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STIM proteins: dynamic calcium signal transducers

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Abstract

Stromal interaction molecule (STIM) proteins function in cells as dynamic coordinators of cellular calcium (Ca^{2+}) signals. Spanning the endoplasmic reticulum (ER) membrane, they sense tiny changes in the levels of Ca^{2+} stored within the ER lumen. As ER Ca^{2+} is released to generate primary Ca^{2+} signals, STIM proteins undergo an intricate activation reaction and rapidly translocate into junctions formed between the ER and the plasma membrane. There, STIM proteins tether and activate the highly Ca^{2+} -selective Orai channels to mediate finely controlled Ca^{2+} signals and to homeostatically balance cellular Ca^{2+} . Details are emerging on the remarkable organization within these STIM-induced junctional microdomains and the identification of new regulators and alternative target proteins for STIM.

Calcium (Ca^{2+}) signals are crucial for the control a broad range of cellular functions, including secretion, excitation, contraction, motility, metabolism, transcription, growth, cell division and apoptosis. The numerous pumps and channels that comprise the machinery for generating cellular Ca^{2+} signals are functionally well defined. However, less understood are the mechanisms that coordinate the operation of this machinery to generate the temporally and spatially precise Ca^{2+} signals that selectively control individual cell functions. Ca^{2+} signalling involves the concerted action of Ca^{2+} release channels in Ca^{2+} storage organelles and Ca^{2+} entry channels in the plasma membrane (BOX 1). The recently identified stromal interaction molecule (STIM) proteins^{1,2}, STIM1 and STIM2, are crucial in coordinating Ca^{2+} release and entry signals and in maintaining cellular Ca^{2+} homeostasis.

Box 1

Principles of Ca^{2+} signalling

The events that maintain cellular Ca^{2+} signalling during homeostasis are shown in part **a** of the figure. Resting cells (left) maintain cytosolic Ca^{2+} in the nM range through sarcoplasmic reticulum Ca^{2+} ATPase (SERCA) and plasma membrane Ca^{2+} ATPase (PMCA) pumps. Following ligand binding to phospholipase C (PLC)-coupled receptors (right), the second messengers inositol-1,4,5-trisphosphate ($\text{Ins}(1,4,5) \text{P}_3$) and diacylglycerol (DAG) are generated through breakdown of phosphatidylinositol-4,5-

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Competing interests statement

The authors declare competing financial interests; see Web version for details.

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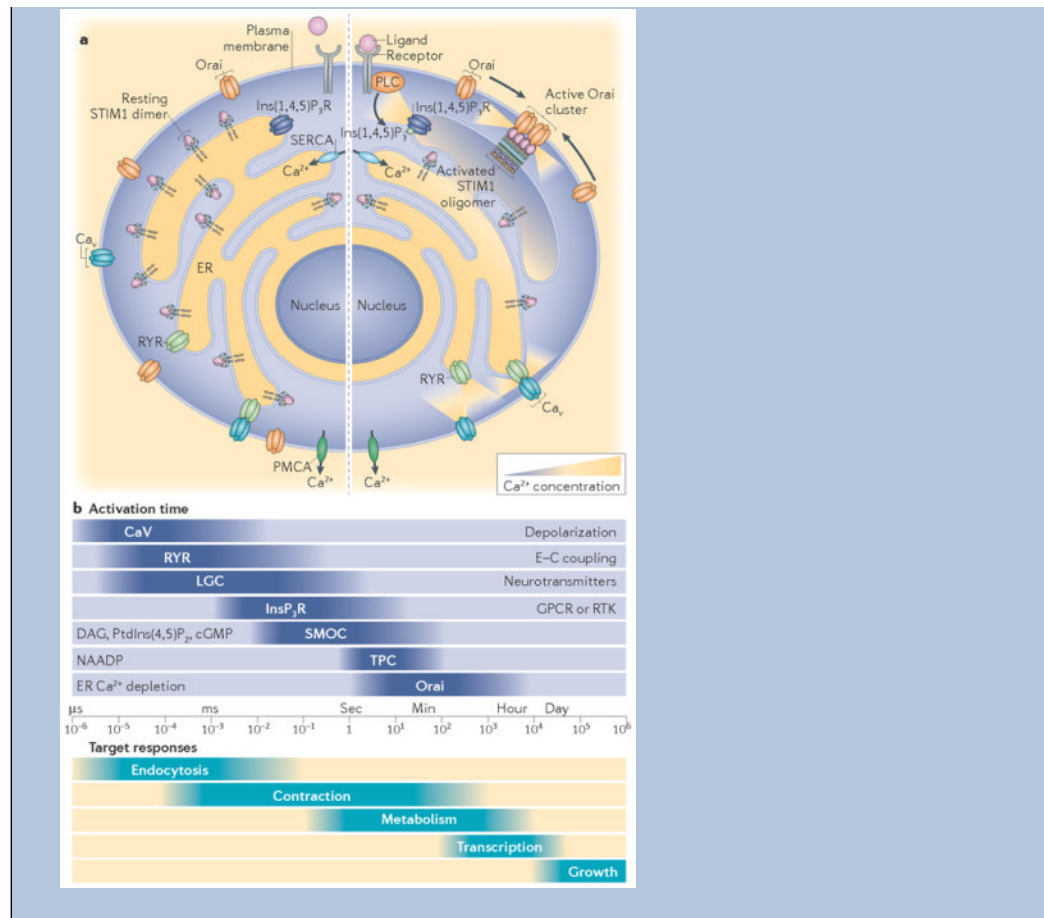
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bisphosphate (PtdIns(4,5)P₂). Ins(1,4,5)P₃ diffuses rapidly within the cytosol to interact with endoplasmic reticulum (ER)-located Ins(1,4,5)P₃ receptors (Ins(1,4,5)P₃Rs), which are channels that release Ca²⁺ from the ER lumen to generate the initial Ca²⁺ signal phase¹⁴⁹. Following depletion of ER Ca²⁺ (right), stromal interaction molecule (STIM) proteins are activated and translocate by diffusion into ER–plasma membrane junctions, where they interact with the plasma membrane. Here, STIM proteins tether and gate Orai1 Ca²⁺ entry channels. ER Ca²⁺ release leading to STIM activation can also be mediated by ryanodine receptor (RyR) activation. In skeletal muscle, RyRs are permanently coupled to plasma membrane voltage-operated Ca²⁺ channel (Ca_V) isoform Ca_V1.1, which is activated by depolarization. In other cells, RyRs are activated by the entry of Ca²⁺ into the cytosol through other Ca_V channel subtypes.

The properties and function of different Ca²⁺ channels are shown in part **b** of the figure. Ca²⁺ signals are generated by several different channels with widely differing timescales of activation (upper panel). As a result, each Ca²⁺ channel mediates temporally distinct Ca²⁺-dependent cellular responses (lower panel). Voltage-operated Ca_V channels are activated by membrane-depolarization in the μ s timescale. In skeletal muscle, RyRs are coupled to Ca_V channels and open rapidly thereafter. In cardiac muscle, smooth muscle and neurons, RyR activation is slightly slower than in skeletal muscle and depends on Ca_V-mediated Ca²⁺ entry. Ligand-gated channels (LGCs) are activated by the binding of extracellular ligands (neurotransmitters such as *N*-methyl-D-aspartate (NMDA), acetyl choline or nucleotides) and display varying activation kinetics depending on the rate of ligand induction or release. The activation rates of Ins(1,4,5) P₃Rs, second messenger-operated channels (SMOCs) and two-pore channels (TPCs) depend on the production rates of their respective ligands. Orai channels have the slowest activation kinetics, which depend on the rate of Ca²⁺ release from the ER and on the diffusion rate of STIM and Orai proteins into ER– plasma membrane junctions. Orai channels can remain active for long time periods under conditions of prolonged store depletion. The cellular responses shown in the lower panel are commensurate with the timescales of channel activation: Ca_V and RyR channels mediate rapid events including exocytosis and contraction, whereas Orai channels regulate long-term events such as gene transcription. cGMP, cyclic GMP; E–C coupling, excitation–contraction coupling; NAADP, nicotinic acid adenine dinucleotide phosphate; RTK, receptor Tyr kinase.



STIM proteins are finely tuned sensors of Ca²⁺ levels in the interior of the endoplasmic reticulum (ER). ER Ca²⁺ levels are needed for generating rapid Ca²⁺ signals in cells, but they are also vital for maintaining the correct protein folding and trafficking environment within the ER. Thus, decreased ER luminal Ca²⁺ levels are a major stress condition. Small changes in ER Ca²⁺ levels trigger STIM proteins to rapidly translocate into specialized junctions where they interact with the plasma membrane. There, STIM proteins directly couple with and gate plasma membrane Orai channels^{3–5}, which mediate an extraordinarily selective entry of Ca²⁺ ions. The entering Ca²⁺ ions provide precise local Ca²⁺ signals that are crucial for controlling long-term cellular responses, including gene expression and growth. The entering Ca²⁺ is also essential for the homeostatic control of cellular Ca²⁺ levels.

Emerging studies reveal much about the molecular sensing properties of STIM proteins: how they become activated, how they unfold and form complexes that can translocate into junctions and how they specifically engage and activate their channel targets. New information reveals that the junctions have an astonishing complexity, comprising an array of previously known and newly identified regulatory proteins. Organized around STIM proteins, this complex of proteins finely choreographs the interactions within junctional domains and controls the flow of Ca²⁺ to generate the exact Ca²⁺ signals that are required by the cell. Recent studies have also revealed that the junctions include several other crucial Ca²⁺ channels and Ca²⁺ pumps that are controlled by STIM proteins. Hence, in addition to generating finely tuned Ca²⁺ signals, STIM proteins are essential modulators of the Ca²⁺ homeostatic machinery in cells. Here, we describe the current understanding on STIM proteins as central communicating intermediaries in cellular signalling and highlight some

fascinating new areas of work that provide insights into their structure and mechanism of activation.

