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STIMulating store-operated Ca^{2+} entry

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Abstract

Calcium influx through plasma membrane store-operated Ca^{2+} (SOC) channels is triggered when the endoplasmic reticulum (ER) Ca^{2+} store is depleted — a homeostatic Ca^{2+} signalling mechanism that remained enigmatic for more than two decades. RNA-interference (RNAi) screening and molecular and cellular physiological analysis recently identified STIM1 as the mechanistic 'missing link' between the ER and the plasma membrane. STIM proteins sense the depletion of Ca^{2+} from the ER, oligomerize, translocate to junctions adjacent to the plasma membrane, organize Orai or TRPC (transient receptor potential cation) channels into clusters and open these channels to bring about SOC entry.

Cells are preoccupied by the control of cytosolic calcium concentration, and with good reason. Calcium ions shuttle into and out of the cytosol — transported across membranes by channels, exchangers and pumps that regulate flux across the ER, mitochondrial and plasma membranes. Calcium regulates both rapid events such as cytoskeleton remodelling or release of vesicle contents, and slower ones such as transcriptional changes. Moreover, sustained cytosolic calcium elevations can lead to unwanted cellular activation or apoptosis.

Among the long-standing mysteries in the Ca^{2+} signalling field is the nature of feedback mechanisms between the ER and the plasma membrane. Studies in the late 1980s and 1990s established that the depletion *per se* of ER Ca^{2+}, but not the resulting rise of cytosolic Ca^{2+}, is the initiating signal that, following $InsP_3$-induced release of ER Ca^{2+}, triggers SOC entry (SOCE) through Ca^{2+} channels in the plasma membrane[1]. The best-characterized SOC current was, and still is, the Ca^{2+} release-activated Ca^{2+} (CRAC) current in lymphocytes and mast cells[2–4]. However, the molecular mechanism remained undefined until recently. The key breakthroughs came from RNAi screening, which first identified STIM proteins as the molecular link from ER Ca^{2+} store depletion to SOCE and CRAC channel activation in the plasma membrane, and then identified Orai (CRACM) proteins that comprise the CRAC channel pore-forming subunit. STIM proteins will be referred to generically as STIM, the *Drosophila melanogaster* protein as Stim and the two mammalian homologues as STIM1 and STIM2.

Origins of STIM

STIM proteins first came to light outside the Ca^{2+} signalling field. STIM1 was originally identified in a library screen by its ability to confer binding of pre-B lymphocytes to stromal cells. Originally named SIM (stromal interacting molecule)[5], the name later morphed into STIM — fortuitously suggestive of its function in stimulating calcium influx across the plasma

Note: Supplementary Information is available on the Nature Cell Biology website.

membrane. *Drosophila* and *Caenorhabditis elegans* have one STIM protein, but representative amphibians, birds and mammals have two STIM homologues (and teleost fish have four)[6,7], suggesting gene duplication at the invertebrate-vertebrate transition. In addition, STIM1 (formerly known as GOK)[8] was implicated in cell transformation and growth by its chromosomal localization (human 11p15.5) to a region associated with paediatric malignancies, and by its ability to induce cell death when transfected into rhabdomyosarcoma and rhabdoid tumour cells[9]. It was originally characterized biochemically as a glycosylated phosphoprotein with ~25% expression in the plasma membrane by surface biotinylation[6,10,11]. The human *STIM2* gene, sharing 61% sequence identity with *STIM1*, localizes in a region (4p15.1) implicated in squamous carcinoma and breast tumours[6]. The sole *Drosophila* homologue, Stim, has 31% identity and 60% sequence similarity to STIM1. STIM proteins from *Drosophila* and mammals are shown diagrammatically in Fig. 1.

Inspection of the primary polypeptide sequence of STIM proteins reveals a modular construction. Two protein–protein interaction domains are separated by a single membrane-spanning segment and, as a type I membrane protein, the amino terminus is predicted to reside either within the ER lumen or facing the extracellular space. Between the N-terminal signal peptide sequence and the sterile alpha motif (SAM) protein interaction domain, two adjacent regions containing negative charges, one typical of an EF-hand Ca^{2+}-binding domain, are found. At the other end, in the cytoplasm, a lengthy bipartite coiled-coil is predicted within a region containing an ezrin/radixin/moesin (ERM) domain leading to the C terminus. This end of the molecule diverges substantially, the *Drosophila* (and *C. elegans*) protein notably lacking a proline-rich region and a lysine-rich tail.

RNAi screens identify STIM and Orai

The key breakthroughs in identifying the molecular constituents of SOC signalling arose from RNAi screening. A critical role of STIM in SOCE was first revealed by two concurrent and independent candidate RNAi screens[12,13], using thapsigargin to deplete the ER Ca^{2+} store. Thapsigargin blocks the ER-resident SERCA (sarcoplasmic/endoplasmic reticulum calcium) pump, causing a decline in luminal Ca^{2+} store content as Ca^{2+} leaks out of the ER passively, unopposed by active re-uptake through the pump. *Drosophila* S2 cells used in one screen[13] have functional CRAC currents closely similar in activation requirements and biophysical characteristics to those in human T lymphocytes[14]. Hits were defined by decreased SOCE and confirmed in single cells by Ca^{2+} imaging and whole-cell recording. The S2 cell screen yielded one hit (Stim)[13], and the human HeLa cell screen identified both mammalian homologues, STIM1 and STIM2 (ref. [12]). It soon became clear that several mammalian cell types rely primarily on STIM1 for SOCE and CRAC channel activation[12,13,15]; an additional screen showed that STIM2 functions in regulating basal Ca^{2+} levels[16].

