

*Current Perspective***Key Components of Store-Operated Ca²⁺ Entry in Non-Excitable Cells**Yosuke Tojyo^{1,2,*}, Takao Morita¹, Akihiro Nezu¹, and Akihiko Tanimura¹¹Department of Pharmacology, ²Laboratory of Biophysics, School of Dentistry, Health Sciences University of Hokkaido, Ishikari-Tobetsu, Hokkaido 061-0293, Japan

Received April 30, 2014; Accepted May 28, 2014

Abstract. Store-operated Ca²⁺ entry (SOCE) is a ubiquitous Ca²⁺ entry pathway in non-excitable cells. It is activated by the depletion of Ca²⁺ from intracellular Ca²⁺ stores, notably the endoplasmic reticulum (ER). In the past 9 years, it has been established that two key proteins, stromal interacting molecule 1 (STIM1) and Orai1, play critical roles in SOCE. STIM1 is a single-pass transmembrane protein located predominantly in the ER that serves as a Ca²⁺ sensor within the ER, while Orai1 is a tetraspanning plasma membrane (PM) protein that functions as the pore-forming subunit of store-operated Ca²⁺ channels. A decrease in the ER Ca²⁺ concentration induces translocation of STIM1 into puncta close to the PM. STIM1 oligomers directly interact with Orai1 channels and activates them. This review summarizes the molecular basis of the interaction between STIM1 and Orai1 in SOCE. Further, we describe current findings on additional regulatory proteins, such as Ca²⁺ release-activated Ca²⁺ regulator 2A and septin, novel roles of STIM1, and modulation of SOCE by protein phosphorylation.

Keywords: store-operated Ca²⁺ entry, STIM1, Orai1, calcium channel

1. Introduction

Activation of phospholipase-coupled receptors induces Ca²⁺ release from intracellular Ca²⁺ stores, mainly the endoplasmic reticulum (ER), and Ca²⁺ entry across the plasma membrane (PM). Inositol 1,4,5-trisphosphate (IP₃) generated by phosphoinositide breakdown is a key messenger of Ca²⁺ release from the ER, while the Ca²⁺ entry into cells is activated by the depletion of Ca²⁺ stored in the ER. The concept of this store-dependent Ca²⁺ influx mechanism was originally described by J. W. Putney (1) in 1986, based on the observations that Ca²⁺ influx induced by muscarinic agonists was closely linked to the emptiness of the ER. It was initially termed the “capacitative Ca²⁺ entry”, but today “store-operated Ca²⁺ entry” (SOCE) is the more popular term in the field of cellular Ca²⁺ signaling.

Since the first description by Putney (1), SOCE has been accepted as a ubiquitous and major Ca²⁺ entry pathway, not only in non-excitable cell types but also

in some excitable cell types. Despite numerous studies focusing on SOCE, two major questions remained unresolved until recently. One question is how the message that Ca²⁺ stores are depleted is conveyed to the store-operated Ca²⁺ (SOC) channels in the PM. A variety of hypothetical mechanisms have been proposed to explain the signal from the ER to the PM. Among them, the idea of a diffusible messenger released into the cytoplasm from depleted Ca²⁺ stores was extensively studied. Randriamampita and Tsien (2) first reported a diffusible messenger partially isolated from store-depleted Jurkat T-cells and named it “calcium influx factor” (CIF); however, the molecular identity of CIF is still unknown. By contrast, Irvine (3) proposed a conformational coupling model analogous to the interaction between dihydropyridine receptors and ryanodine receptors in skeletal muscle. In this model, the IP₃ receptors (IP₃R) directly interact with SOC channels in the ER–PM junctions. Although this model is an attractive idea, there is little or no critical evidence for the direct coupling of SOC channels with IP₃R.