Store-operated Ca^{2+} entry: concept and history

The concept of Ca^{2+} signalling was first recognized by Ringer who revealed the importance of extracellular Ca^{2+} in maintaining contraction of isolated hearts⁶. The regulation of intracellular Ca^{2+} levels and the generation of Ca^{2+} signals are much newer concepts⁷. Ca^{2+} signals are a combination of both Ca^{2+} entry across the plasma membrane and Ca^{2+} release from intracellular stores, predominantly from the ER in higher eukaryotic cells. The causal relationship and coordinated control of these two processes was not appreciated until Putney proposed the model of capacitative Ca^{2+} entry in 1986 (REF. 8) (TIMELINE). After the discovery that inositol-1,4,5-triphosphate ($\text{Ins}(1,4,5)\text{P}_3$) activates Ca^{2+} release from ER Ca^{2+} stores⁹, the apparently ‘privileged’ movement of Ca^{2+} from outside to replenish stores led Putney to propose that “a specialized region exists where ER and plasma membrane are closely apposed and Ca^{2+} diffusion laterally is therefore geometrically restricted”⁸. This concept was visionary. However, the idea of a direct capacitative pathway allowing extracellular Ca^{2+} to directly enter depleted Ca^{2+} stores needed refinement. Studies showed that Ca^{2+} store depletion activates Ca^{2+} entry initially into the cytosol^{10,11}, a process that is triggered solely by decreased Ca^{2+} store content and does not require $\text{Ins}(1,4,5)\text{P}_3$ production¹². Thus, Putney presented a revised model in 1990 depicting the activation of Ca^{2+} channels in the plasma membrane as a direct consequence of Ca^{2+} store depletion¹³, the process now referred to as store-operated Ca^{2+} entry (SOCE). Simultaneously, small agonist-induced Ca^{2+} currents were being defined in haematopoietic cells^{14,15}, and in 1992 Hoth and Penner¹⁶ described the Ca^{2+} release-activated Ca^{2+} current (CRAC current), which is a small, inwardly rectifying, highly Ca^{2+} -selective current, in mast cells. In keeping with the recognition by Putney that Ca^{2+} entry is solely related to the Ca^{2+} content of the ER, the CRAC current is activated regardless of whether Ca^{2+} is released from stores following $\text{Ins}(1,4,5)\text{P}_3$ receptor ($\text{Ins}(1,4,5)\text{P}_3\text{R}$) activation, ER Ca^{2+} pump blockade, Ca^{2+} ionophore application or luminal Ca^{2+} chelation. Theories to explain how sensing of ER Ca^{2+} stores and the activation of specific channels could be coupled ranged from ER-derived diffusible messengers to direct conformational coupling between ER and plasma membrane proteins as envisaged by Berridge¹⁷. Close ER–plasma membrane interactions were clearly important for coupling to occur^{18,19}, and, ultimately, with the identification of STIM proteins in 2005 (REFS 1,2) and Orai channels a year later^{3–5}, the original ER–plasma membrane junctional coupling model of Putney⁸ was proven correct.

The discovery of the involvement of STIM proteins in SOCE came from two studies performing RNA interference (RNAi) screens. The first study, which examined Ca^{2+} responses in *Drosophila melanogaster* S2 cells, led to the identification of the single *D. melanogaster* STIM protein¹, the other study, which monitored Ca^{2+} signalling in HeLa cells, identified the pair of human STIM proteins². STIM proteins are now clearly recognized as the store Ca^{2+} sensors that trigger SOCE (BOX 1; FIG. 1a). Three genome-wide screens in S2 cells were successful in identifying the SOCE channel^{3–5} that is now known as Orai (FIG. 1b). In mammals, there are three *Orai* genes encoding store-operated channels with significantly different properties. The gene encoding Orai1 was identified by linkage analysis as being mutated in individuals with a rare immunodeficiency, in which T cells display defective SOCE³. Subsequently, other mutations in *ORAI1* (REF. 20) and *STIM1* (REF. 21) have been linked to human immunodeficiencies. Overall, the revelation that STIM proteins could mediate SOCE^{1,2} provided the mechanistic coupling paradigm predicted within the models of Putney^{8,13} and Berridge¹⁷.

STIM1 and STIM2

Dynamic intermembrane communicators

STIM proteins are type 1A single-span membrane proteins that are largely conserved across species from *D. melanogaster*¹ to *Caenorhabditis elegans*²². STIM proteins are likely to have evolved earlier than Ins(1,4,5)P₃Rs²³ and may have originally had a greater role in Ca²⁺ homeostasis than in Ca²⁺ signalling. In vertebrates, STIM1 and STIM2 are expressed ubiquitously throughout cell types²⁴. In most tissues STIM1 levels are higher than STIM2 levels^{24,25}, however, STIM2 expression predominates in the brain²⁶ and dendritic cells²⁷. STIM1 and STIM2 have high homology through most of their length, with variations in their amino-terminal and carboxy-terminal segments²⁸ (see [Supplementary information S1](#) (figure)). The functions of STIM1 and STIM2 are subtly distinct with important physiological implications.

Triggering and translocation of STIM1

STIM proteins are located predominantly in the ER^{29–31}, and they undergo rapid and reversible translocation into close ER– plasma membrane junctions to couple with and activate Orai channels following store depletion^{28,32} (BOX 1). Under resting conditions, STIM1 is distributed throughout the ER^{2,33,34} probably in a dimeric form^{35–40} (BOX 1). The dimers undergo rapid oligomerization and move into plasma membrane junctions within a few seconds following store depletion^{2,34} (BOX 1). The luminal N-terminal domain of STIM1 contains a tightly clustered assembly of short α -helices comprising two EF-hand domains and a sterile α -motif (SAM) domain^{41,42} (FIG. 1a,d). This region enables STIM1 to sense small changes in luminal Ca²⁺ levels and triggers intermolecular interactions. The cytoplasmic C terminus of STIM1 contains extensive coiledcoil regions that can span the ER– plasma membrane junctional gap, which is estimated to be ~15 nm^{2,33,34,43,44}. The C-terminal region includes an ~100 amino acid segment named the STIM–Orai activating region (SOAR), which mediates direct coupling with Orai channels⁴⁵ (FIG. 1a,d–f). This is similar to the CRAC activation domain (CAD)⁴⁶ or Orai1-activating small fragment⁴⁷ (OASF) (FIG. 1a). Crystallization approaches now provide the first evidence for the α -helical structure of these domains⁴⁰.

Although the role of STIM1 is now mostly defined in the ER, STIM1 was originally identified⁴⁸ on the surface of stromal cells mediating interactions with pre-B cells. Orai channel activation does not require plasma membrane STIM1, and thus tagged STIM1 that cannot insert into the plasma membrane⁴⁹ still fully activates Orai channels^{31,35}. Interestingly, replacement of the Ca²⁺-sensing luminal N-terminal domain of STIM1 with protein domains that oligomerize in response to a Ca²⁺-independent signal revealed that simple STIM1 crosslinking is sufficient for its activation⁴⁴. Moreover, the ER membrane-attached STIM1 C terminus alone is capable of clustering in junctions and activating Orai⁵⁰. However, these observations belie the complex conformational transition that the resting STIM protein must undergo to present itself within junctions.

STIM1 is a finely tuned ER Ca²⁺ sensor

The two EF-hand domains within STIM1 operate together in a tightly associated complex with the SAM domain, which comprises five α -helices, to finely sense luminal Ca²⁺ levels^{41,42,51–53} (FIG. 1d). At resting luminal Ca²⁺ levels (that is, ~400 μ M) the Ca²⁺-binding canonical EF-hand (cEF) domain of STIM1 forms a tight, stable EF-hand–SAM configuration. Decreased luminal Ca²⁺ levels cause Ca²⁺ to dissociate from the cEF-hand domain. This leads to unfolding and destabilization of the EF-hand–SAM complex as hydrophobic residues are exposed within both the EF-hand domain and the SAM domain⁴², which triggers activation and oligomerization of STIM1 (REFS 35,37,46,50,54) (FIG. 2).

The Ca^{2+} dissociation-induced EF-hand–SAM domain interactions are reversed when luminal Ca^{2+} returns to resting levels⁴². STIM1 rapidly retreats from ER–plasma membrane junctions and Orai channels become deactivated^{2,33,55,56}. The luminal EF-hand–SAM domain of STIM1 binds Ca^{2+} with a dissociation constant (K^d) of $\sim 200 \mu\text{M}$ ^{41,42,57,58}. The dependence of STIM1 oligomerization on Ca^{2+} has a Hill coefficient of almost 4 which is similar to that for Orai channel activation⁴⁴. Thus, STIM-induced Orai channel activation is triggered in a narrow range of luminal Ca^{2+} depletion. Mutation of acidic residues in the cEF-hand domain to lower its Ca^{2+} affinity causes STIM1 oligomerization just as if luminal Ca^{2+} levels had been lowered⁴². Expression of STIM1 with such cEF-hand domain mutations results in activated STIM1 localized almost entirely within junctions and constitutively active CRAC channels^{2,30,43,59}. Mutation of equivalent EF-hand residues in the non- Ca^{2+} -binding hidden EF-hand (hEF) loop causes similar oligomerization and constitutive Ca^{2+} entry⁴². This tandem functioning of the two EF-hand domains contributes to the narrow Ca^{2+} -dependency of STIM1 activation.

Distinct roles for STIM2

STIM2 is a significantly weaker activator of Orai channels than STIM1 (REF. 60) but is a more sensitive sensor of ER luminal Ca^{2+} (REF. 57). STIM2 has a strong C-terminal ER-retention sequence and is exclusively localized in the ER, whereas STIM1 lacks this sequence, and therefore $\sim 10\%$ of STIM1 localizes to the plasma membrane^{30,61,62}. Unlike STIM1 (REFS 30,59), overexpressed STIM2 has a strong negative effect on endogenous SOCE^{30,57} and mediates slower Orai1 activation than STIM1 (REFS 63,64). The K^d of STIM2 for Ca^{2+} (that is $\sim 400 \mu\text{M}$) is 2-fold higher than that of STIM1, which possibly reflects the differences in EF-hand domain structures. Hence, STIM2 is more sensitive to small changes in luminal Ca^{2+} levels⁵⁷, and its overexpression results in higher constitutive Ca^{2+} entry and CRAC channel activity^{65,66}. The STIM2 EF-hand–SAM domain undergoes slower unfolding and aggregation following Ca^{2+} withdrawal^{52,53}. This delay in STIM2 aggregation may reflect increased stability conferred by the short flexible N-terminal sequences^{52,64}. The slower rate of STIM2 unfolding and aggregation is likely to account for the slower kinetics of store-operated channel activation by STIM2 (REFS 63,64) and the negative dominance of overexpressed STIM2 on SOCE³⁰. Thus, whereas STIM2 is sensitized to small changes in ER Ca^{2+} levels, the slow activation and poor Orai-coupling efficacy may be important in preventing uncontrolled activation of store-operated channels. The differences in the sensitivity of STIM1 and STIM2 to ER luminal Ca^{2+} levels are important for the maintenance of Ca^{2+} oscillations that are generated in response to distinct $\text{Ins}(1,4,5) \text{P}_3$ -activating agonists⁶⁷, as described below.

A molecular model for STIM1 activation

STIM proteins undergo a remarkable series of molecular rearrangements triggered by and intimately linked to their ER Ca^{2+} -sensing ability. STIM and Orai protein coupling seems sufficient for SOCE induction (FIGS 1b–f,2a). Thus, Orai channels are activated by cytoplasmically expressed C-terminal fragments of STIM1 (REFS 36,45–47,56,66) and even by STIM1 domains targeted to mitochondria⁶⁸. Moreover, SOAR added to vesicles extracted from yeast expressing human Orai1 can directly activate the channel⁶⁹. Here we describe recent progress in understanding the molecular role of STIM1.