STIM triggers SOCE but does not form the CRAC channel itself. Following the success of the candidate screening approach, the search for the CRAC channel continued as genome-wide RNAi screening in S2 cells was performed independently by three research groups[17–19]. This led to the identification of several additional genes required for Ca^{2+} signalling, including the previously unheralded *Orai* (also known as *CRACM*, formerly known as *olf186-F*), a *Drosophila* gene with three mammalian homologues. A parallel genetic screen by one of the three groups led to the remarkable discovery that a point mutation (corresponding to R91W) in human *Orai1* on chromosome 12 causes a rare but lethal disorder SCID (severe combined immunodeficiency disorder) — the first identified channelopathy of the immune system[17]. Orai proteins have four predicted transmembrane segments, but apart from being a multi-span transmembrane protein Orai has no other similarity to known ion channels. Evidence that Orai forms the CRAC channel itself came from two further discoveries. First, the unique biophysical fingerprint of the CRAC channel in patch clamp studies is duplicated, but with currents that

are amplified 10 to 100-fold, when STIM and Orai are overexpressed together in heterologous cells[19–22]. Second, Orai and Orai1 were confirmed as CRAC channel pore-forming subunits by the marked alteration of ion selectivity that results from point mutation of a key glutamate residue in the loop between transmembrane segments 1 and 2 (refs [23–25]).

Sensing luminal ER Ca^{2+}

The essential function of STIM as a non-channel intermediary of SOCE and CRAC channel activation has been explored by mutagenesis and subcellular localization. The presence of an EF-hand motif near the N terminus, localized in the ER lumen, suggested a role in sensing ER Ca^{2+}. It was soon discovered that expression of EF-hand mutants engineered to prevent Ca^{2+} binding resulted in constitutive Ca^{2+} influx and CRAC channel activation, but without depletion of the ER Ca^{2+} store[12,26]. This ability of the EF-hand mutants to bypass the requirement for Ca^{2+} store depletion convincingly demonstrated that the Ca^{2+}-unbound state of STIM leads to CRAC channel activation. The resting concentration of Ca^{2+} within the ER lumen is hundreds of micromolar — much higher than in the cytoplasm — and is maintained by ongoing activity of the SERCA pump. Ca^{2+} binding to the isolated EF-SAM portion of STIM1 was shown to have a dissociation constant in the range of hundreds of micromolar and a 1:1 binding stoichiometry[27]. Low-affinity binding of Ca^{2+} to the EF-hand domain is compatible with the proposal that Ca^{2+} is bound when the ER store is full and STIM releases Ca^{2+} when the ER is depleted, thus initiating a process that leads to CRAC channel activation.

Signal initiation by oligomerization

In the basal state when ER Ca^{2+} stores are filled, STIM is a dimer stabilized by C-terminal coiled-coil interactions, as shown by several lines of evidence. Intact STIM proteins heteromultimerize under basal conditions[6,28,29], and Stim forms dimers[30]. Moreover, the isolated C-terminal portion of STIM1 forms dimers[31], whereas the isolated N-terminal ER-SAM domain is monomeric at basal ER Ca^{2+} concentration. When the ER Ca^{2+} store is depleted, further STIM1 oligomerization occurs within seconds in intact cells[32,33], and this is mediated by the SAM domain adjacent to the EF-hand domain, as shown by the formation of multimers at reduced Ca^{2+} levels in biochemical and nuclear magnetic resonance structural studies of the isolated N-terminal portion of STIM1 (refs [34, 35]). In intact cells, oligomerization precedes and triggers translocation of STIM to the plasma membrane and activation of CRAC channel activity[32,33,36].

Conveying the message to the plasma membrane

After Ca^{2+} store depletion, the EF-hand domain releases bound Ca^{2+} and oligomers of STIM physically translocate ‘empty-handed’, thereby conveying the message to the plasma membrane that the ER store has been depleted (Fig. 2). Native STIM1 and tagged constructs were first shown by light microscopy, and then by electron microscopy, to form clusters (also termed ‘puncta’ or ‘hotspots’, with surface areas in the order of 1–10 μm^2) immediately adjacent to the plasma membrane, following Ca^{2+} store depletion[12,20,26,28,37]. Whereas native untagged STIM1 colocalized with ER-resident proteins in the resting state when the Ca^{2+} store was filled, it was shown to translocate to the plasma membrane following store depletion[26]. Moreover, activating EF-hand mutant STIM1 proteins were localized predominantly at the plasma membrane even when the Ca^{2+} store remained full[12,26]. The marked redistribution of STIM1 after Ca^{2+} store depletion was not accompanied by any obvious changes in the bulk ER structure[12,26,37]. Instead, as seen from Förster resonance energy transfer (FRET) measurements, ER STIM1 oligomerizes and subsequently accumulates at specific predetermined foci in the peripheral ER[32,33,38,39], representing points of close ER–plasma membrane apposition[37]. Total internal reflectance fluorescence (TIRF) microscopy placed yellow fluorescent protein (YFP)-tagged STIM1 clusters to within 100–200 nm of the cell

surface[12,28,32,37] (Supplementary Information, Movie 1), and electron microscopy visualization of horseradish peroxidase (HRP)-tagged STIM1 demonstrated that it migrates to within 10–25 nm of the plasma membrane[37]. Tagging may prevent surface expression of STIM1 (Box 1), but tagged proteins are effective in evoking SOCE and CRAC currents indicating that acute insertion of STIM1 into the plasma membrane is not a required step. Clustering of STIM1 adjacent to the plasma membrane is rapid (occurring before CRAC channels open), spatially reproducible and rapidly reversible on signal termination and refilling of the ER Ca^{2+} store[32,38–40]. In addition to the refilling of the ER Ca^{2+} store, the disassembly of STIM1 clusters may also result from Ca^{2+} influx and the resulting rise in cytosolic Ca^{2+}, implying a local negative feedback mechanism that may contribute to CRAC channel inactivation [41].