The second question is that of the identity of the endogenous SOC channels mediating SOCE. The transient receptor potential (TRP) channels, especially the TRPC family, have been focused on as a candidate for

*Corresponding author. tojyo@hoku-iryu-u.ac.jp

Published online in J-STAGE on July 17, 2014

doi: 10.1254/jphs.14R06CP

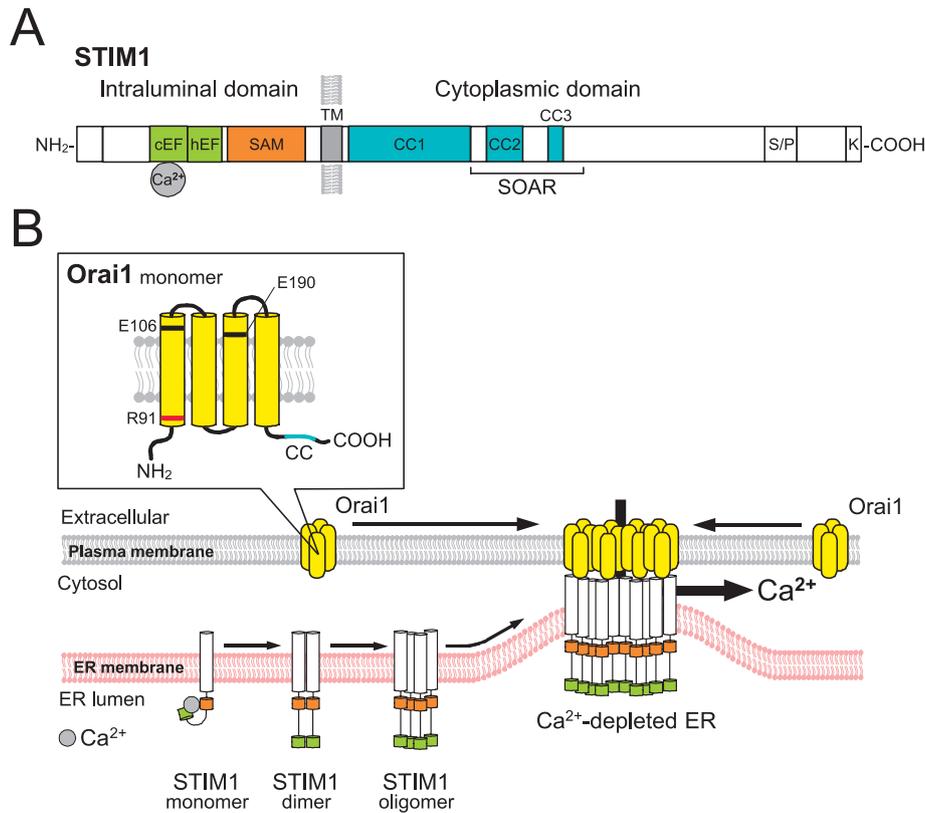


Fig. 1. Current model of store-operated Ca²⁺ entry. **A)** Domain structure of STIM1. The N-terminus of STIM1 includes a canonical Ca²⁺-binding EF-hand (cEF), a hidden EF-hand (hEF), and a sterile α motif (SAM), followed by the transmembrane domain (TM). The SAM domain contributes to STIM1 oligomerization. The C-terminus includes three coiled-coil domains (CC1-3), a serine/proline-rich domain (S/P), and a lysine-rich domain (K). CC2 and CC3 are part of the STIM-Orai activating region (SOAR), which is involved in the activation of Orai channels (24). **B)** Coupling between STIM1 and Orai1. Depletion of Ca²⁺ in the ER causes Ca²⁺ dissociation from the STIM1 N-terminal cEF-hand, resulting in STIM1 oligomerization and translocation to ER-PM junctions. There, the STIM1 oligomers interact with diffusible Orai1 tetramers in the PM and activate Orai1 channels, leading to Ca²⁺ entry.

the SOC channels. However, the role of the TRPC family in SOCE remains controversial, because TRPC channels have relatively low selectivity for Ca²⁺ and often exhibit distinct properties from Ca²⁺ release-activated Ca²⁺ (CRAC) current, which is the electrophysiological current associated with SOCE (4).

In the past 9 years, two key molecules playing crucial roles in SOCE regulation were discovered: the Ca²⁺-sensor protein STIM1 (stromal interacting molecule 1) in the ER, and the SOC channel component Orai1. The discoveries of these proteins were the major breakthroughs in our understandings of the molecular mechanism of the SOCE pathway. In this review, we summarize the molecular basis of the interaction between STIM1 and Orai1 and the roles of these two proteins in SOCE. Further, we describe current studies on additional regulatory proteins and modulation of SOCE by phosphorylation.