Transition from resting to activated state

Within the Ca^{2+} -replete ER, the resting dimeric state of STIM1 (REFS 36,37,40,47) is maintained through interactions mediated by the coiled-coil 1 (CC1) and SOAR domains³⁷ (FIG. 2a). These coiled-coil sequences, which comprise a large segment of the STIM1 C-terminal region (that is, amino acids 238–423), mediate STIM1–STIM1 interactions both

before and after depletion of Ca^{2+} stores^{37,40}. Earlier studies suggested that oligomerization of STIM1 is mediated mainly by EF-hand–SAM domain interactions⁴². However, when a truncated form of STIM1 lacking the STIM1 cytoplasmic domain (that is, the EF-hand–SAM domain-containing N terminus and transmembrane region) is expressed, the oligomerization of this STIM1 fragment in response to store depletion is unstable³⁷. Thus, although EF-hand–SAM domains are necessary for sensing Ca^{2+} levels and triggering STIM1 oligomerization, they may not be the major crosslinking domains between STIM1 proteins. The STIM1 cytoplasmic coiled-coil regions are important for oligomerization in the resting state⁵⁴, and deletion of the amino acids 249–390 of the coiled-coil segment prevents constitutive STIM1 interactions³⁵. The resting STIM1 dimers are held together primarily by interactions between both the CC1 and SOAR regions³⁷ (FIG. 1d). In this state, the EF-hand–SAM domains are likely to be monomeric and contribute little to STIM1–STIM1 interactions. Following store depletion, the EF-hand–SAM domains undergo interactions to trigger the oligomerization of STIM1 (REFS 41,42), and these relatively unstable N-terminal interactions are stabilized by C-terminal interactions. Although the CC1 region alone may not support store depletion-induced STIM1 oligomerization, the coiled-coil domains within SOAR allow STIM1 to undergo robust multimerization following store depletion forming stable oligomers, which translocate into ER–plasma membrane junctions to activate Orai channels³⁷. Thus, the SOAR region of STIM1 has a dual role within the STIM molecule: it mediates the transition of STIM1 into an oligomeric active conformation³⁷, and it binds directly to and activates Orai channels^{45,46}.

STIM–Orai interaction sites

The short coiled-coil C-terminal cytoplasmic domain of Orai1 is an important STIM1-interacting site (FIG. 1b,c), and its disruption prevents STIM1-mediated activation of Orai channels⁵⁶. A cluster of acidic residues within this Orai1 segment (that is, amino acids 272–291; ELNELAEFARLQDQLDHRGD) (FIG. 1d) was identified as necessary for store-dependent binding of STIM1 and channel activation⁷⁰. The amphipathically coiled, acidic Orai1 segments may interact electrostatically with the highly conserved, short polybasic region within the SOAR domain (that is, amino acids 382–387; KIKKKR)^{68,71} (FIG. 1f). Mutations to neutralize this basic region in either intact STIM1, C-terminal STIM1 fragments (STIM1CT) or SOAR, prevent coupling to and activation of Orai1 channels^{68,71}. Disruption of the adjacent CC2 region in STIM1 (FIG. 1a) also prevents coupling to Orai1 channels⁷². However, despite mutation of the polybasic STIM1 residues and Orai1 acidic residues, STIM1–Orai1 interactions can still be measured by fluorescence resonance energy transfer (FRET)⁷¹, which supports the evidence that STIM1 has a second site of interaction with Orai1 in its N-terminal segment⁴⁶. The second site is likely to be within the region spanning amino acids 73–91 of Orai1 close to its first transmembrane spanning segment⁴⁶, a region that is crucial for channel gating and STIM1 interaction^{73,74}.

Intramolecular control of SOAR

Whereas the short SOAR^{45,46} fragments of STIM1 constitutively bind to and activate Orai channels, these sequences are much less active within longer, cytosolically expressed C-terminal constructs^{46,66,68,74,75}. This suggests that a conformational transition may occur to either expose SOAR or relieve it from functional constraint. The nature of this important priming reaction is intriguing. Mutation of a series of acidic residues (in particular amino acids 318–322; EEELE) upstream of SOAR leads to constitutive STIM1 activation⁶⁸. Moreover, co-expression of the STIM1 fragment (amino acids 283–343) that contains these acidic residues interferes with Orai1 activation by STIM1. The concept of a possible electrostatic ‘intramolecular clamp’ that prevents STIM1 activation at rest was supported by FRET studies of a larger segment of STIM1CT (amino acids 233–474; the region termed OASF)⁷⁴ (FIG. 1a). These studies revealed that OASF undergoes significant unfolding into

an extended state as it interacts with the Orai1 channel. This unfolding does not depend on Ca^{2+} passage through Orai channels. Intriguingly, mutations to neutralize the cluster encompassing the acidic residues 318–322 in the CC1 domain caused some unfolding of OASF. In addition, considerable unfolding was induced by mutations that replace hydrophobic residues in several of the coiled-coil segments of OASF, which suggests that hydrophobic interactions also contribute to its folded inactive conformation^{74,76}.

New insights from STIM1 atomic structure

Recent crystallographic analyses provide valuable insights into the structure and intramolecular control of SOAR activation⁴⁰. SOAR was observed as a dimeric structure by ultracentrifugation, in agreement with earlier reports showing that active C-terminal STIM1 fragments are dimers^{45,74}, although a tetrameric configuration had also been reported⁴⁶. The crystallized structure revealed four α -helices within the SOAR peptide (that is, amino acids 345–444), two long helices (S α 1, S α 4) and two very short helices (S α 2, S α 3) (FIG. 1d,e). The atomic structure identified residues through which cross-peptide interactions mediate dimerization of SOAR: specific residues in the N-terminal region of S α 1 (within the 347–354 amino acid segment) from one SOAR molecule interact through either hydrophobic or hydrogen bonds with residues in the C-terminal region of S α 4 (within the 429–436 amino acid segment) in the other SOAR molecule. Importantly, mutating these dimerization regions within SOAR or full-length STIM1 prevented interaction with and activation of Orai1 (REF. 40), suggesting that the active functional unit within STIM1 is a SOAR dimer. Furthermore, the atomic structure revealed that the long rigid S α 1 helix within SOAR (which comprises residues 345–391) exposes the polybasic Orai-interacting residues (which are amino acids 382–387) at its apex, with Lys382, Lys385 and Lys386 amphipathically oriented towards the centre of the dimer (FIG. 1f). Hypothetically, this structure could provide a cleft within which the two amphipathic acidic groups of the Orai C-terminal helix might electrostatically interact (FIG. 1b,c,f). A further important conclusion is that the acidic residues (which are residues 318–322) within the inhibitory domain that are located close to the C-terminal end of the S α 1 helix would be unable to directly interact with the polybasic active sites (that is, amino acids 382–387) within the dimer by virtue of the intervening long (47 residues) S α 1 helix, militating against the electrostatic clamp model. The *C. elegans* STIM sequence encompassing residues 214–410 (REF. 40), including the complete CC1 and SOAR regions, was also successfully crystallized. The structural data indicate that the above described inhibitory region in CC1 is α -helical and undergoes tight interactions through several hydrogen bonding and hydrophobic interactions with the N-terminal start of SOAR and the C-terminal end of SOAR — the two regions in SOAR furthest away from the middle ‘apical’ polybasic active site. The corresponding inhibitory sequence identified in human STIM1 (which are residues 310–337), when eliminated from full-length STIM1, leads to constitutive interaction with and activation of Orai1, which is consistent with the previous findings⁶⁸. The unfolding of the STIM1 coiledcoil region that is required for Orai channel activation⁷⁴ may thus correspond to the detachment of the inhibitory helix from SOAR. The acidic residues in this sequence known to prevent STIM1 activation⁶⁸ may in fact mediate its interaction with SOAR⁴⁰.

A consolidated activation model

The new structural data provide strong evidence for the central role of the SOAR dimer in both presentation of the active Orai-interacting site of STIM1 and in the unfolding and activation of the entire STIM1 C-terminal domain (FIG. 2a). The model of one STIM dimer interacting with each Orai1 subunit of the active Orai1 channel is in good agreement with recent stoichiometric data showing that eight STIM1 molecules are coupled to activate each tetrameric Orai1 channel³⁸.

The schematic in FIG. 2a attempts to bring together what is currently known about the STIM protein activation and Orai coupling processes. The SOAR dimer is shown as a central core structure throughout STIM1 activation. In the STIM1 resting state, the predicted three α -helices of CC1 are shown in a folded configuration with the Ca1 N terminus close to the C terminus of SOAR as suggested by FRET analysis of OASF⁷⁴. The inhibitory Ca3 helix is shown to interact with SOAR at its far C- and N-terminal regions as suggested by the recent structural data for *C. elegans* STIM-1 (REF. 40). During activation, Ca²⁺ dissociation-induced interactions between the EF-hand and SAM domains may trigger unfolding and elongation of the STIM1 C-terminal coiled-coil domains, separating the Ca3 inhibitory helix of CC1 from SOAR to provide an activated SOAR domain^{40,68,74}. The unfolding of the STIM protein may also allow interactions of its Lys-rich C-terminus with plasma membrane lipids⁴⁶ and exposure of SOAR, which enables the activation of Orai channels^{40,77}. Although convincing evidence exists for the interactions between the polybasic SOAR region and the acidic regions of Orai1 (REFS 40,68,71), a direct electrostatic interaction has not yet been shown. SOAR also interacts with the N-terminal region of Orai1 (that is, amino acids 73–91)⁴⁶. This segment of Orai1 is crucial for channel gating, and the Orai1-K85E mutation alters STIM1-mediated gating without altering SOAR–Orai1 interactions⁷³. Indeed, recent studies revealed that the pore properties and gating of Orai1 channels are intimately associated⁷⁸.

Thus, by binding to Orai1, STIM1 not only induces channel opening but also increases the Ca²⁺ selectivity of the channel⁷⁸, which suggests that STIM1 functions in a similar fashion to a regulatory subunit of the Orai1 channel. This is remarkable considering that it exists in a separate membrane. Theoretically, the SOAR–Orai interactions may involve tethering at the Orai1 C terminus and ‘regulatory’ interactions at the N-terminus of Orai1, leading to channel gating and control of ion selectivity. In addition, the Lys-rich far C-terminal end of STIM1 strongly interacts with acidic phospholipids in the plasma membrane^{33,46,79}, which stabilizes its attachment and allows it to trap and activate Orai channels.

The function of the remainder of the large (~200 residues) C-terminal tail of STIM proteins remains unclear. The regions are dissimilar in STIM1 and STIM2 (see Supplementary information S1 (figure)) and absent in *D. melanogaster* STIM and may function sterically as intramolecular shields occluding the SOAR region⁷⁷ to maintain STIM proteins in an inactive state. The attachment of STIM1 to the plasma membrane through its Lys-rich C terminus may assist in relieving this occlusion.