Oligomerization of STIM is necessary and sufficient for its translocation to ER–plasma membrane junctions[36]. STIM1 clustering and translocation begins when ER luminal Ca^{2+} concentration falls below ~300 μM[16,36], consistent with low-affinity binding of Ca^{2+} to the EF-hand–SAM region of STIM1 (ref. [27]). STIM1 clustering at the plasma membrane was found to be heavily dependent on ER Ca^{2+} concentration (a Hill coefficient of ~4, far steeper than the Ca^{2+} concentration dependence of binding to the EF-hand domain of STIM1), suggesting that STIM1 oligomers, but not monomers or dimers, accumulate near the plasma membrane. Moreover, CRAC channel activation closely matched the steep Ca^{2+} dependence of STIM1 aggregation and translocation to the peripheral ER, suggesting sequential processes triggered by aggregation[36]. Importantly, chemically induced oligomerization of STIM1 also resulted in translocation and activation of CRAC channels without ER Ca^{2+} store depletion[36]. These key experiments confirm that STIM oligomerization is sufficient to induce translocation to the plasma membrane and CRAC channel activation.

During even modest depletion of the ER Ca^{2+} store, STIM2 formed clusters at the plasma membrane, even as STIM1 remained localized in the bulk ER[16]. A distinct function of STIM2 in basal Ca^{2+} regulation, STIM2 having a lower ‘effective affinity’ for luminal ER Ca^{2+} than STIM1 (400 and 200 μM, respectively), is probably linked to differences in coupling between the EF-hand domain and the adjacent SAM domain[34].

So how does STIM migrate within the ER under basal conditions and then accumulate at the plasma membrane following store depletion? When the ER Ca^{2+} store is filled, STIM1 colocalizes with microtubules and appears to have a role in organizing and remodelling the ER, as the tubulovesicular distribution of STIM1 and the ER itself are disrupted when microtubules are depolymerized[28,42,43] and ER extension is stimulated by STIM1 overexpression[43]. STIM1 binds to the microtubule plus-end tracking protein EB1 (end binding protein 1) and associates with tubulin at the growing plus-end of microtubules[43]. TIRF microscopy imaging revealed rapid comet-like movement of ER STIM1 along fibrillar microtubule tracks near the cell surface[28,43–45] (Supplementary Information, Movie 1). As a plus-end microtubule-tracking protein, STIM1 can cover long distances within the cell. STIM1 proteins in tubular ER structures have been reported to associate with lipid raft regions of the plasma membrane, colocalizing with the ganglioside GM1 and caveolin[45,46]. A role for microtubules in SOCE and CRAC channel function remains controversial, some studies showing partial inhibition upon microtubule depolymerization[44,47] and others reporting no effect[28,48]. Microtubules interact with, and appear to guide, the intracellular trafficking of STIM1 as it migrates within the ER under basal conditions, but are neither required for cluster formation nor for functional activation of SOCE or CRAC channels when STIM1 is overexpressed[28,44].

Box 1 Surface expression, plasma membrane insertion and tags

Native STIM1 is localized primarily in the ER but is also detected by surface biotinylation, indicative of plasma membrane expression of a sizeable fraction (20–30%) of the total pool [10,26,82,83]. Moreover, it was reported to insert into the plasma membrane following store depletion by thapsigargin treatment[26,83]. Neither STIM2 nor EF-hand mutant STIM1 was detected under conditions that revealed plasma membrane expression through surface accessible biotinylated STIM1[15,82]. However, surface expression experiments using anti-tag antibodies, electron microscopy and the failure to observe acute quenching of pH-sensitive forms of GFP by acidic pH did not show covalently tagged STIM1 in the plasma membrane (when tagged with GFP, YFP, HRP or HA— haemagglutinin — at the N-terminus[12,20,32,37,50,84] or CFP — cyan fluorescent protein — at the C-terminus[84]). Thus, it appears that trafficking of STIM1 to the plasma membrane may be perturbed by protein tags. Consistently, a very small tag (hexahistidine) at the N terminus of STIM1 was shown to externalize following thapsigargin treatment, whereas co-expressed C-terminal CFP-tagged STIM1 remained within the ER[84]. Direct fusion events of STIM1- containing ER tubules to lipid raft domains of the plasma membrane have been reported using C-terminal-tagged YFP imaged by TIRF microscopy[45]. Membrane insertion will clearly need to be evaluated using other methods. Collectively, these results imply that STIM1 is in the plasma membrane and the ER, that tags (N-terminal YFP, GFP, HRP, HA and C-terminal CFP) can prevent surface localization and that the native protein may insert acutely following store depletion. It now seems clear that regardless of whether STIM1 is present in the plasma membrane or inserts acutely into it following store depletion, translocation to the plasma membrane without STIM1 insertion is sufficient to activate the CRAC channel, as STIM1 or Stim tagged with YFP or with other fluorescent proteins functions perfectly well in activating robust SOC influx and CRAC current[12,20,30,32,37,50]. Moreover, the presence of plasma membrane STIM1 is not required for ER-resident-tagged STIM1 to function in triggering SOCE[28]. Plasma membrane STIM1 may be involved with other functions such as cell adhesion, as originally suggested in the first report on ‘SIM’, where it was defined as a protein required for adhesion to stroma[5], and as suggested in very recent evidence favouring a role of plasma membrane STIM1 in SOC channel activation[82]. In addition, STIM1 in the plasma membrane may be essential for activation of arachidonate-regulated Ca^{2+} (ARC) channels[85] that include ORAI1 and ORAI3 as essential components[86].