2. STIM1

STIM1 is a protein identified originally as a membrane molecule in stromal cells, and it is widely expressed from *Drosophila* to mammalian cells. In 2005, on the basis of limited RNA interference (RNAi) screens in *Drosophila* S2 cells (5) or human HeLa cells (6), two laboratories almost simultaneously reported that STIM1 is an essential protein to trigger SOCE. Mammalian cells have STIM1 and its homologue STIM2, while *Drosophila* has a single STIM gene. Since the knockdown of STIM1, but not that of STIM2, strongly reduced SOCE (5), a large number of studies have focused on STIM1.

STIM1 is a single-pass transmembrane protein located predominantly in the ER, although a small fraction of STIM1 is located in the PM (7). STIM1 contains two EF-hand domains, a canonical EF-hand domain (cEF), and a non-Ca²⁺ binding hidden EF-hand domain (hEF), and an adjacent sterile α motif (SAM) in the N-terminus

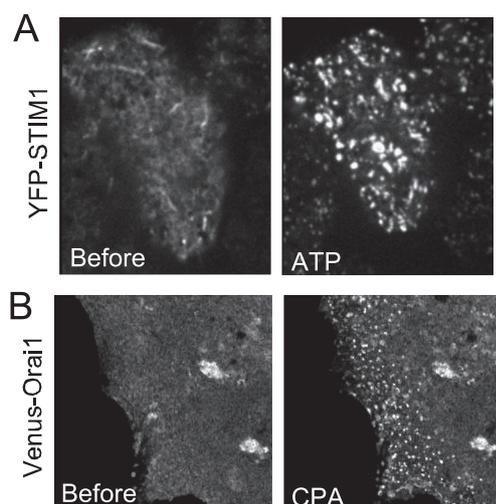


Fig. 2. Rearrangement of STIM1 and Orai1 following store depletion. In panel A, HSY cells expressing YFP-STIM1 were stimulated with ATP in Ca^{2+} -free medium. In panel B, COS-7 cells expressing Venus-Orai1 were stimulated with cyclopiazonic acid (CPA), an ER Ca^{2+} pump inhibitor, in Ca^{2+} -free medium. Modified from Ref. 38 with permission.

located in the lumen of the ER (Fig. 1A). Ca^{2+} binds only to the cEF-hand domain. The N-terminus of STIM1 functions as the sensor of Ca^{2+} concentration in the ER. When Ca^{2+} stores are full in resting status, STIM1 is uniformly distributed throughout the ER membrane. Depletion of Ca^{2+} stores by Ca^{2+} release induces dissociation of Ca^{2+} from the cEF-hand domain of STIM1, resulting in the unfolding of the EF-SAM domain and in a conformational change in STIM1. This leads to rapid oligomerization of STIM1 molecules through interaction of their SAM domains. The STIM1 oligomers migrate to the periphery of the cells, accumulate into punctate structures at ER-PM junctions, and activate SOC (Orai1) channels in the PM (Fig. 1B). This rearrangement of STIM1 has been observed by confocal microscopy in cells expressing YFP-STIM1 (Fig. 2A). The oligomerization is reversible: refilling with Ca^{2+} restores the diffuse localization of STIM1 and results in termination of SOCE (8, 9). Mutation of the crucial cEF-hand acidic residues results in loss of Ca^{2+} binding and causes the same oligomerization as reducing the ER Ca^{2+} concentration, constitutively activating CRAC channels without store depletion (6, 10).

STIM2 is also a single transmembrane protein located exclusively in the ER, but its physiological significance is poorly understood. STIM2 can translocate to ER-PM junctions with only small decreases in ER Ca^{2+} concentration (11), and its over-expression results in constitutive Ca^{2+} entry and CRAC channel activity (12, 13). Since

STIM2 is more sensitive to small changes in Ca^{2+} levels, Brandman et al. (11) suggest that STIM2 may play a role in regulating basal cytosolic and ER Ca^{2+} concentrations by finely modulating SOCE.