Control of the Ca²⁺ signalling junction

STIM proteins are central components in ER–plasma membrane junctions as they mediate crucial Ca²⁺ communication among the ER lumen, the cytoplasm and the extracellular space. Under resting conditions, these junctions account for approximately 5% of the plasma membrane surface^{34,80} and can become considerably larger upon STIM protein activation³⁴. The junctions are complex structures and include several recently identified STIM-interacting proteins that control junctional assembly, disassembly and function (FIG. 2b).

Junctional assembly: STIM turn-on events

The interaction between oligomerized STIM and Orai proteins can itself drive the formation of junctions^{68,69}. However, the emerging picture is that junctions are highly organized domains that contain an array of protein modulators (FIG. 2b). Although STIM–Orai interactions are important, the interaction between the Lys-rich C-termini of STIM proteins and plasma membrane acidic phospholipids provides another major driving force in junctional assembly^{33,46,79} (FIG. 2a). Although the seven C-terminal Lys residues of a single STIM1 protein (and eight C-terminal Lys residues in STIM2) provide only weak lipid

interactions⁵⁸, oligomerized STIM proteins provide a robust multivalent interaction with plasma membrane surface lipids. The *D. melanogaster* STIM protein lacks the Lysrich C-terminus, and therefore Orai activation does not require lipid-interactions, although lipid association may assist junctional localization⁴⁶.

The Ca²⁺-binding integral ER membrane protein juncate, known to assist in ER–plasma membrane interactions, was shown to be present in STIM–Orai containing junctions and to be important in mediating SOCE^{81,82}. Indeed, juncate is an interacting partner within the STIM1–Orai1 complex and can recruit STIM1 into the ER–plasma membrane junctions⁸³. Interestingly, juncate contains a Ca²⁺-binding EF-hand domain, which, when mutated to prevent Ca²⁺ binding, causes CRAC channel activation even without store depletion⁸³ by promoting the formation of ER–plasma membrane junctions (FIG. 2b). Juncate does not seem to be widely distributed throughout the ER but remains close to the junctions and may represent an important additional factor contributing to STIM protein recruitment and Orai channel activation (REF. 83). Its role may be to mediate CRAC activation in response to local Ca²⁺ depletion events and/or to amplify the STIM-mediated activation of Orai channels.

Although in most cells STIM–Orai junctional assembly occurs slowly (within 10–30 seconds), in skeletal muscle the kinetics of activation of Orai channels by Ca²⁺ depletion can be much faster, and it seems that STIM and Orai proteins remain junctionally coupled within cisternae^{84,85}. Interestingly, the unique actin-binding domain within the STIM1 splice variant STIM1L may mediate this junctional pre-coupling⁸⁶. Thus, local control of assembly and the degree of ‘pre-assembly’ of junctions are crucial factors in controlling SOCE kinetics.

Turning off STIM–Orai coupling

The activation and deactivation of STIM–Orai coupling is a steady-state event that is controlled by the local junctional environment. The entry of Ca²⁺ into the junctional space through Orai channels provides powerful feedback control on STIM-induced Orai channel activation. STIM and Orai proteins function together as a coupled channel assembly⁷⁸, and turn-off events reflect properties of both proteins. In addition, several regulatory proteins within this complex were recently identified^{87–89}. Turning off the activated STIM–Orai complex involves two major events: rapid Ca²⁺-dependent inactivation (CDI) of the Orai channel, and the subsequent slow dissociation of the STIM–Orai junctional complex. CDI of CRAC channels has long been recognized^{90,91}, and several recent studies revealed that a short segment of acidic residues in the STIM1 cytoplasmic inhibitory domain region (ID region; which comprises residues 475–483) (FIG. 1a) is necessary for CDI^{89,92,93}. Orai1 activation by SOAR fragments that lack this region does not exhibit CDI^{46,93}, and replacement of a few or all of these acidic residues in STIM1 prevents CDI^{89,92,93}. Ca²⁺ interacts with the ID region, however, it is not clear whether Ca²⁺ binding to the ID region is required for CDI⁸⁹. Calmodulin is a powerful mediator of CDI⁹⁴ and seems to induce this effect through direct interaction with the Orai1 channel. This interaction site was shown to reside at a region within the N-terminal cytoplasmic domain (that is, residues 69–91), close to the second STIM1-interaction site⁸⁹ (FIG. 2b). The slow dissociation of the STIM–Orai complex and the disaggregation of STIM proteins is mainly mediated by increased ER luminal Ca²⁺ levels, which lead to STIM protein disaggregation²⁸. However, careful manipulation of cytosolic and ER Ca²⁺ levels reveals that localized cytoplasmic Ca²⁺ in ER–plasma membrane junctions also has a role in STIM1 de-oligomerization⁹⁵.

Several other proteins seem to be important in controlling the turn-off of STIM–Orai coupling. The cytoplasmic protein CRAC regulatory protein 2A (CRACR2A) was recently identified as being tightly associated with the STIM–Orai1 complex⁸⁷. CRACR2A is a

soluble, cytoplasmic protein with two predicted EF-hand motifs (FIG. 2b). *In vitro* binding reveals strong interactions with the same Orai1 N-terminal sequence (that is, residues 64–93) that binds calmodulin. The Orai1–CRACR2A interaction is inhibited by Ca^{2+} binding to CRACR2A, and a mutation in the CRACR2A EF-hand domain that prevents Ca^{2+} binding eliminates the Ca^{2+} -mediated inhibition of this interactions⁸⁷. Furthermore, CRACR2A also binds directly to STIM1. CRACR2A may function as a stabilizer of STIM1–Orai interactions, but only when junctional Ca^{2+} levels are low. As Ca^{2+} levels increase at ER–plasma membrane junctions, CRACR2A dissociates from and destabilizes the STIM1–Orai1 complex, thereby terminating the interaction (FIG. 2b). Similarly to CRACR2A, calmodulin also binds Ca^{2+} via its high affinity EF-hand domains, and both CRACR2A and calmodulin may function synergistically when junctional Ca^{2+} levels increase, with calmodulin inactivating Orai and CRACR2A enhancing STIM1–Orai1 dissociation.

The cytoplasmic protein golli, which is an alternatively spliced isoform of myelin basic protein (MBP), negatively controls SOCE⁹⁶ and also enhances $\text{Ca}_v1.2$ channel activity⁹⁷. Golli directly interacts with the C-terminal domain of STIM1, an interaction that may reflect cytoplasmic Ca^{2+} levels. As golli colocalizes with STIM1–Orai1 complexes after store depletion⁹⁸, it may also promote Ca^{2+} -dependent turning off of STIM1–Orai1 coupling (FIG. 2b).

SOCE-associated regulatory factor (SARAF) has also recently been implicated in the deactivation of the STIM1–Orai1 complex⁸⁸. SARAF, which was identified by high-throughput functional screening, is a singlepass ER membrane protein that is closely associated with STIM1 and that moves with STIM1 into junctions following store depletion (FIG. 2b). Although SARAF does not contain Ca^{2+} -sensing EF-hand domains, it seems to be important in mediating the effects of cytosolic Ca^{2+} on Orai uncoupling and inhibition of SOCE. A cluster of basic residues in the cytoplasmic tail of SARAF might interact with plasma membrane lipids in a similar manner to STIM1. Perhaps as a key player in deactivating SOCE, SARAF may be crucial in controlling store refilling and protecting cells from ER Ca^{2+} overload. Clearly, the inactivation of SOCE and disassembly of STIM–Orai junctions are under the tight control of Ca^{2+} and are mediated by several ancillary proteins.

New STIM protein triggers, targets and partners

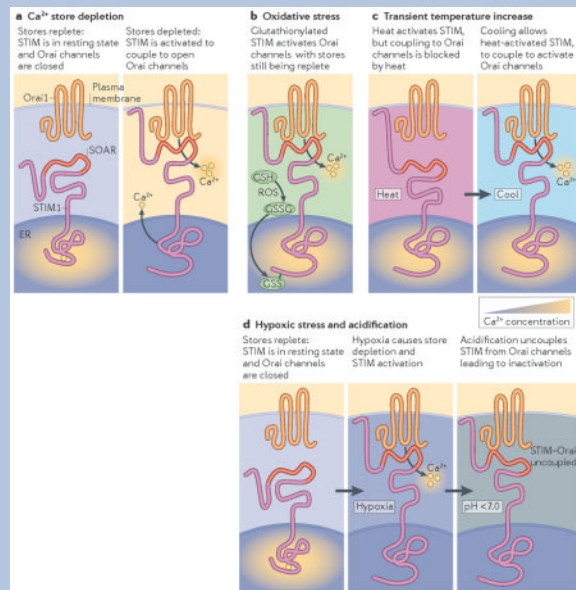
Recent information revealed that in addition to sensing decreased ER Ca^{2+} levels, STIM proteins are triggered by several other distinct stress conditions⁹⁹, including oxidative stress¹⁰⁰, temperature change^{101,102}, hypoxia and acidification¹⁰³. Details of these additional stress-sensing roles of STIM proteins are shown in BOX 2. Recent studies also revealed various new targets and partners for STIM proteins that operate within ER–plasma membrane junctions. Thus, STIM proteins interact with and control a range of other channels, pumps and scaffolding proteins suggesting that STIM proteins have many other Ca^{2+} signalling targets besides Orai channels (FIG. 2b).

Box 2

Multiple sensing roles of STIM1

Stromal interaction molecule (STIM) proteins are activated in response to several different cellular stress conditions. ER Ca^{2+} store depletion is itself a major cellular stress condition leading to protein misfolding and STIM activation (see the figure, part **a**). Reactive oxygen species (ROS) were shown to induce STIM1 aggregation, STIM translocation to ER–plasma membrane junctions and activation of Orai channels without Ca^{2+} store depletion¹⁰⁰ (see the figure, part **b**). ROS-induced *S*-glutathionylation of Cys56 in the amino terminus of STIM1 decreases Ca^{2+} binding by the EF-hand domain

and triggers STIM1 activation. Increased temperature from 37°C to 41°C also triggers STIM1 activation independently of Ca²⁺ store depletion¹⁰¹ (see the figure, part c). However, the higher temperature (left panel) prevents coupling to and activation of Orai channels, which can occur only after cooling (right panel). The role of STIM1 in temperature sensing may be important in priming haematopoietic cells during fever¹⁰¹. It is also possible that high temperature controls STIM–Orai uncoupling to protect cells from temperature-induced Ca²⁺ overload^{99,102}. Hypoxic stress and ensuing decreased ATP levels cause Ca²⁺ store depletion and activation of STIM proteins (see the figure, part d). However, coupling to Orai channels is prevented in this case through hypoxia-induced cytoplasmic acidification which may prevent the electrostatic STIM–Orai interactions that are crucial for Orai channel activation (FIG. 1). Thus, STIM–Orai uncoupling in response to decreased pH may be a protective mechanism against hypoxia-induced Ca²⁺ overload¹⁰³. GSH, reduced glutathione; GSSG, oxidized glutathione disulphide; SOAR, STIM–Orai activating region.