Organizing the elementary unit of Ca^{2+} signalling

After ER Ca^{2+} store depletion, STIM clusters are found in the ER, close enough to the cell surface to allow for direct interaction with plasma membrane proteins. There are important precedents for ER–plasma membrane protein–protein signalling interactions, including excitation-contraction coupling in muscle. However, unlike STIM-mediated Ca^{2+} signalling, the primary signal in excitation-contraction coupling is from the plasma membrane to the sarcoplasmic reticulum, through a molecular coupling between plasmalemmal voltage-gated Ca^{2+} channels and ryanodine receptor Ca^{2+} release channels in the sarcoplasmic reticulum. In contrast, CRAC channel activation involves physical migration of ER-resident STIM to ER–plasma membrane junctions and subsequent aggregation of the Ca^{2+} influx channel. Not only does STIM serve as both Ca^{2+} sensor and messenger, it also organizes Orai subunits into adjacent plasma membrane clusters[30,49,50]. The clusters of messenger STIM and corresponding Orai channel proteins have been termed the ‘elementary unit’ of SOCE and CRAC channel activation, as Ca^{2+} influx through CRAC channels occurs precisely at STIM–Orai clusters[49]. Induced aggregation of Orai channels serves to concentrate local Ca^{2+} signalling at the particular regions of the cell where STIM and Orai cluster. Based on cluster size and number, estimates of the single-channel CRAC conductance, total number of channels per cell[19] and on direct fluorescence measurements[31], STIM and Orai clusters contain hundreds to thousands of molecules packed into a few square micrometres in the overexpression systems.

A particularly notable example of altered subcellular localization of STIM and Orai occurs at the region of contact — the 'immunological synapse' — between lymphocytes and antigen-presenting cells. When a T lymphocyte contacts an antigen-presenting cell, local signalling events lead rapidly to a cascade of tyrosine phosphorylation, $InsP_3$ generation and release of Ca^{2+} from the ER store. STIM1 and Orai1 colocalize to the immunological synapse, resulting in localized Ca^{2+} entry into the T cell region immediately next to the synapse[51]. STIM1–Orai1 clustering at the synapse was confirmed and also detected at the distal pole of the T cell in cap-like membrane aggregates[52]. Interestingly, following the initial phase of T cell activation, all three Orai isoforms and STIM1 are upregulated and Ca^{2+} signalling is enhanced, implying a positive feedback mechanism that sensitizes T cells to antigen during the first day of an immune response[51].

Orai channel activation by STIM

Stim activates Orai[19], and STIM1 couples functionally to all three Orai homologues in expression systems[20,53,54], forming very large CRAC-like currents with subtle differences in biophysical properties after store depletion. Evidence for a physical interaction between Stim and Orai was first obtained by co-immunoprecipitation studies demonstrating an increased interaction strength after Ca^{2+} store depletion[25]. A direct nanoscale molecular interaction was suggested by FRET measurements between donor and acceptor fluorescent protein tags at the C termini of STIM1 and Orai1 (refs [33, 42, 52, 55]). Abundant evidence indicates that the cytosolic C terminus of STIM functions as the effector domain to open Orai and TRPC channels. Most importantly, the truncated C-terminal portion of either Stim or STIM1 ('C-STIM') is sufficient, when expressed as a cytosolic protein, to constitutively activate native CRAC current, as well as expressed Orai, Orai1 and TRPC1 channels[30,31,33,56–58], bypassing the requirement for Ca^{2+} store depletion. Cytosolic C-STIM was found by colocalization, co-immunoprecipitation and FRET analysis to interact with Orai independent of ER Ca^{2+} store depletion, but without forming clusters typical of full-length STIM after store depletion[30,31,33]. Together, these studies identify the C terminus as the effector of STIM and show that CRAC channel activation does not necessarily require cluster formation. Further deletion analysis and fragment-expression experiments have recently defined even smaller portions of STIM1 as the effector[59–61]. A minimal activating sequence of approximately 100 amino acids spanning the distal coiled-coil of STIM1 was shown to activate the Orai1 current[60,61] and to bind directly to purified Orai1 protein[60]. Interestingly, the Orai1 current induced by the minimal fragment of STIM1 reportedly lacks the normal CRAC channel property of rapid Ca^{2+}-dependent inactivation[60,62].

Stoichiometry: STIM in Orai channel subunit assembly

Many ion channels are stable tetramers that surround a conducting pore, but examples of trimers, pentamers and hexamers are also well known. STIM1 oligomerization is sufficient to cause its translocation adjacent to the plasma membrane where activation of Orai1 channels occurs[36] (Fig. 3). How many STIM and Orai molecules are required to activate the channel? To date, four studies have investigated the oligomerization status of Orai. In biochemical experiments (glycerol gradient centrifugation, native gel systems and cross-linking)[30,63], Orai appears predominantly as a stable dimer. Yet strong functional evidence favouring a tetramer also exists; tandem tetramers of Orai1 were resistant to dominant-negative suppression by a non-conducting Orai1 monomer[64]. Two recent studies took the different, single-molecule approach of counting the number of photobleaching steps to determine the number of GFP (green fluorescent protein)-tagged Orai subunits per channel[30,31]. Both studies showed that a tetramer predominates when the channel is activated. However, using biochemical analysis and single-molecule photobleaching, we found that when Orai was expressed alone (without Stim or activating C-Stim) it appeared as a stable dimer and no change in oligomerization status

was seen in resting cells expressing Stim and Orai[30]. In contrast, another study found that STIM1 and Orai1 co-expression always resulted in tetrameric Orai1 in both closed and open states[31]. Further studies are needed to determine the kinetics and reversibility of the dimer-to-tetramer transition. A similar photobleaching approach applied to C-STIM1 suggests that two STIM1 molecules are required to activate the tetrameric Orai channel[31].