3. Orais

In 2006, Feske et al. (14) discovered the SOC channel molecule Orai1 using a combination of gene mapping of a family with a severe combined immunodeficiency (SCID) due to a defect in SOCE and whole genome RNAi screen in *Drosophila* S2 cells. Soon thereafter, two other laboratories also reported the same molecule using whole genome RNAi screens (15, 16). Although the molecule was initially called either of Orai1 or CRACM1 (CRAC modulator 1), today the term Orai1 is more extensively accepted. The SCID patients are homozygous for a single missense mutation, whereby a conserved arginine (R) residue is replaced with a tryptophan (W) at position 91 (14) (Fig. 1B). Cells from SCID patients were defective in SOCE and CRAC current. Expression of wild-type Orai1 in T-cells and fibroblasts from SCID patients restored SOCE and CRAC current, establishing that Orai1 plays a critical role in the regulation of SOCE.

In addition to Orai1, mammalian cells have genes for two additional homologs, Orai2 and Orai3. All three Orai proteins are tetraspanning PM channels with intracellular N- and C-termini and two extracellular loop regions (Fig. 1B). Mutation of conserved acidic residues (E106D and E190Q) in Orai1 or RNAi knockdown of Orai1 completely eliminates SOCE and CRAC current (16, 17), indicating that Orai1 is a predominant type forming a pore of CRAC channels. However, when over-expressed in a cultured cell line, Orai2 and Orai3 can also form pore subunits of CRAC channels (18), and it is likely that Orai2 and/or Orai3 may play a role as compensative types for the lack of Orai1.

Endogenous Orai channels have been thought to be formed by a tetramer of Orai subunits. A recent study reported that the crystals of the purified full-length Orai protein from *Drosophila* display a hexameric channel structure (19). However, it is unclear whether the *Drosophila* Orai protein assembles into a channel in the same manner as its mammalian homolog. Thompson and Shuttleworth (20) showed that the tetrameric *Drosophila* Orai channel displays highly Ca^{2+} -selective conductance properties consistent with endogenous CRAC channels, whereas the hexameric construct forms a non-selective cation channel. This suggests that endogenous Orai1 channels have a constant tetrameric structure.

4. Interaction between STIM1 and Orai1

The Orai1 protein is located uniformly in the PM in resting cells but rapidly forms clusters after store depletion (Fig. 2B) and becomes co-localized with STIM1 puncta (21, 22), indicating a direct interaction between STIM1 and Orai1. Although over-expression of STIM1 alone elicits only a poor increase in SOCE, its over-expression together with Orai1 dramatically increases SOCE or CRAC current (12, 18). This finding has been interpreted as an indication that the STIM1 and Orai1 proteins might be the essential and sufficient constituents of SOCE. Zhou et al. (23) reported that recombinant STIM1 cytoplasmic fragments directly gate the Orai1 channel *in vitro*. In this study, human Orai1 was expressed in yeast cells, and sealed membrane vesicles containing functional Orai1 channels were isolated from the cells. *In vitro* Ca^{2+} flux assays clearly showed that the STIM1 fragments induced a release of Ca^{2+} through the Orai1 channels of the isolated membrane vesicles. The STIM1 fragments did not elicit Ca^{2+} release from vesicles containing mutant Orai1. This elegant study by Zhou et al. (23) strongly suggests that STIM1 can gate Orai1 directly without the participation of any other proteins. It has been reported that all Orai channels are activated via a STIM–Orai activating region (SOAR) contained in the cytoplasmic domain of STIM1 (24) (Fig. 1A).

Nonetheless, the possibility that additional proteins may modulate the function of the Orai1–STIM1 interaction is not excluded. Varnai et al. (25) suggest that Orai1 is part of a larger macromolecular complex with a protrusion into the cytoplasm. This indicates the presence of additional molecular components within the STIM1–Orai1 complex. Further, CRAC regulator 2A (CRACR2A), a novel EF-hand protein, was identified as an important component of the Orai1–STIM1 complex (26). CRACR2A seems to act as a stabilizer of the interaction between Orai1 and STIM1. A recent study using a genome-wide RNAi screen in HeLa cells found that filamentous septin proteins function as crucial regulators of SOCE (27). The knockdown of septins with siRNA resulted in a significant reduction in SOCE and CRAC current and strongly inhibited the NFAT nuclear translocation induced by SOCE. The reorganization of phosphatidylinositol-4,5-bisphosphate (PIP_2) associated with the rearrangement of septin filaments appears to contribute to the stabilization of the STIM1–Orai1 complexes in puncta. This study suggests that septins may play a role in modulating Orai distribution in the PM and in clearing PIP_2 from ER–PM junctional sites.