Control of other channel targets

Although Orai channels are the most established STIM protein targets, other channels are either directly controlled by or closely associated with STIM proteins. The family of transient receptor potential channels (TRPCs) has long been linked to SOCE²⁸. The fly TRP homologues were reported to be Ca²⁺ store-dependent^{104,105}, and mammalian TRPCs seem to couple with Ins(1,4,5)P₃Rs and mediate SOCE¹⁰⁶. The involvement of STIM proteins in activating TRPC has been controversial²⁸. As non-selective cation channels, TRPCs mediate cation currents with properties distinct from those of the CRAC current. Whereas some prominent studies have described direct STIM-induced activation of TRPCs^{75,107–109}, other studies have presented strong evidence against this hypothesis^{110–112}. TRPC activation by phospholipase C (PLC)-coupled receptors may result from direct actions of changes in diacylglycerol (DAG) or phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5) P₂) levels rather than Ins(1,4,5)P₃-mediated Ca²⁺ store depletion. However, close functional connections between TRPCs and the STIM–Orai machinery have been suggested from studies revealing that Ca²⁺ entering through Orai channels induces plasma membrane insertion of TRPCs¹¹³.

STIM proteins also interact with and control the function and turnover of $\text{Ca}_v1.2$ voltage-operated Ca^{2+} channels^{40,112,114}. In contrast to its effects on Orai channels, STIM1 inhibits $\text{Ca}_v1.2$ channel function. Store depletion causes STIM1, $\text{Ca}_v1.2$ and Orai1 channels to accumulate within the same junctional areas¹¹², but STIM1-mediated $\text{Ca}_v1.2$ channel inhibition does not require Orai channel activation. The reciprocal actions of STIM1 on Orai1 and $\text{Ca}_v1.2$ channels are both mediated by the cytoplasmic SOAR region of STIM1 (REFS 112,114), which interacts with the C-terminal tail of the $\text{Ca}_v1.2$ channel α_{1C} subunit¹¹⁴. In addition to inhibiting channel function, the interaction of STIM1 causes a dramatic increase in $\text{Ca}_v1.2$ channel internalization, which results in its decreased surface expression¹¹⁴. STIM1 also influences Orai channel turnover by increasing the insertion and retention of Orai channels in the oocyte plasma membrane¹¹⁵. During meiosis, STIM protein activation is turned off, which leads to prominent internalization of Orai channels and the prevention of SOCE¹¹⁶. Thus, STIM protein activation has reciprocal effects on turnover of Orai and $\text{Ca}_v1.2$ channels. As $\text{Ca}_v1.2$ channels and Orai channels are widely expressed in excitable and non-excitable cells, their reciprocal control by STIM proteins is likely to have important functional consequences.

The STIM-dependent ARC channel has been reported to comprise a unique configuration of Orai1 and Orai3 subunits^{117–119}. The ARC channel is not activated by Ca^{2+} store depletion but by receptor-induced arachidonic acid generation. ARC channels were found to depend exclusively on *cis*-located STIM1 within the plasma membrane. However, it is unclear how STIM1, the structure of which has evolved to enable interaction with and activation of Orai channels in a junctional *trans*-membrane configuration, can also activate Orai channels within a *cis*-membrane configuration.

Control of other major regulatory proteins

The ER–plasma membrane junctional domain, as defined by the presence of STIM proteins, is becoming recognized as a Ca^{2+} signalling microdomain in which not only Ca^{2+} channels but also Ca^{2+} pumps are recruited. Evidence indicates that sarcoendoplasmic reticulum Ca^{2+} ATPase (SERCA) pumps in the ER are functionally close to STIM–Orai junctions¹²⁰. Furthermore, FRET imaging studies revealed that SERCA2A is recruited into junctions together with STIM1 (FIG. 2b), whereas after store refilling STIM1, Orai1 and SERCA2A dissociate¹²¹. Studies combining targeted aequorins to measure local Ca^{2+} with imaging of labelled STIM1, Orai1 and SERCA2B revealed local Ca^{2+} pumping and the formation of a triple complex of STIM1, Orai1 and SERCA2B^{122,123}.

The plasma membrane Ca^{2+} ATPase (PMCA) pump also seems to be closely controlled within the STIM–Orai junction (FIG. 2b). PMCA is modulated by CRAC channel activation, independent of increases in local cytosolic Ca^{2+} levels¹²⁴, and it was recently shown to be inhibited in the immunological synapse in T cells^{125,126}. PMCA inhibition was proposed to be mediated by mitochondrial Ca^{2+} loading at a location remote from the STIM–Orai interacting site where PMCA and mitochondria closely interact¹²⁵. However, more recently it was revealed that activated STIM1 proteins within STIM–Orai junctions interact directly with PMCA and induce an inhibitory effect on Ca^{2+} pumping independent of mitochondrial function¹²⁶.

The identification of partner of STIM1 (POST) provides clues about the role of STIM1 in organizing crucial Ca^{2+} regulatory proteins and other functionally important proteins¹²⁷. POST is a 10-transmembrane spanning ‘adaptor’ protein existing, like STIM1, predominantly in the ER, although ~5–10% of POST is present in the plasma membrane. Following Ca^{2+} store depletion, POST binds strongly to STIM1 and moves with STIM1 into precisely the same ER–plasma membrane junctions (FIG. 2b). POST is not required for STIM–Orai interactions and altered expression does not change CRAC channel activation.

Store depletion leads to POST-dependent binding of STIM1 to SERCA and PMCA pumps. This provides a mechanistic basis for the above described functioning of SERCA pumps within STIM–Orai junctions (FIG. 2b). Moreover, POST was shown to inhibit the Ca^{2+} pumping activity of PMCA independently of mitochondria¹²⁷, and this is consistent with the inhibitory effect of STIM1 on PMCA¹²⁶. Thus, POST-mediated organization and control of PMCA and SERCA pumps within STIM–Orai junctions is a powerful means to control and direct the entering Ca^{2+} (FIG. 2b). Intriguingly, POST also interacts with the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX), consistent with reports that STIM1 promotes reverse-mode NCX activity in smooth muscle¹²⁸. In addition, POST was found to bind the Na^+/K^+ ATPase and the nuclear transporters importin- β and exportin¹²⁷, and may explain why STIM1 can immunoprecipitate with importin- β and exportin⁶².

STIM1 also interacts with the ER chaperones calnexin⁶² and ERp57 (REF. 129). Moreover, STIM1 may exert control over plasma membrane adenylyl cyclases¹³⁰. Recent work revealed direct binding between Orai1 and the adenylyl cyclase isoform AC8, which indicates that SOCE exerts local control of cyclic AMP (cAMP) production. Overall, STIM proteins seem to interact with and control several distinct classes of Ca^{2+} -regulatory proteins and other signalling proteins.

STIM proteins, Ca^{2+} oscillations and gene expression

Transient oscillations in cytosolic Ca^{2+} provide long-term signals while avoiding the cytotoxic effects of prolonged Ca^{2+} increases¹³¹ and are crucial in transcriptional control¹³². The coordinated function of ER Ca^{2+} release and SOCE is essential in generating Ca^{2+} oscillations^{133,134} and controlling transcription (FIG. 3). During oscillations, SOCE replenishes ER Ca^{2+} stores and oscillations cannot be sustained without SOCE¹³⁴. During individual oscillations, STIM1 migrates into junctions and activates Orai channels⁶⁰. Although more sensitive to store depletion⁵⁷, STIM2 was not found to enter junctions during individual oscillations⁶⁰. However, both STIM1 and STIM2 are important in long-term transcriptional responses mediated by Ca^{2+} oscillations (FIG. 3). Nuclear factor of activated T cells (NFAT)-driven interleukin 2 (IL-2) production is decreased in STIM2-deficient T cells, and STIM2 is necessary for long-term SOCE-driven gene expression²⁵. In B cells, STIM2 is required for NFAT-driven transcription, although STIM2 is only modestly involved in mediating Ca^{2+} signals¹³⁵. A recent study revealed that Ca^{2+} release spikes in response to G protein-coupled receptor activation induce extensive store depletion and drive STIM1 (the activation of which has a high Ca^{2+} release threshold) to mediate Ca^{2+} entry⁶⁷. By contrast, more subtle Tyr kinase-induced oscillations involve moderate store depletion and drive both STIM1 and STIM2 (the activation of which has a lower Ca^{2+} release threshold) to activate Ca^{2+} entry⁶⁷.

Although SOCE contributes only a small fraction of each global Ca^{2+} spike, local contributions of SOCE to Ca^{2+} oscillations seem to be crucial in mediating transcriptional control^{136,137}. Blocking both Orai-mediated entry and PMCA-mediated exit of Ca^{2+} across the plasma membrane with lanthanides allows oscillations to continue in a ‘closed system’ purely through Ca^{2+} release¹³⁸. In mast cells, leukotriene C4-induced Ca^{2+} oscillations seem to be identical in the presence or absence of SOCE blockers. However, when the SOCE component is eliminated, leukotriene C4-induced Ca^{2+} oscillations fail to induce *c-fos* expression and, thus, a c-FOS-mediated transcriptional response in activated mast cells¹³⁶. This implicates the local Ca^{2+} environment (which is also known as the ‘ Ca^{2+} signature’) created by SOCE as being crucial for c-FOS activation. Similar requirements for SOCE were demonstrated for NFAT activation^{139,140}. Nuclear translocation of NFAT requires dephosphorylation by calcineurin^{141,142}, and continued NFAT activation requires extended cytosolic Ca^{2+} level increases to maintain calcineurin activation. Calcineurin localized

within near-plasma membrane scaffolds^{143,144} may be selectively activated in the immediate environment of SOCE (FIG. 3).

Conclusions and implications

The pace of discovery in understanding the function of STIM proteins continues to accelerate. New information reveals the remarkable molecular coordination between STIM proteins and their growing number of interacting partners and targets. Also emerging is an understanding of the broader sensing and coupling properties of STIM proteins. As ‘stress-transducers’, STIM proteins can respond to cellular stressors, such as Ca²⁺, oxidative and hypoxic conditions, temperature fluctuation and changes in pH (BOX 2), and this extends their potential as homeostatic regulators.

Perhaps the most important goal of future studies will be the discovery of target and regulatory proteins that closely coexist with STIM proteins within junctions. The organization of these junctional domains with the participation of major Ca²⁺ channel and pump proteins suggests that the local Ca²⁺ signalling environment has a profound impact on cell function. The application of super-resolution imaging and FRET technology may provide enhanced understanding of the structure, operation and role of these domains.

