. Low platelet counts were attributed to SOCE induced pre-activation of the platelets, leading to enhanced platelet clearance. GoF mutations in STIM1 have also been described as the causative agent in patients suffering from York platelet syndrome or Stormorken syndrome—diseases that are characterized by increased bleeding due to thrombocytopenia and platelet defects.[217–219]

Loss-of-function (LoF) mutations or conditional knockout of STIM1 or Orai1 expression in different mouse models exhibit significant defects in SOCE in platelets.[211–213,215] Although these platelets with impaired SOCE showed relatively minor defects in integrin activation and granule release, they had a defect in their ability to switch to a pro-coagulant state. As a direct result of the role of STIM1 and Orai1 in promoting platelet activation and blood clotting, STIM1 or Orai1 deficiency results in resistance to pulmonary and arterial thrombosis.[211,213] Indeed, when STIM1 was conditionally knocked out of the platelets in a mouse model, thrombi were instable and fibrin formation at sites of vascular injury was slowed.[211] Interestingly, this phenotype was not associated with significantly increased bleeding risk, suggesting STIM1 and Orai1 may be novel targets for anti-platelet therapy for the treatment of thrombosis-associated diseases.

SOCE has also been implicated in vascular remodeling in patients with pulmonary arterial hypertension (PAH).[220] PAH is a progressive and fatal disease for which there are some treatments, but no cures. Excessive proliferation of pulmonary arterial smooth muscle cells (PASMC) is a common causative agent in the development and progression of PAH. Expression of STIM2, Orai2, and transient receptor potential channel 6 (TRPC6) is upregulated in PASMC with a proliferative phenotype compared to those with a contractile phenotype.[220] Increased $[Ca^{2+}]_c$ is a key signal initiating the switch from a contractile to proliferative phenotype in PASMC. The data indicate that upregulation of STIM2 and Orai2 in PASMC results in enhanced SOCE, and therefore an increase in proliferating PASMC. Furthermore, clinically, STIM2 and Orai2 expression levels have been found to be elevated in PASMC from patients with PAH compared to normal controls. This study identifies potential new molecular targets for the treatment of PAH and raises the possibility that by downregulating STIM2 or Orai2, PASMC proliferation and progression of PAH could be prevented or reduced.

Musculoskeletal diseases associated with abnormal SOCE

Although Ca^{2+} influx via SOCE is not necessary for skeletal muscle fiber excitation-contraction coupling, it does appear important for fatigue resistance.[221–223] In mouse and human myotubes, deletion of STIM1 or STIM2 reduced Ca^{2+} stores, impaired SOCE, and decreased fatigue resistance.[221,223] Similar results were obtained in transgenic mice with skeletal myocyte-specific expression of a dominant-negative mutant of Orai1 (*Orai1*^{E108Q}).[224] Muscle fibers expressing *Orai1*^{E108Q} fatigued more rapidly than controls upon repeated stimulation. Pharmacological inhibition of SOCE in the presence of extracellular Ca^{2+} has been shown to increase muscle fatigue.[90] Furthermore, muscles isolated from Mitsugumin 29 (MG29) null mice were more susceptible to muscle fatigue and this susceptibility can be attributed to a dysfunction in SOCE.[90] In sarcalumenin null mice, MG29 expression was significantly increased, resulting in enhanced SOCE and elevated muscle fatigue resistance.[225]

In addition to playing a role in skeletal muscle fatigue resistance, SOCE also serves as a signal to regulate muscle development and differentiation through Ca^{2+}-dependent pathways.[226,227] The expression of STIM1 and Orai1, and SOCE have been found to be significantly increased during differentiation of mouse and human myoblasts into myotubes in culture.[228] While knockdown of STIM1 or Orai1 reduced SOCE and myoblast differentiation, overexpression of STIM1 and Orai1 increased and quickened myoblast differentiation.[228] The roles STIM1 and SOCE myocyte development were further supported in experiments using conditional deletion of STIM1 in mouse skeletal muscle. This study revealed that STIM1-mediated SOCE was required for neonatal muscle growth and differentiation.[229]