5. Regulation of other channels by STIM1

STIM1 can interact with certain TRPC channels (28), implying that TRPCs function as STIM1-regulated channels. Zeng et al. (29) demonstrated that the gating of TRPC channels by STIM1 occurs through electrostatic interactions between positively charged lysine residues in STIM1 and negatively charged aspartate residues in TRPCs. They suggested that STIM1 gates Orai1 and TRPC channels by two distinct mechanisms and that TRPCs might form a different type of SOC channel with different properties from CRAC channels. However, this idea is controversial. A number of laboratories have failed to observe activation of TRPC channels by store depletion (4). In addition, it has been demonstrated that TRPC channels are activated by mechanisms dependent on phospholipase C, but not dependent on STIM1 and Orai1 (30). Further studies are required to conclude whether STIM1 is involved in the activation of TRPC channels.

The arachidonic acid–regulated Ca^{2+} channel (ARC channel), which is a Ca^{2+} store-independent pathway for agonist-activated Ca^{2+} entry, has been described in various cell types (31). ARC channels display many features in common with CRAC channels but are activated without store depletion. Recently, Shuttleworth (31) proposed a unique model for the activation of ARC channels. In the model, regulation of ARC channel activity involves the discrete population of STIM1 that is constitutively present in the PM, but not the oligomerization of STIM1 in the ER upon store depletion. In addition, the pore of ARC channels is comprised of both Orai1 and Orai3 subunits, and arachidonic acid generation is assumed to induce the interaction between the PM STIM1 and ARC channel. However, it is unclear how the STIM1 in the PM can activate ARC channels.

We previously found a unique La^{3+} -resistant, non-capacitative Ca^{2+} entry in DT40 cells induced by low concentrations of anti-IgM antibodies, and we named it B-cell receptor–mediated SOC (B-SOC) (32). The B-SOC was in contrast with thapsigargin-induced Ca^{2+} entry, which is highly sensitive to La^{3+} , an inhibitor of SOC channels. Interestingly, La^{3+} -resistant Ca^{2+} entry was completely abolished in STIM1-deficient cells and restored by over-expression of STIM1, while double knockdown of Orai1 and Orai2 had no effect. Orai3 is not expressed in DT40 cells. Therefore, the B-SOC pathway may be mediated by STIM1-dependent Ca^{2+} channels distinct from Orai channels.

6. Other target proteins of STIM1

STIM1 interacts with the plasma membrane Ca^{2+} pump (PMCP) and attenuates the cytosolic Ca^{2+} clearance

mediated by PMCP during T-cell activation (33). This interaction may occur through a conformational coupling mechanism analogous to that between STIM1 and Orai1. Recently, we found that over-expression of STIM1 in rat submandibular acinar cells increased the resting cytosolic Ca^{2+} concentration and increased agonist-induced Ca^{2+} release from stores (34). This alteration of the Ca^{2+} response may be explained by the inhibition of Ca^{2+} clearance resulting from the interaction of over-expressed STIM1 with PMCP. Although the effect of over-expression should be carefully interpreted, it seems likely that STIM1 also interacts with major regulatory proteins other than CRAC channels at the ER-PM junctions.

Csutora et al. (35) reported a novel role for STIM1 as a trigger of CIF production in the ER following Ca^{2+} store depletion. They suggest that after Ca^{2+} dissociation from the STIM1 EF-hand, the STIM1 SAM domain may change conformation to interact with and activate the CIF-producing machinery via glycosylation sites in the SAM domain. In their hypothetical model, Ca^{2+} -independent phospholipase A_2 (iPLA₂) is a PM target of CIF, and the activated iPLA₂ activates SOC channels through generation of lysophospholipids. They demonstrated that knockdown or over-expression of STIM1 results in impairment or increase of CIF production. However, the molecular identity of CIF remains mystery, and the precise molecular mechanism by which STIM1 triggers CIF production is unclear.