STIM proteins have novel and unexpected physiological and pathophysiological roles in several tissues (BOX 3). Animal studies revealed that the phenotypes of *Stim1* and *Stim2* knockout mice are surprisingly different. The use of conditional tissue-specific knockout mouse models will provide important information on the sometimes subtle tissue-specific functions of STIM proteins. For example, STIM proteins have novel and surprising roles in growth and development of skeletal muscle^{145,146}, cardiac muscle¹⁴⁷ and smooth muscle¹⁴⁸. With new structural details emerging on STIM proteins, the generation of transgenic mouse models carrying *Stim* genes mutationally modified at key regulatory and interactive sites will help understand the role and pathophysiology of STIM proteins and provide new information on functional differences between STIM1 and STIM2. The Ca²⁺ sensing properties, activation kinetics and target coupling efficiency of the two STIM proteins are quite different. The exquisite sensitivity, ubiquitous expression and breadth of control over Ca²⁺ signals mediated by STIM proteins predict that we are only beginning to understand the implications and importance of their cellular function.

Box 3

Physiological and pathophysiological roles of STIM and Orai proteins

Lung

Stromal interaction molecule 1 (STIM1)–Orai1 complexes mediate store-operated Ca²⁺ entry (SOCE) in airway smooth muscle cells¹⁵⁰. STIM1-dependent Ca²⁺ release-activated Ca²⁺ (CRAC) channel activation participates in hypoxia-induced AMP-activated protein kinase (AMPK) activation¹⁵¹. STIM1–Orai1 upregulation correlates with pulmonary smooth muscle cell proliferation. Increased Orai1 insertion contributes to the inflammatory response in cystic fibrosis¹⁵². Orai1 knockdown attenuates endothelial cell migration and angiogenesis¹⁵³.

Heart

STIM1-dependent SOCE contributes to cardiac hypertrophy^{147,154}. STIM1 knockdown diminishes diastolic Ca²⁺ levels and Orai1 knockdown abrogates hypertrophic signalling¹⁵⁵.

Liver

Hormone-induced cytosolic Ca^{2+} oscillations require Ca^{2+} entry through SOCE¹⁵⁶. STIM1 contributes to hepatocyte injury during cholestasis¹⁵⁷.

Kidney

In glomerular mesangial cells, SOCE requires STIM1 and regulates glomerular haemodynamics¹⁵⁸.

Reproductive tract

STIM1 is required for sheath cell and spermatheca contractile activity, which contributes to fertility in *Caenorhabditis elegans*¹⁵⁹. STIM1–Orai1-dependent SOCE contributes to oocyte maturation and fertilization in mammals¹⁶⁰.

Bone

Orai1 is required for the formation of multinuclear osteoclasts^{161,162}. Orai1 knockout animals exhibit low bone density, implicating additional roles in osteoblast function¹⁶³.

Skeletal muscle

STIM1 and Orai1 are expressed in skeletal muscle¹⁶⁴. STIM1 ablation reduces SOCE, which causes muscle weakness and neonatal lethality^{21,165}. STIM1 and STIM2 are necessary for excitation–contraction coupling and myoblast differentiation¹⁶⁶. The fast STIM–Orai1 activation in skeletal muscle depends on the actin-binding STIM1L splice variant⁸⁶.

Brain

STIM proteins and Orai channels are implicated in neuronal development and memory¹⁶⁷. Loss of STIM2 affects cognitive function but protects from neuronal damage after ischaemic stroke²⁶.

Breast

During lactation, the expression of Orai1 and STIM2 is increased, whereas STIM1 is downregulated¹⁶⁸. The STIM1–Orai1 complex is required for breast tumour cell migration and metastasis¹⁶⁹. Orai1–SPCA2 (secretory pathway Ca^{2+} ATPase 2)-mediated store-independent Ca^{2+} entry influences mammary tumorigenesis¹⁷⁰. STIM1-, STIM2- and Orai3-induced SOCE contributes to breast cancer progression^{171,172}.

Pancreas

STIM1 is localized exclusively to the lateral and basal regions in pancreatic acinar cells¹⁷³, whereas Orai1 is restricted to the apical region¹⁷⁴. SOCE is implicated in insulin secretion from pancreatic β -cells¹⁷⁵.

Intestine

In colonic epithelial cells, STIM1 is required for cAMP production triggered by polyunsaturated fatty acids¹⁷⁶. CRAC channels do not contribute to oscillatory Ca^{2+} signalling in worm intestines¹⁵⁹.

T cells

Mutations in either *Orai1* or *STIM1* results in defective SOCE that leads to severe combined immunodeficiency^{3,21,25}. Mice lacking either STIM1 or STIM2 are protected from developing multiple sclerosis¹⁷⁷.

B cells

SOCE-induced Ca^{2+} signalling contributes to the generation of interleukin 6 (IL-6), IL-10 and immunoglobulin M (IgM)¹³⁵. Loss of STIM1 and STIM2 exacerbates

autoimmune encephalomyelitis in mice. STIM1 overexpression sensitizes developing B cells to negative selection¹⁷⁸.

Mast cells

Mast cells lacking either STIM1 (REF. 179) or Orai1 (REF. 180) exhibit defective cytokine production and release, which prevents allergic responses. No defects in proliferation or differentiation were observed in these cells.

Platelets

Constitutive STIM1 activation causes premature platelet activation¹⁸¹. STIM1 is required for physiological platelet activation in arterial thrombus formation^{182,183}. The STIM1–Orai1 complex is essential for occlusive thrombus formation during stroke^{182,184}.

Granulocytes

STIM1–Orai1 mediated increases in Ca^{2+} levels are key for the modulation of NADPH oxidase activity and the rise in Ca^{2+} levels are crucial for phagocytic activity¹⁸⁵. In murine eosinophils, Orai1 contributes to allergic rhinitis¹⁸⁶.

Myeloid cells

STIM1 ablation causes phagocytosis defects without affecting cytokine induction. Mice that lack STIM1 are resistant to experimental immune thrombocytopenia, autoimmune haemolytic anaemia and acute pneumonitis¹⁸⁷. Dendritic cells predominantly express STIM2, but not STIM1, and STIM2 moves to the immunological synapse²⁷.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Glossary

Ca^{2+} signalling	The process through which spatially and temporally controlled changes in Ca^{2+} levels are induced in response to external and/or internal cellular activation events
Stromal interaction molecule (STIM)	The Ca^{2+} sensing endoplasmic reticulum (ER) transmembrane protein that becomes activated upon decrease of ER luminal Ca^{2+} . Higher eukaryotes contain two similar proteins, STIM1 and STIM2. STIM2 is exclusively expressed in the ER, whereas ~5–10% of STIM1 is located in the plasma membrane. Activated STIM proteins translocate into ER–plasma membrane junctions where they activate plasma membrane Orai Ca^{2+} channels
Ca^{2+} homeostasis	The process of global Ca^{2+} maintenance within the entire cell and/or sub-compartments therein. The term refers to the regulation of Ca^{2+} within cells through the operation of all Ca^{2+} pumps, channels, binding proteins and other regulatory proteins within every cellular compartment, including the

	cytosol, endoplasmic reticulum, mitochondria, Golgi apparatus and endolysosomal system
Endoplasmic reticulum (ER)	The extensive subcellular tubular network that has crucial roles in lipid and protein synthesis and serves also as the major Ca^{2+} storage organelle
Orai channels	A family of plasma membrane Ca^{2+} entry channels comprising three mammalian homologues termed Orai1, Orai2 and Orai3. Orai proteins have four transmembrane domains and form tetrameric channels. Orai1 tetramers are the pore-forming units of Ca^{2+} release-activated Ca^{2+} (CRAC) channels, gated by stromal interaction molecule (STIM) proteins. Orai2 and Orai3 can also form STIM-responsive channels, although the physiological roles of these proteins are less clear
Capacitative Ca^{2+} entry	This term was introduced to describe the activation of Ca^{2+} entry across the plasma membrane in response to the depletion of Ca^{2+} levels in the endoplasmic reticulum. Currently known as storeoperated Ca^{2+} entry (SOCE)
Inositol-1,4,5-trisphosphate (Ins(1,4,5)P_3)	A product derived from breakdown of phosphatidylinositol-4,5- bisphosphate by phospholipase C. It functions as a cytosolic second messenger by binding to Ins(1,4,5) P_3 receptors, which are located on the endoplasmic reticulum membrane and operate as ER Ca^{2+} release channels
Store-operated Ca^{2+} entry (SOCE)	The activation of a Ca^{2+} channel in the plasma membrane in response to the depletion of Ca^{2+} levels in the endoplasmic reticulum. Formerly known as capacitative Ca^{2+} entry
Ca^{2+} release-activated Ca^{2+} current (CRAC current or I_{CRAC})	The electrophysiologically defined current mediated by the Orai family of channels
Mast cells	Leukocyte cells with large secretory granules that contain histamine and various protein mediators. Mast cells contain an unusually high density of Ca^{2+} release-activated Ca^{2+} (CRAC) channels, which function to mediate mast cell activation
EF-hand domains	Highly conserved Ca^{2+} -binding domains comprising two helices (E and F after the 5 th and 6 th helices of parvalbumin) that are linked by a short acidic Ca^{2+} -binding loop
STIM–Orai activating region (SOAR)	A ~100 amino acid segment within the cytosolic domain of stromal interaction molecule 1 (STIM1) that is the minimal sequence for mediating interaction with and activation of Orai channels
Pre-B cells	Cells in a stage of B cell development in the bone marrow that are characterized by complete immunoglobulin heavy-chain rearrangement in the absence of immunoglobulin light-chain rearrangement. They express the pre-B cell receptor, which comprises a pseudo light chain and a heavy chain.