These defects noted can also be directly linked to human skeletal muscle diseases. For example, LoF or null mutations in Orai1 and STIM1 are associated with global muscular hypotonia in most patients.[38,174,230,231] Partial iris hypoplasia and/or mydriasis in some Orai1- and STIM1-deficient patients is yet another sign of muscular hypotonia and further underscores the importance of defects in SOCE in the development of myopathies in humans.[200,230]

More recently, mutations in the STIM1 intralumenal Ca^{2+}-binding EF hand domain have been identified as a genetic cause of tubular-aggregate myopathy (TAM).[232] TAM is characterized by the abnormal build-up of protein in type I and type II fibers that forms clumps of tube-like structures called tubular aggregates. The disease onset is in childhood or early adulthood and presents itself as slow and progressive lower-limb weakness. The TAM-related STIM1 mutants are constitutively active, forming clusters with Orai1 even when the stores are filled. This leads to higher basal $[Ca^{2+}]$ and dysregulated Ca^{2+} homeostasis in muscles from TAM patients.[232]

Increased Ca^{2+} influx and protease activity for which SOCE may be involved is also proposed to play a key role in Duchenne muscular dystrophy (DMD).[233] In adult *mdx* mice, a well-characterized DMD animal model, Orai1 expression was significantly increased. Additionally, SOCE and $[Ca^{2+}]_{SR}$ were elevated in the dystrophic muscles. RNAi mediated knockdown of *Orai1* eliminated the differences in SOCE and $[Ca^{2+}]_{SR}$ between *mdx* and control muscle fibers, suggesting Orai1-mediated SOCE contributes to the altered Ca^{2+} homeostasis and subsequent enhanced proteolytic activity in dystrophic muscles.

Disruption of SOCE in the pathogenesis of diabetic complications

Abnormal SOCE has also been identified in diabetes. For example, STIM1 is significantly reduced in mouse coronary endothelial cells (MCEC) isolated from diabetic mice.[234] As a result of reduced STIM1 expression, diabetic MCECs exhibit reduced $[Ca^{2+}]_{ER}$ and consequently, diminished endothelium-dependent coronary vasodilation. These changes could contribute to cardiovascular complications common in diabetic patients. Consequent to STIM1 over-expression in diabetic MCECs, $[Ca^{2+}]_{ER}$ and endothelium-dependent relaxation of the coronary artery were restored to levels observed in control MCECs. The mechanism by which STIM1 is reduced in diabetic MCECs is unknown, but may be a secondary consequence of hyperglycemia, which has been shown to reduce STIM1 protein expression in control MCECs.[234] Although the mechanisms are not completely understood, changes in SOCE may also be linked to various complications resulting from diabetes, such as diabetic cardiomyopathy, diabetic vasculopathy, or diabetic nephropathy. A more thorough discussion of diabetes-associated changes in SOCE and its role in disease complications is provided elsewhere.[235]

Conclusions and future directions

STIM proteins are highly versatile, multifunctional members of a family of Ca^{2+}-binding proteins. The identification of the molecular basis of SOCE with STIM1 as the ER Ca^{2+} sensor and Orai1 as the channel pore has provided exciting new understanding of the roles of these proteins in Ca^{2+} signal transduction and contributed novel insights into the pathogenesis of human diseases. A rapidly growing body of evidence indicates that STIM1 and STIM2 dynamically control Ca^{2+} signal transduction by regulating several different channel proteins, including Orai1, TRPC1, ARC, T- and L-type voltage-gated Ca^{2+} channels, and K_{ATP} channels.[84,86,109,157] Given that proteomic and biochemical analyses have revealed a broad array of potential and novel STIM1 and STIM2 binding proteins, future studies will be necessary to identify and characterize other cellular functions mediated by interaction of STIM1 and STIM2 with their binding partners.

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