Box 2 Knockouts, STIMopathies and knockdown

Corresponding to its widespread expression pattern and documented role in Ca^{2+} signalling, *Stim1* knockout has devastating effects *in vivo*. *Stim1*−/− mice generated in various ways perished *in utero* or soon after birth of respiratory failure, although early embryos appeared to develop normally[80,87–89]. STIM2-deficient animals were born, but died at 4–5 weeks[88]. A difference in STIM1 and STIM2 function has been confirmed *in vivo* in T cells from single- and double-knockout mice[88]. Consistent with RNAi studies on cells in culture[12,13], deletion of *Stim1* resulted in a more profound reduction of SOCE in primary T cells and embryonic fibroblasts than deletion of *Stim2* (ref. [88]). CRAC currents were nearly abolished in T cells when *Stim1* was deleted but were scarcely reduced by *Stim2* knockout. Yet effects on cytokine production were profound in both *Stim1*- and *Stim2*-knockout T cells. One possible explanation is that STIM2 continues to provide sufficient Ca^{2+} influx to promote gene expression responses at late times when STIM1 has redistributed back to the bulk ER. CD4-CRE mice doubly deficient in both STIM1 and STIM2 developed a lymphoproliferative disorder due to impaired function of regulatory T cells[88]. *Stim1* knockout also profoundly reduced SOCE in fetal liver-derived mast cells and acute degranulation was nearly abolished[87]. Secretion of cytokines was strongly reduced, and in heterozygous *Stim1*+/− mice anaphylactic responses were mildly reduced. A gene-trap knockout to generate *Stim1*−/− mice resulted in neonatal lethality with growth retardation and a severe skeletal myopathy[89]. Functional analysis of myotubes from gene-trap-knockout *Stim1gt/gt* mice indicated reduced SOCE, attenuated CRAC-like current and increased fatiguability during repetitive action potentials. A role of STIM1 in platelets relating to cerebrovascular function was also inferred[80]. SOCE, agonist-dependent Ca^{2+} influx and platelet aggregation was reduced in platelets from STIM1-deficient mice, also generated by insertion of an intronic gene-trap cassette. Of potential clinical significance, bone-marrow chimaeric mice with STIM1 deficiency in haematopoietic cells, including platelets, had improved outcomes in a brain infarction model of stroke using transient occlusion of a cerebral artery to induce neuronal damage[80]. STIM1 also has a vital role in FCγ receptor-mediated functions in macrophages, including the secretion of inflammatory cytokines and phagocytosis[90]. In the same study, mice lacking STIM1 in haematopoietic cells were shown to be resistant in experimental models of thrombocytopenia and autoimmune hemolytic anaemia. Furthermore, transgenic mice expressing an EF-hand mutant STIM1 exhibited a bleeding disorder associated with elevated Ca^{2+} and reduced survival of platelets that led to reduced clotting in this induced 'STIMopathy' (ref. [91]). Collectively, these studies show that Ca^{2+} signalling was dramatically reduced in T lymphocytes, fibroblasts, mast cells, skeletal muscle, platelets and macrophages from Stim1 knockouts.

Recently, an immune deficiency syndrome due to mutation of STIM1 was identified in a human family[92]. This first human STIMopathy arises from a truncation mutation in the SAM domain that introduces a premature STOP codon and results in undetectable levels of *Stim1* mRNA and protein expression. SOCE was absent in patient fibroblasts, and the patients exhibited a complex syndrome of combined immunodeficiency with infections and autoimmunity associated with hepatosplenomegaly, hemolytic anaemia, thrombocytopenia and reduced numbers of regulatory T cells. In addition, patients exhibited muscular

hypotonia and an enamel dentition defect, similarly to patients with immunodeficiency due to mutations in Orai1.

STIM1 has also been implicated by RNAi knockdown of SOCE or CRAC channel function in a variety of primary cells and cell lines, including: Jurkat T[13], HEK293 (refs [13, 93]), HeLa[12,94], PC12 (ref. [95]), neuroblastoma/glioma[75] and breast cancer[96] cell lines; airway and vascular smooth muscle[75,97–101]; hepatocytes[102] and liver cell lines[103–105]; and salivary and mandibular gland cells[70]. In addition, parallels in STIM1 expression and SOCE function are reported in primary megakaryocytes and platelets[106], resting and activated human T cells[51] and vascular smooth muscle[107,108]; STIM1 translocation has been studied in a pancreatic cell line[40].

TRPC channel activation by STIM1

Are all SOC channels actually STIM-Operated Ca^{2+} channels with a variety of different pore-forming subunits? The coupling between STIM1 and Ca^{2+}-permeable channels appears to be promiscuous, a property that may potentially help to resolve an often tendentious discussion of what is, and what is not, a SOC channel. From a biophysical perspective, it has been puzzling to note the very different properties of ion selectivity and rectification of SOC channels in various cell types. CRAC channels in the immune system are very selective for Ca^{2+}, have an inwardly rectifying current-voltage relationship and an unusually low single-channel conductance in the femtosiemens range; in contrast,TRPC channels are less Ca^{2+}-selective and have more linear current-voltage relationships and higher single channel conductances in the picosiemens range. The promiscuity of STIM1 coupling potentially resolves this conundrum. ‘Classic’ CRAC current is identified as originating from Orai channels, whereas the less selective SOC influx may be due to TRPC family members. So far, STIM1 has been implicated in activating Ca^{2+}-permeable TRPC1, C2, C3, C4, C5 and C6 (but not TRPC7) channels[45, 46,56,61,65–70]. As shown previously for native CRAC channels, the C terminus of STIM1 activates TRPC1 and TRPC3 channels and interacts with TRPC1, C2, C4 and C5 (as shown by co-immunoprecipitation)[56,57], leading to the conclusion that STIM1 heteromultimerizes TRPC channels and confers function as SOC channels[57]. For TRPC1, channel activation is mediated through a charge–charge interaction that requires the C-terminal lysine-rich domain of STIM1 (ref. [71]). TRPC1 is reportedly recruited by STIM1 to lipid raft domains in the surface membrane, resulting in a store-dependent mode of TRPC1 channel activation[45,46]. A further level of complexity was suggested on the basis of RNAi suppression, dominant-negative suppression and biochemical pulldown experiments that point to interactions between TRPC and Orai channels[68,70,72,73]. However, the functional relationship between STIM, TRPC and Orai proteins will need further investigation to confirm the extent of inter-family heteromultimerization and to ensure that TRPC channels are activated directly by STIM1 following store depletion and not through an indirect route.