	Cells are phenotypically CD19 ⁺ cytoplasmic immunoglobulin M ⁺ (IgM ⁺) or are sometimes defined as B220 ⁺ CD43 ⁻ cell surface IgM ⁻ (which is known as the Hardy classification)
Hill coefficient	Quantifies the cooperativity of ligand binding by an allosteric protein and indicates the minimal number of interacting binding sites. A Hill coefficient of 1 indicates independent binding even when ligands are bound to different binding sites, and a coefficient of >1 reflects positive cooperativity
Junctate	A Ca ²⁺ -binding, integral endoplasmic reticulum (ER) membrane protein that induces and/or stabilizes peripheral coupling between the ER and the plasma membrane
Ca²⁺-dependent inactivation (CDI)	The process whereby an increase in cytosolic Ca ²⁺ levels leads to inactivation of the Ca ²⁺ release-activated Ca ²⁺ (CRAC) current. CDI is mediated by residues located in stromal interaction molecule 1 (STIM1) (in the cytosolic domain) and Orai1 (within the amino terminus)
Inhibitory domain region (ID region)	A short sequence in the carboxy-terminal region of stromal interaction molecule 1 (STIM1) containing acidic residues that is important for mediating Ca ²⁺ -dependent inactivation of Orai channels
Calmodulin	A highly conserved cytosolic Ca ²⁺ -binding protein that mediates several Ca ²⁺ -dependent responses in cells by interaction with and modification of calmodulin-binding target proteins. Calmodulin is expressed in all eukaryotic cell types
CRAC regulatory protein 2A (CRACR2A)	A recently described stromal interaction molecule (STIM)-Orai-binding protein shown to regulate the stability of the Ca ²⁺ signalling junction
Golli	A member of the myelin basic protein (MBP) family that interacts with stromal interaction molecule 1 (STIM1) and regulates store operated Ca ²⁺ entry (SOCE)
SOCE-associated regulatory factor (SARAF)	A recently defined stromal interaction molecule (STIM)-binding protein that mediates the dissociation of STIM proteins from Ca ²⁺ signalling junctions
Transient receptor potential channels (TRPCs)	A family of seven non-selective cation channels defined on the basis of homology with the <i>Drosophila melanogaster</i> TRP channel. TRPCs have been extensively investigated as potential mediators of store-operated Ca ²⁺ entry (SOCE). However, this notion is highly controversial mainly because they are activated downstream of phospholipase C and mediate non-selective cation currents easily distinguishable from Ca ²⁺ release-activated Ca ²⁺ (CRAC)
Phospholipase C (PLC)	A signalling enzyme that breaks down phosphatidylinositol-4,5-bisphosphate into inositol-1,4,5-trisphosphate and diacylglycerol in response to the activation

	of both G protein-coupled receptor and Tyr kinase-coupled receptor
Diacylglycerol (DAG)	Lipid product of phospholipase C that activates and modulates the function of several proteins including various ion channels
Phosphatidylinositol-4 5-bisphosphate, (PtdIns(4,5)P₂)	A negatively charged phospholipid found primarily in the inner leaflet of the plasma membrane. As the substrate for phospholipase C, PtdIns(4,5)P ₂ breakdown leads to the release of Ins(1,4,5)P ₃ and diacylglycerol
ARC channel	An arachidonic acid-responsive channel activity comprising a unique combination of Orai1 and Orai3 subunits
Sarcoendoplasmic reticulum Ca²⁺ATPase (SERCA)	A family of Ca ²⁺ pumps comprising three members, all of which pump Ca ²⁺ from the cytosol into the endoplasmic reticulum (ER) or the sacroelplamic reticulum lumen. Each family member has several splice variants. Due to the leakiness of the ER to Ca ²⁺ , SERCA is constitutively active
Aequorins	Ca ²⁺ -sensitive photoproteins isolated from luminescent jellyfish that are used to detect the Ca ²⁺ content in different subcellular compartments
Plasma membrane Ca²⁺ATPase (PMCA)	A family of Ca ²⁺ pumps comprising four members that pump Ca ²⁺ from the cytosol to the extracellular milieu. Each family member has at least two splice variants. PMCA pumps primarily function to maintain cytosolic Ca ²⁺ levels
Immunological synapse	A region that can form between two cells of the immune system that are in close proximity. The name derives from similarities to the synapse that is found in the nervous system. The immunological synapse refers to the interaction between a T cell or natural killer cell and an antigen-presenting cell
Partner of STIM1 (POST)	A 12-transmembrane spanning protein found mostly in the endoplasmic reticulum membrane, but also in the plasma membrane. POST binds activated stromal interaction molecule 1 (STIM1) and modulates the function of multiple target proteins
Calnexin	Ca ²⁺ -binding transmembrane endoplasmic reticulum chaperone protein that functions to support correct folding of new proteins

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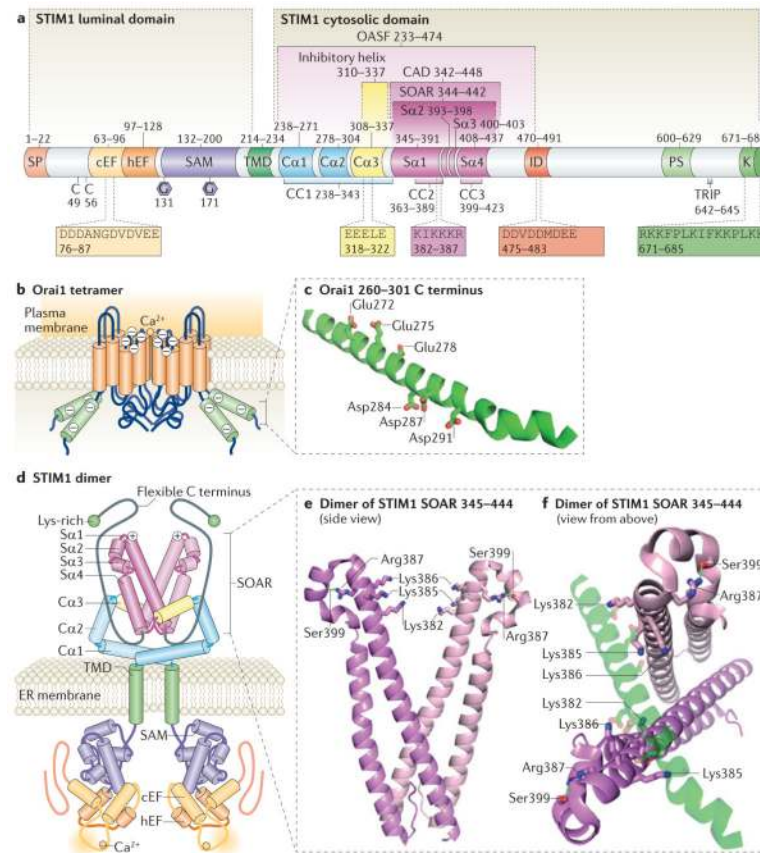


Figure 1. Structure and activation of STIM1

a | The molecular domains of stromal interaction molecule 1 (STIM1). Endoplasmic reticulum (ER) STIM1 contains a luminal and a cytosolic domain. The amino-terminal signal peptide (SP) is cleaved during translation. The ER luminal N-terminal domain includes a conserved Cys pair, a Ca^{2+} -binding canonical EF-hand domain, (cEF), a non- Ca^{2+} -binding hidden EF-hand (hEF) domain, a sterile α -motif (SAM) with two Asn-linked glycosylation sites (shown as hexagons) and a single transmembrane domain (TMD). The cytosolic carboxy-terminal domain is considered to include three coiled-coil regions⁷⁴ (called CC1, CC2 and CC3). CC1 is divided into three α -helices (termed Ca1, Ca2 and Ca3) on the basis of sequence analysis predictions by using JPred3 (REF. 188). The structure of Ca3 is also determined on the basis of homology with the recently solved *Caenorhabditis elegans* STIM structure⁴⁰. SOAR (STIM–Orai activating region) is the minimal sequence required for the activation of Orai1 (REF. 45). SOAR contains four α -helices, termed Sa1, Sa2, Sa3 and Sa4 (REF. 40). The segments CAD (Ca^{2+} release-activated Ca^{2+} (CRAC) activation domain)⁴⁶ and OASF (Orai1-activating small fragment)⁴⁷ are larger than SOAR, contain the CC1 region and also activate Orai1. SOAR includes the polybasic region, with the sequence KIKKKR (amino acids 382–387), which is crucial for the interaction with Orai1 (REFS 40,68,71). Ca3 contains an inhibitory helix that inhibits SOAR function^{40,68,71}. The acidic EEELE (residues 318–322) region is required for the action of the inhibitory helix^{40,68}. Downstream of SOAR resides an acidic inhibitory domain (ID) that mediates fast Ca^{2+} -dependent inactivation of Orai1 (REFS 89,92,93). The C-terminal tail contains a Pro/Ser-rich domain (PS), a microtubule interacting domain (TRIP) and a Lys-rich domain responsible for phospholipid interaction at the plasma membrane. **b** | The tetrameric structure of the Orai1 channel is shown, highlighting the

TMDs (shown in orange), extracellular and intracellular sequences (blue) and C-terminal predicted α -helices (shown in green). Negatively charged residues are indicated in the C-terminal helices and in the Ca^{2+} selectivity filter at the predicted mouth of the pore. **c** | The predicted α -helical structure of the Orai1 C terminus and potential sites for SOAR binding are illustrated on the basis of secondary structure prediction by using JPred3 (REF. 188). Side chains from acidic residues Glu272, Glu275, Glu278, Asp284, Asp287 and Asp291 are shown as amphipathic helices that may electrostatically interact with basic residues in the SOAR dimer. **d** | Proposed structure of a resting STIM1 dimer. The predicted α -helices in CC1 are indicated in the figure and are shown in a folded configuration. The inhibitory Ca_v3 helix (highlighted in yellow) is bound to SOAR (shown in red), which comprises four α -helices as indicated. The SOAR polybasic region is shown (+). The STIM1 dimer is held together predominantly by interactions between the CC1 and SOAR regions^{37,40}. The C-terminal flexible region (shown in grey) together with the C-terminal Lys-rich domain (shown in green) may provide some steric shielding of SOAR⁷⁷. α -helices are shown as cylinders; flexible regions as lines. **e** | The side view of the SOAR structure reveals that Lys382, Lys385 and Lys386 are located in the polybasic Orai-interacting region, and that these residues are oriented towards the centre of the cleft. Lys384 (not shown) is oriented in another direction. The Arg387 residue may be involved in hydrogen bonding with Ser399 (indicated by a green line). Coordinates were obtained from Protein data bank entry 3TEQ⁴⁰. **f** | SOAR structure shown in (e) rotated 90° to illustrate the potential sites of association with the Orai1 C terminus depicted in (c). The hypothetical electrostatic binding of the Orai1 C terminus within the cleft between the SOAR monomers is shown.