7. Regulation of SOCE by phosphorylation/dephosphorylation

Some studies suggest that phosphorylation of STIM1 or Orai1 is associated with regulation of SOCE. We previously reported that the kinase inhibitor staurosporine (ST) increased SOCE without affecting Ca^{2+} release from the ER in rat parotid cells, whereas serine/threonine phosphatase inhibitors, such as okadaic acid and calyculine A, attenuated SOCE (36, 37). Since the increase in Ca^{2+} entry by ST was inhibited by phosphatase inhibitors (37), our studies suggest a phosphorylation-dependent regulation of SOCE. Further, we recently found that ST maintains the activation of SOCE even after the refilling of Ca^{2+} stores in COS-7 cells (38). Since this study suggested that in ST-treated cells the STIM1-Orai1 complex did not completely dissociate even after the refilling of Ca^{2+} stores, we assume that ST may stabilize the interaction between Orai1 and STIM1 through inhibition of phosphorylation.

Kawasaki et al. (39) showed that Orai1 is a target of phosphorylation by protein kinase C (PKC). PKC β phosphorylated the N-terminal serine residues (S27 and

S30) of Orai1 and suppressed SOCE and CRAC channel function. Substitution of endogenous Orai1 with a S27A/S30A mutant resulted in increased SOCE and CRAC currents. The paper suggests that PKC-mediated phosphorylation may be an important mechanism in the modulation of SOCE. In addition, Smyth et al. (40) found that SOCE and CRAC current are strongly suppressed during cell division. This suppression of SOCE is directly associated with the phosphorylation of STIM1. Two serine residues (S486 and S668) in the C-terminus of STIM1 are phosphorylated during cell division, resulting in inhibition of STIM1 rearrangement and in suppression of SOCE. The molecular mechanisms of SOCE modulation by phosphorylation/dephosphorylation should be investigated in the future studies.

8. Conclusion

This review described the molecular basis of the two key components, STIM1 and Orai1, mediating the SOCE pathway. STIM1 and Orai1 serve as ER Ca^{2+} sensors and SOC channels, respectively. Direct interaction between STIM1 and Orai1 at the ER-PM junction is a major mechanism for conveying ER information to the SOC channels. The molecular characteristics of STIM1-Orai1 coupling are becoming clearer by information of the crystallographic structures. Additional proteins, such as septin and CRACR2A, have been identified as a stabilizer and a modulator of the STIM1-Orai1 interaction, but the precise molecular mechanisms by which they regulate the STIM1-Orai1 interaction are not fully understood. Studies suggest that ion channels or membrane components other than Orais are regulated by STIM1, but further information is required to assess the physiological significance of their interaction with STIM1. It has been suggested that phosphorylation/dephosphorylation is important for the regulation of SOCE, but the molecular basis of STIM1 and Orai1 phosphorylation and the mechanism underlying phosphorylation-dependent regulation remain to be elucidated.

The SOCE pathway plays a crucial role in a variety of biological functions, including immune responses, platelet activation, gene expression, and cell migration and proliferation. Patients with impaired SOCE due to STIM1 or Orai1 deficiency have severe clinical phenotypes, including immunodeficiency, myopathy, and ectodermal dysplasia (41). Further clarification of STIM1 and Orai1 functions in vivo will provide new strategies for the development of novel drugs to aid in the treatment of human diseases.