Conclusion and remaining questions

In this review, the modular organization and five distinct functions of STIM that link ER Ca^{2+} store depletion to the activation of SOC channels in the plasma membrane are highlighted: Ca^{2+} sensing, signal initiation by oligomerization, translocation to ER–plasma membrane junctions, inducing Orai subunits to cluster and activating Ca^{2+} influx. This entire intracellular spatiotemporal signalling sequence can occur within a minute in small cells. Molecular feedback mediated by STIM1 shuttling between the ER and the plasma membrane ensures that Ca^{2+} influx occurs locally across the plasma membrane, refilling the ER Ca^{2+} store near the site of signal initiation in large cells and producing sustained and global Ca^{2+} signalling in small cells. Local Ca^{2+} signalling is likely to have important consequences for amplifying the Ca^{2+} signal near lipid raft domains as, for example, in the region of the immunological synapse.

Knockout and RNAi knockdown experiments suggest important functions and potential therapeutic targets that involve Ca^{2+} signalling and homeostasis during development and in several different organ systems and cell types (Box 2).

Despite rapid progress since the initial discovery of STIM's role in SOCE, we still do not understand several aspects of how STIM functions. We are uncertain how ER-resident STIM migrates rapidly along microtubules, how it accumulates at the plasma membrane following store depletion and how it is retrieved when the Ca^{2+} store is refilled. Many questions are posed. Are there additional scaffolding functions of STIM to anchor microtubules or to keep the ER intact? Is STIM translocation active or passive? What defines ER–plasma membrane junctions and how stable is the organization? When STIM arrives at the plasma membrane, does it induce Orai to accumulate using a diffusion trap? Or something else? Is the assembly of Orai dimers into a tetramer a dynamic and rapidly reversible process during channel gating? Finally, how exactly does STIM open the Ca^{2+} channel formed by Orai?

Other than STIM and Orai, there may be additional molecular components to the mechanism. After all, there were dozens of hits in genome-wide screens for SOCE function; to date, however, attention has been focused on STIM and Orai. Is there a 'CRAC-osome', and if so what is it? Suggestions that there are other components include size constraints involved in migration of Orai1 into clusters[39], evidence old and recent that a Ca^{2+} influx factor might be involved[74–76] and evidence that several proteins can be recruited to clusters[77]. On the other hand, overexpression of STIM and Orai alone, without a third protein, amplifies the number of functional CRAC channels to a remarkably high expression level ($> 10^5$ per cell) in many cell types, indicating that expression levels of other proteins are not limiting, at least up to very high channel densities. Furthermore, EF-hand mutant STIM1 and C-STIM N-terminal fragments bypass ER Ca^{2+} store depletion in activating robust CRAC current, arguing strongly against the hypothesis that a Ca^{2+} influx factor-like substance liberated by store depletion is required. How is it that STIM1 interacts with Orai1 and also with structurally unrelated TRPC channels? And concerning the function of STIM2, how is it that mammals have two STIMs, whereas flies function successfully with one? How does STIM1 (but not STIM2) insert into the plasma membrane? In addition to resolving mechanistic questions, future work may target STIM–channel interactions in the design of new immunosuppressant drugs[78,79] or to ameliorate cerebrovascular damage following stroke[80]. Finally, and again referring to early discoveries relating to its chromosomal localization[9,81], it will be of interest to learn if STIM has a role in tumorigenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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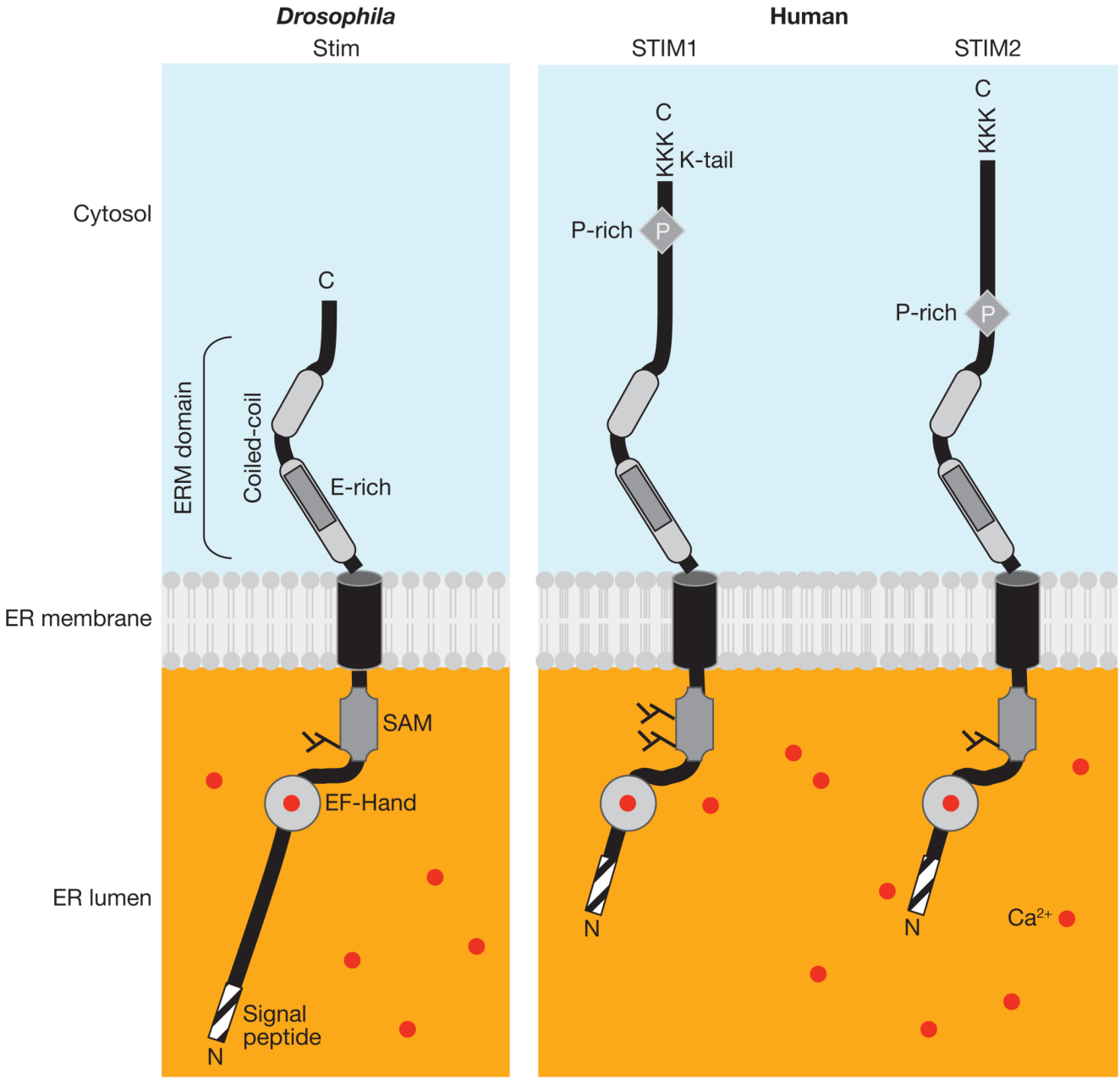