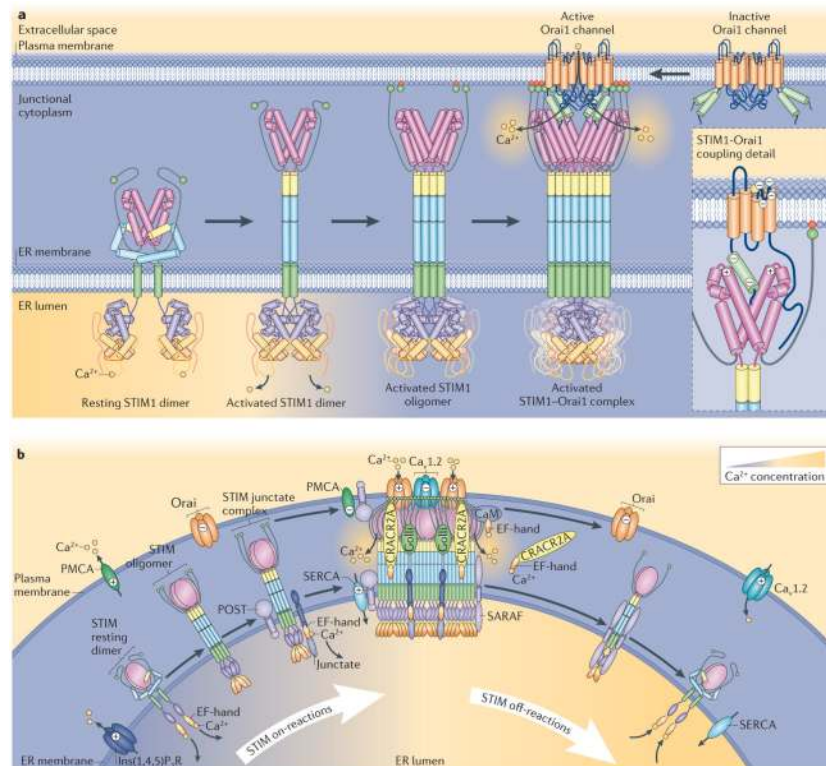


Figure 2. STIM activation and organization of the Ca^{2+} signalling junction

a | Hypothetical model of stromal interaction molecule 1 (STIM1) activation and coupling to Orai1. The resting STIM1 dimer in the Ca^{2+} -replete endoplasmic reticulum (ER) is shown on the left. The activation of the STIM1 dimer is initiated by Ca^{2+} dissociation from the STIM1 dimer. This causes EF-hand–SAM domains within the STIM1 dimer to interact, which induces an extended configuration of the cytoplasmic coiled-coil domains⁷⁴, dissociation of the Ca3 inhibitory helix from SOAR (STIM–Orai activating region)^{40,68,74}, and the carboxy-terminal flexible domains recede and expose SOAR. STIM1 continues to oligomerize and migrates into ER–plasma membrane junctions, and the polybasic C termini bind and anchor STIM1 to negatively charged phospholipids in the plasma membrane⁴⁶ (shown in red) and active SOAR is fully exposed. Large aggregates of anchored STIM1 within ER–plasma membrane junctions are able to tether and activate Orai1 proteins. Each SOAR dimer interacts with one Orai1 protein, therefore eight STIM1 molecules form an active complex with one tetrameric Orai1 channel. **b** | Role of regulatory and target proteins in the STIM-activated Ca^{2+} signalling junction. In response to inositol-1,4,5-trisphosphate ($\text{Ins}(1,4,5)\text{P}_3$) receptor ($\text{Ins}(1,4,5)\text{P}_3\text{R}$)-mediated ER Ca^{2+} depletion, Ca^{2+} dissociates from STIM1, and STIM1 aggregates and translocates into ER–plasma membrane junctions. During activation, STIM1 initially interacts with plasma membrane lipids through its Lys-rich domain assisted by interaction with junctate in or near ER–plasma membrane junctions^{81,82}. This interaction is stabilized by the Ca^{2+} -free form of Ca^{2+} release-activated (CRAC) regulatory protein 2A (CRACR2A)⁸⁷ and golgi^{96,98}. During targeting, STIM1 interacts with and activates Orai1 channels and inhibits $\text{Ca}_v1.2$ channels^{112,114}. STIM1 recruits partner of STIM1 (POST) to the junction, and this adaptor protein recruits both plasma membrane Ca^{2+} ATPase (PMCA) and sarcoendoplasmic reticulum Ca^{2+} ATPase (SERCA) to the Ca^{2+} signalling junction¹²⁷. POST¹²⁷ and STIM1 (REF. 126) inhibit PMCA-mediated Ca^{2+} efflux from the cell and thereby increase Ca^{2+} availability for signalling. SERCA recruited into junctions by POST may also assist ER Ca^{2+} loading.

During STIM deactivation, increased cytosolic Ca^{2+} -dependent loss of both CRACR2A⁸⁷ and goli⁹⁸ destabilizes the STIM complex. Ca^{2+} -binding to calmodulin (CaM) promotes Orai channel inactivation. The STIM-binding protein store-operated Ca^{2+} entry (SOCE)-associated regulatory factor (SARAF)⁸⁸ also mediates dissociation of STIM proteins, resulting in their configuration into a resting state.

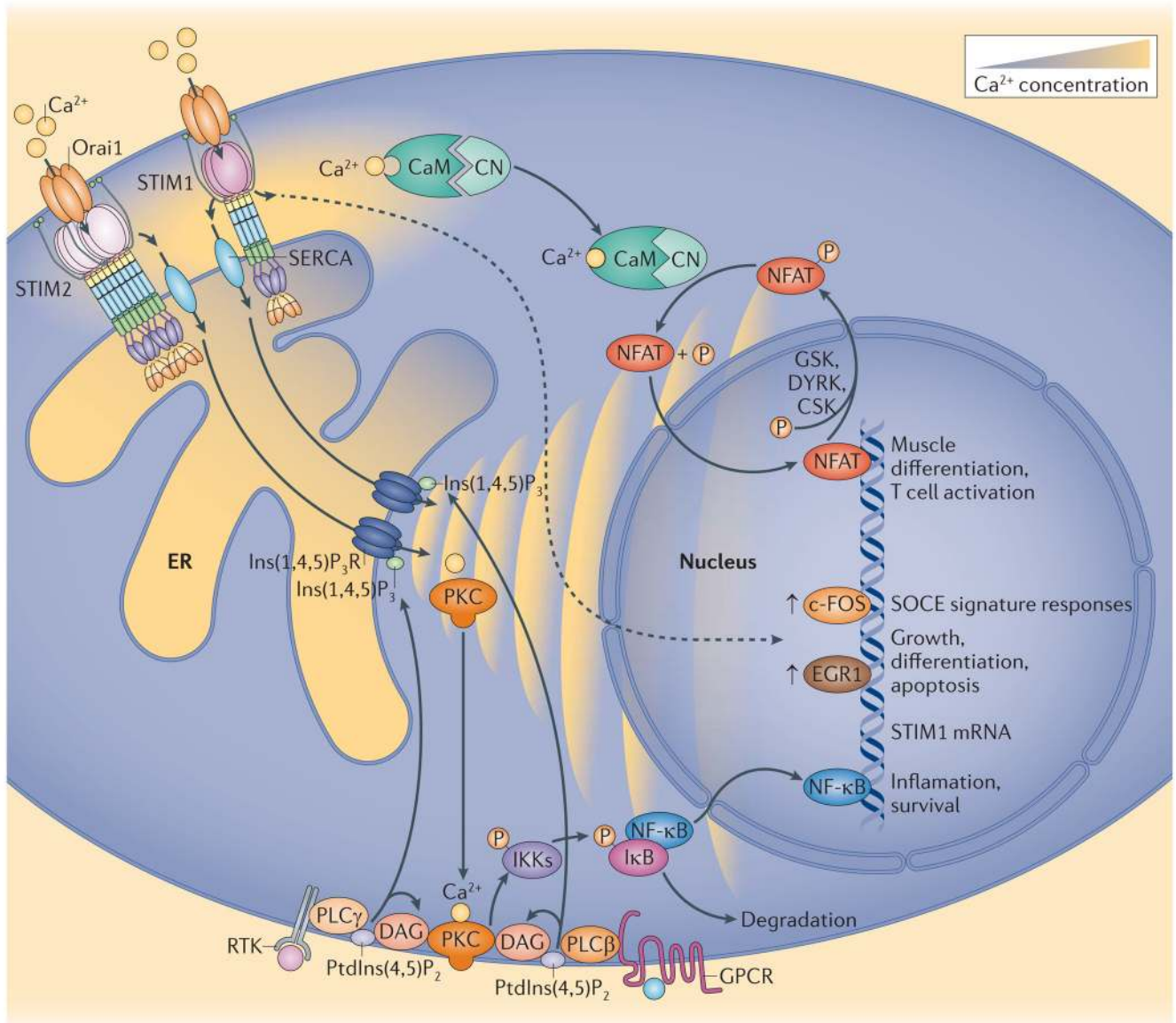
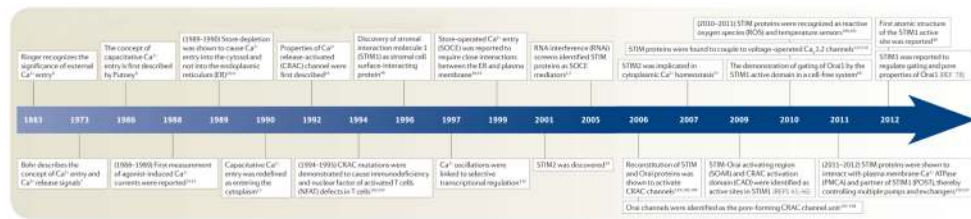


Figure 3. STIM proteins control gene expression by generating spatiotemporally controlled Ca^{2+} signals

Activation of either receptor Tyr kinase-coupled or G protein-coupled receptors (RTK or GPCR, respectively) result in phospholipase C (PLC)-mediated production of inositol-1,4,5-trisphosphate (Ins(1,4,5)P₃) and diacylglycerol (DAG). RTK-mediated PLC γ activation can be slower and more gradual than GPCR-mediated PLC β responses⁶⁷. The rapid ER Ca²⁺ depletion mediated by GPCR leads to stromal interaction protein 1 (STIM1)-mediated but not STIM2-mediated Orai channel activation. By contrast, ‘shallow’ Ca²⁺ release through RTK may cause both STIM1- and STIM2-mediated Orai channel activation. Close apposition of sarcoplasmic reticulum Ca²⁺ ATPase (SERCA) with junctions allows the entry of Ca²⁺ to refill stores and maintain oscillations while contributing only minimally to global changes in cytosolic Ca²⁺ concentration⁶⁰. However, the local increase in Ca²⁺ near the STIM1–Orai junction is crucial for the activation of both c-FOS¹³⁶ and nuclear factor of activated T cells (NFAT)^{139,140}. Hence, Ca²⁺-Calmodulin (CaM) activates calcineurin (CN), which dephosphorylates NFAT, resulting in nuclear translocation. Casein kinase (CK), dual

specificity Tyr phosphorylation-regulated kinase 2 (DYRK) and glycogen synthase kinase (GSK) phosphorylate NFAT, which results in a cycling of NFAT across the nuclear membrane as long as the Ca^{2+} signal continues. Store-operated Ca^{2+} entry (SOCE) can also lead to both nuclear factor- κB (NF- κB) activation^{189,179} and early growth response protein 1 (EGR1) upregulation¹⁹⁰, both of which directly stimulate STIM1 transcription (not shown)^{191,192}. Ca^{2+} -mediated NF- κB activation may be mediated by protein kinase C (PKC), which phosphorylates (P) inhibitor of NF- κB (I κB) kinases (IKKs). IKKs phosphorylate I κB , resulting in its dissociation from NF- κB .



Timeline.

Major events in the discovery and characterization of store-operated Ca^{2+} entry