References

- 1 Putney JW Jr. A model for receptor-regulated calcium entry. *Cell Calcium* 1986;7:1–12.
- 2 Randriamampita C, Tsien RY. Emptying of intracellular Ca^{2+} stores releases a novel small messenger that stimulates Ca^{2+} influx. *Nature* 1993;364:809–814.
- 3 Irvine RF. “Quantal” Ca^{2+} release and the control of Ca^{2+} entry by inositol phosphates: a possible mechanism. *FEBS Lett.* 1990; 263:5–9.
- 4 Putney JW Jr. Capacitative calcium entry: from concept to molecules. *Immuno Rev.* 2009;231:10–22.
- 5 Roos J, DiGregorio PJ, Yeromin AV, Ohlsen K, Lioudyno M, Zhang S, et al. STIM1, an essential and conserved component of store-operated Ca^{2+} channel function. *J. Cell Biol.* 2005;169: 435–445.
- 6 Liou J, Kim ML, Heo WD, Jones JT, Myers JW, Ferrell JE Jr, et al. STIM is a Ca^{2+} sensor essential for Ca^{2+} -store-depletion-triggered Ca^{2+} influx. *Curr Biol.* 2005;15:1235–1241.
- 7 Spassova MA, Soboloff J, He L-P, Xu W, Dziadek MA, Gill DL. STIM1 has a plasma membrane role in the activation of store-operated Ca^{2+} channels. *Proc Natl Acad Sci U S A.* 2006; 103:4040–4045.
- 8 Baba Y, Hayashi K, Fujii Y, Mizushima A, Watarai H, Wakamori M, et al. Coupling of STIM1 to store-operated Ca^{2+} entry through its constitutive and inducible movement in the endoplasmic reticulum. *Proc Natl Acad Sci U S A.* 2006;103:16704–16709.
- 9 Liou J, Fivaz M, Inoue T, Meyer T. Live-cell imaging reveals sequential oligomerization and local plasma membrane targeting of stromal interaction molecule 1 after Ca^{2+} store depletion. *Proc Natl Acad Sci U S A.* 2007;104:9301–9306.
- 10 Zhang SL, Yu Y, Roos J, Kozak JA, Deerinck TJ, Ellisman MH, et al. STIM1 is a Ca^{2+} sensor that activates CRAC channels and migrates from the Ca^{2+} store to the plasma membrane. *Nature.* 2005;437:902–905.
- 11 Brandman O, Liou J, Park WS, Meyer T. STIM2 is a feedback regulator that stabilizes basal cytosolic and endoplasmic reticulum Ca^{2+} levels. *Cell.* 2007;131:1327–1339.
- 12 Soboloff J, Spassova MA, Tang XD, Hewavitharana T, Xu W, Gill DL. Orai1 and STIM1 reconstitute store-operated calcium channel function. *J Biol Chem.* 2006;281:20661–20665.
- 13 Wang Y, Deng X, Zhou Y, Hendron E, Mancarella S, Ritchie MF, et al. STIM protein coupling in the activation of Orai channels. *Proc Natl Acad Sci U S A.* 2009;106:7391–7396.
- 14 Feske S, Gwack Y, Prakriya M, Srikanth S, Puppel S-H, Tanasa B, et al. A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. *Nature.* 2006;441:179–185.
- 15 Zhang SL, Yeromin AV, Zhang X H-F, Yu Y, Safrina O, Penna A, et al. Genome-wide RNAi screen of Ca^{2+} influx identifies genes that regulate Ca^{2+} release-activated Ca^{2+} channel activity. *Proc Natl Acad Sci U S A.* 2006;103:9357–9362.
- 16 Vig M, Peinelt C, Beck A, Koomoa DL, Rabah D, Koblan-Huberson M, et al. CRACM1 is a plasma membrane protein essential for store-operated Ca^{2+} entry. *Science.* 2006;312:1220–1223.
- 17 Prakriya M, Feske S, Gwack Y, Srikanth S, Rao A, Hogan PG. Orai1 is an essential pore subunit of the CRAC channel. *Nature.* 2006;443:230–233.
- 18 Mercer JC, DeHaven WI, Smyth JT, Wedel B, Boyles RR, Bird GS, et al. Large store-operated calcium selective currents due to co-expression of Orai1 or Orai2 with the intracellular calcium sensor, Stim1. *J Biol Chem.* 2006;281:24979–24990.
- 19 Hou X, Pedi L, Diver MM, Long SB. Crystal structure of the calcium release-activated calcium channel Orai. *Science.* 2012; 338:1308–1313.
- 20 Thompson JL, Shuttleworth TJ. How many Orai’s does it take to make a CRAC channel? *Science Rep.* 2013;3:1961.
- 21 Smyth JT, DeHaven WI, Bird GS, Putney JW Jr. Ca^{2+} -store-dependent and –independent reversal of Stim1 localization and function. *J Cell Sci.* 2008;121:762–772.