Figure 1.

Domains of STIM proteins. *Drosophila* and human STIM proteins are situated in the ER membrane. Modules of STIM1, STIM2 and Stim include: the signal peptide, the predicted EF-hand and SAM domains, the transmembrane region and two regions predicted to form coiled-coil structures comprising the ERM domain. Proline-rich domains (P) and the lysine-rich C-terminal regions are unique to the mammalian STIM family members. *Drosophila* Stim contains an N-terminal sequence in the ER that is not present in either STIM1 or STIM2. The *N*-linked glycosylation sites at the SAM domain, experimentally verified for STIM1 (refs [11, 85]), are also indicated. Background colours represent basal Ca^{2+} concentrations of ~50 nM in the cytosol and > 400 μM in the ER lumen. Ca^{2+} ions are shown as red dots, including Ca^{2+} bound to the EF-hand domain.

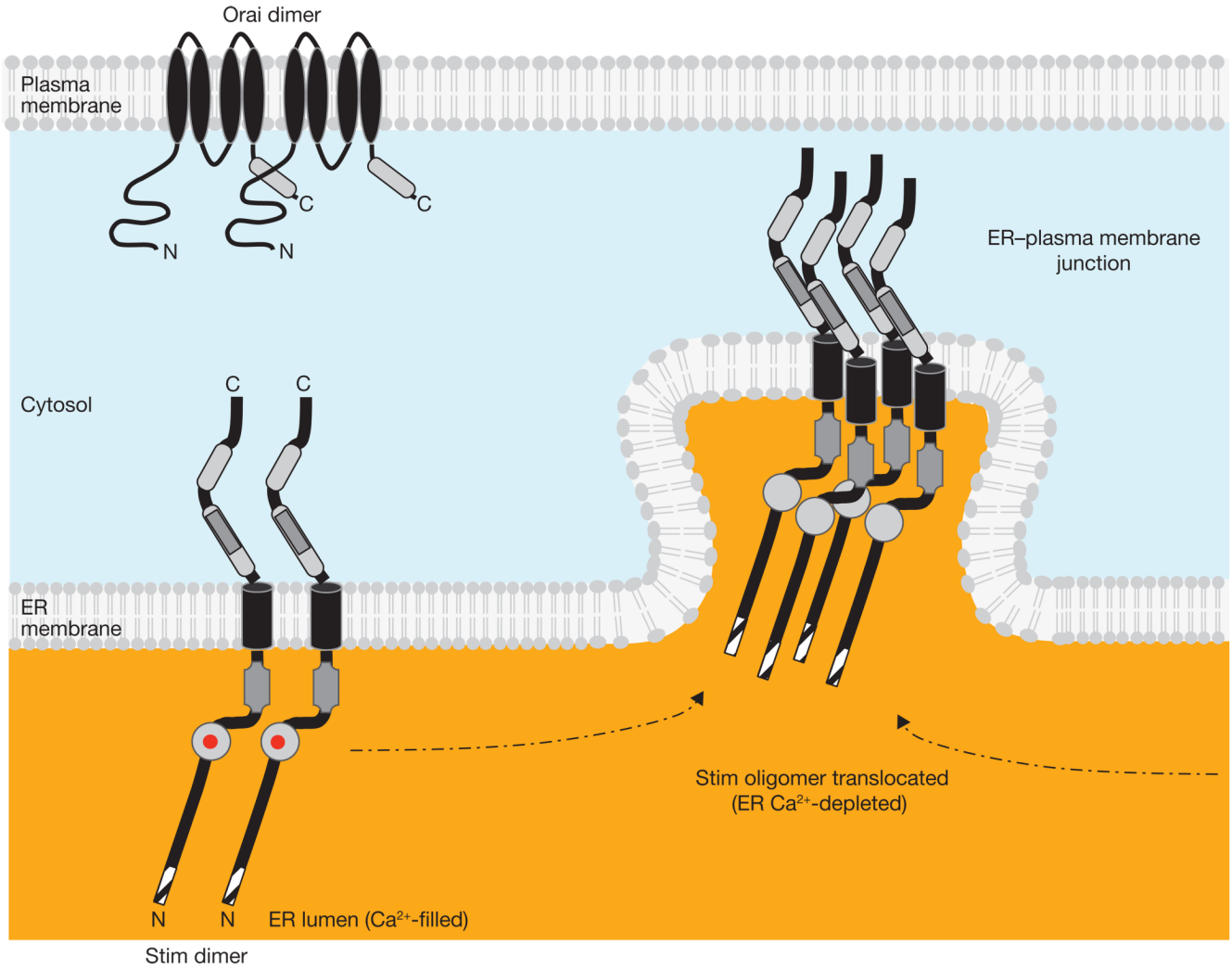

Figure 2.

STIM Ca^{2+} sensor, signal initiation and messenger functions. STIM molecules are shown in the basal state as dimers (left); the Ca^{2+} sensor EF-hand domain has bound Ca^{2+} (red dots) when the ER Ca^{2+} store is filled. Background colours represent basal Ca^{2+} concentrations of ~50 nM in the cytosol and > 400 μM in the ER lumen. ER Ca^{2+} store depletion causes Ca^{2+} to unbind from the low affinity EF-hand of STIM; this is the molecular switch that leads to STIM oligomerization and translocation (dashed arrows) to ER–plasma membrane junctions. Non-conducting Orai channel subunits are shown as dimers.

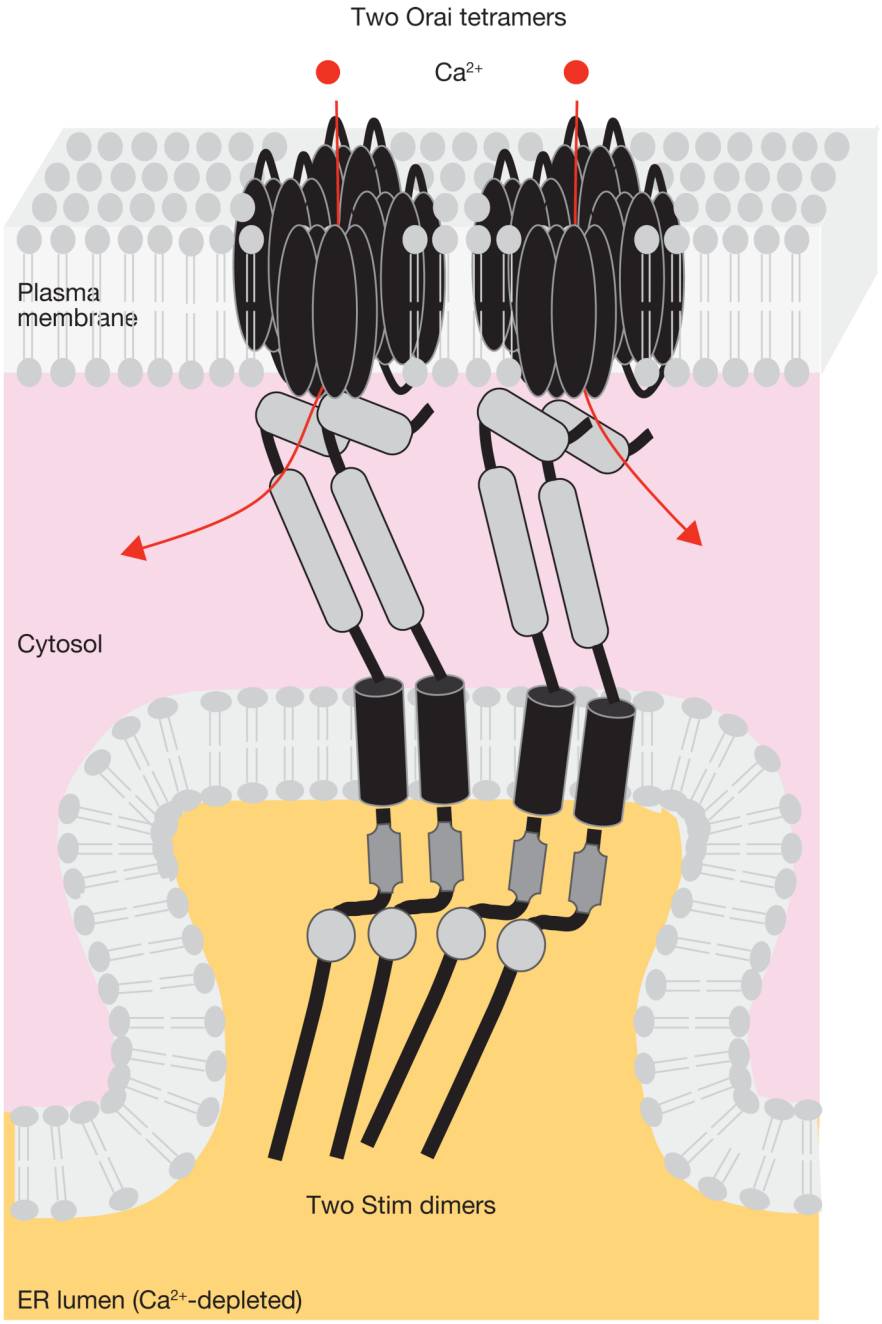

Figure 3.
STIM-mediated organization of the elementary unit and activation of Orai channels. STIM accumulation induces Orai channels to cluster in the adjacent plasma membrane. The C-terminal effector domain of STIM induces Orai channels to open by direct binding of the distal coiled-coil domain, and Ca^{2+} enters the cell (red arrows). Two STIMs can activate a single CRAC channel consisting of an Orai tetramer; channel activation of Orai may involve a preliminary step of assembling Orai dimers into a functional tetramer. Background colours represent changes in cytosolic Ca^{2+} concentration (from that in the basal state as represented in Fig 2 and Fig 3) to > 1 μM due to Ca^{2+} entry, and ER luminal Ca^{2+} to < 300 μM.