- 22 Park CY, Hoover PJ, Mullins FM, Bachhawat P, Covington ED, Raunser S, et al. STIM1 clusters and activates CRAC channels via direct binding of a cytosolic domain to Orai1. *Cell.* 2009; 136:876–890.
- 23 Zhou Y, Meraner P, Kwon HT, Machnes D, Oh-hora M, Zimmer J, et al. STIM1 gates the store-operated calcium channel ORAI1 *in vitro*. *Nat Struct Mol Biol.* 2010;17:112–116.
- 24 Yuan JP, Zeng W, Dorwart MR, Choi Y-J, Worley PF, Muallem S. SOAR and the polybasic STIM1 domains gate and regulate the Orai channels. *Nat Cell Biol.* 2009;11:337–343.
- 25 Varnai P, Toth B, Toth DJ, Hunyady L, Balla T. Visualization and manipulation of plasma membrane-endoplasmic reticulum contact sites indicates the presence of additional molecular components within the STIM-Orai1 complex. *J Biol Chem.* 2007;282:29678–29690.
- 26 Srikanth S, Jung HJ, Kim KD, Souda P, Whitelegge J, Gwack Y. A novel EF-hand protein, CRACR2A, is a cytosolic Ca^{2+} sensor that stabilizes CRAC channels in T cells. *Nat Cell Biol.* 2010; 12:436–446.
- 27 Sharma S, Quintana A, Findlay GM, Mettlen M, Baust B, Jain M, et al. An siRNA screen for NFAT activation identifies septins as coordinators of store-operated Ca^{2+} entry. *Nature.* 2013;499: 238–242.
- 28 Worley PF, Zeng W, Huang GN, Yuan JP, Kim JY, Lee MG, et al. TRPC channels as STIM1-regulated store-operated channels. *Cell Calcium.* 2007;42:205–211.
- 29 Zeng W, Yuan JP, Kim MS, Choi YJ, Huang GN, Worley PF, et al. STIM1 gates TRPC channels, but not Orai1, by electrostatic interaction. *Mol Cell.* 2008;32:439–448.
- 30 DeHaven WI, Jones BF, Petranka JG, Smyth JT, Tomita T, Bird GS, et al. TRPC channels function independently of STIM1 and Orai1. *J Physiol.* 2009;587:2275–2298.
- 31 Shuttleworth TJ. STIM and Orai proteins and the non-capacitative ARC channels. *Front Biosci.* 2012;17:847–860.
- 32 Morita T, Tanimura A, Baba Y, Kurosaki T, Tojyo Y. A Stim1-dependent, noncapacitative Ca^{2+} -entry pathway is activated by B-cell-receptor stimulation and depletion of Ca^{2+} . *J Cell Sci.* 2009;122:1220–1228.
- 33 Ritchie MF, Samakai E, Soboloff J. STIM1 is required for attenuation of PMCA-mediated Ca^{2+} clearance during T-cell activation. *EMBO J.* 2012;31:1123–1133.
- 34 Morita T, Nezu A, Tojyo Y, Tanimura A. Increase in muscarinic stimulation-induced Ca^{2+} response by adenovirus-mediated Stim1-mKO1 gene transfer to rat submandibular acinar cells *in vivo*. *Biochem Biophys Res Commun.* 2013;439:433–437.
- 35 Csutora P, Peter K, Kilic H, Park KM, Zarayskiy V, Gwozdz T, et al. Novel role for STIM1 as a trigger for calcium influx factor production. *J Biol Chem.* 2008;283:14524–14531.
- 36 Tojyo Y, Tanimura A, Matsumoto Y, Sugiya H. Staurosporine enhances Ca^{2+} entry induced by depletion of intracellular Ca^{2+}

- stores in rat parotid acinar cells. *Cell Calcium*. 1995;17:32–40.
- 37 Tojyo Y, Tanimura A, Matsumoto Y. Suppression of capacitative Ca^{2+} entry by serine/threonine phosphatase inhibitors in rat parotid acinar cells. *Jpn J Pharmacol*. 1995;69:381–389.
- 38 Tojyo Y, Morita T, Nezu A, Tanimura A. Staurosporine maintains the activation of store-operated Ca^{2+} entry even after the refilling of Ca^{2+} stores. *Cell Calcium*. 2013;53:349–356.
- 39 Kawasaki T, Ueyama T, Lange I, Feske S, Saito N. Protein kinase C-induced phosphorylation of Orai1 regulates the intracellular Ca^{2+} level via the store-operated Ca^{2+} . *J Biol Chem*. 2010;285:25720–25730.
- 40 Smyth JT, Petranka JG, Boyles RR, DeHaven WI, Fukushima M, Johnson KL, et al. Phosphorylation of STIM1 underlies suppression of store-operated calcium entry during mitosis. *Nature Cell Biol*. 2009;11:1465–1472.
- 41 Feske S. CRAC channelopathies. *Pflügers Arch*. 2010;460:417–435.