Calcium (Ca\textsuperscript{2+}) entry into non-excitable cells is mainly carried by store-operated channels (SOCs), which serve essential functions ranging from regulation of transcription to cell growth. The best-characterized store-operated current, I\textsubscript{CRAC}, is the calcium release-activated calcium (CRAC) current initially discovered in T-lymphocytes and mast cells. The search for the molecular components of the CRAC channel lasted over 20 years. Recently STIM1 has been identified as the Ca\textsuperscript{2+} sensor in the endoplasmic reticulum (ER) that accumulates into punctae close to the plasma-membrane following store-depletion. The identification of STIM1 has been closely followed by the discovery of Orai1 as the CRAC channel pore in human T-cells. Upon punctae formation STIM1 activates Ca\textsuperscript{2+} influx via Orai1 channels. This review covers functional details concerning the activation cascade of the STIM1/Orai1 complex from ER Ca\textsuperscript{2+} sensing to Ca\textsuperscript{2+} influx through Orai1 channels. This review covers functional details concerning the activation cascade of the STIM1/Orai1 complex from ER Ca\textsuperscript{2+} sensing to Ca\textsuperscript{2+} influx through Orai1 channels. Furthermore, functional domains within STIM1 and Orai1 in comparison to their structural homologs STIM2 as well as Orai2 and Orai3, respectively, are displayed together with recent findings on the pore architecture and selectivity filter of Orai channels. A broad tissue expression of STIM and Orai proteins together with substantial effects in STIM1/Orai1 knock-out mice suggests an essential physiological role in store-operated Ca\textsuperscript{2+} signalling in human health and disease.

**Discovery of STIM and Orai Proteins**

Cytosolic calcium signals are adjusted by intracellular sources such as the endo-/sarcoplasmic reticulum, Ca\textsuperscript{2+} binding proteins and via plasma-membrane Ca\textsuperscript{2+} permeable channels. The intracellular free calcium, Ca\textsuperscript{2+}, is essential for various cellular processes ranging from contraction and secretion to transcription, cell growth and cell division.\textsuperscript{1,2} The endoplasmic reticulum (ER) functions as a store for the intracellular pool of Ca\textsuperscript{2+} which is released when ER-localized inositol-1,4,5-triphosphate (IP\textsubscript{3}) receptors are activated, a process triggered by the phosphorylase C product IP\textsubscript{3}, allowing for Ca\textsuperscript{2+} efflux into the cytosol.\textsuperscript{3} Due to the limited capacity of the ER to store Ca\textsuperscript{2+}, it needs to be refilled following depletion. More than two decades ago, in 1986, James Putney has proposed a ‘capacitative Ca\textsuperscript{2+} entry’ hypothesis in which the depletion of ER Ca\textsuperscript{2+} stores itself activates Ca\textsuperscript{2+} channels in the plasma-membrane to refill internal stores (later renamed store-operated calcium entry or SOCE).\textsuperscript{4} Putney’s hypothesis has received strong support from Ca\textsuperscript{2+}-imaging experiments in various cell types where inhibitors of the ER-Ca\textsuperscript{2+} uptake like thapsigargin have been shown to activate Ca\textsuperscript{2+} entry from the extracellular milieu without the involvement of cell surface receptors or IP\textsubscript{3}.\textsuperscript{5} Subsequently the use of the patch-clamp technique has enabled to identify and characterize a store-operated Ca\textsuperscript{2+} current, the so called Ca\textsuperscript{2+} release—activated Ca\textsuperscript{2+} (CRAC) current, or I\textsubscript{CRAC}, in mast cells\textsuperscript{6} as well as in Jurkat T-cells.\textsuperscript{7,8} The distinctive biophysical characteristics of the CRAC channel comprise a high selectivity for Ca\textsuperscript{2+}, a pronounced inwardly rectifying current/voltage relationship and a very low single channel conductance.\textsuperscript{9}

Many studies have clearly demonstrated that the CRAC channel is required for the response of T-cells to antigens,\textsuperscript{10,11} for proliferation and cytokine secretion.\textsuperscript{12} However, despite intense studies over 20 years, the decoding of the molecular choreography of the CRAC channel has turned out to be very challenging. For example, proteins of the canonical transient receptor potential (TRP) family have been for a long time in the focus as components of CRAC or SOC channels.\textsuperscript{9} A variety of CRAC blockers have so far been identified,\textsuperscript{13} however, due to their non-specificity in the context with other channels and signalling pathways, these compounds have been less valuable for the identification of CRAC channels.\textsuperscript{14} Emphasized studies on the elucidation of the CRAC components and their signalling machinery have remained inefficient until high-throughput RNAi screens (searching for molecules involved in SOC influx of Drosophila S2 and HeLa cells) have led to the identification of a protein that is required for store-operated Ca\textsuperscript{2+} entry: the stromal interaction molecule 1 (STIM1).\textsuperscript{15,16} STIM1 functions as an ER calcium-sensor\textsuperscript{10,15} that translocates to defined punctae close to the plasma-membrane following store-depletion thereby activating CRAC currents.\textsuperscript{10} The elucidation of STIM1 as a Ca\textsuperscript{2+} sensor has been closely followed by the identification of the CRAC channel’s pore-forming subunit, named Orai1/CRACM1.\textsuperscript{17-19} Up to now, three human homologs, termed Orai1-3 have been identified.

These proteins contain four transmembrane segments with both N- and C-termini located intracellularly.\textsuperscript{18} Unveiling Orai1 as a CRAC component has been additionally carried out by a modified linkage analysis, where a naturally occurring single point mutation at position 91 (R91W) results in impaired T-cell signalling, which leads to severe combined immunodeficiency (SCID) syndrome. This single point mutation disrupts I\textsubscript{CRAC} in human T-cells of SCID patients.\textsuperscript{19}
patients, however, the mechanism leading to complete loss of function is still unresolved.

Molecular Structure of STIM1 and Orai Proteins

**STIM1 domains.** STIM1, initially characterised as a phospho-protein, includes a single transmembrane domain located in the ER in resting cells (Fig. 1A). The N-terminus of STIM1 stretches into the ER lumen, while the C-terminus extends into the cytosol. Within the former, a single EF-hand Ca²⁺-binding motif acts as luminal Ca²⁺ sensor. A sterile-alpha motif (SAM), including two N-linked glycosylation sites, is followed by the transmembrane domain and a cytosolic C-terminus with two coiled-coil regions overlapping with an ezrin-radixin-moesin (ERM)-like domain. Subsequent glutamate-, serine/proline-, serine/threonine- and lysine-rich regions are an ezrin-radixin-moesin (ERM)-like domain. Subsequent glutamate-, serine/proline-, serine/threonine- and lysine-rich regions are additionally located at the C-terminus (Fig. 1A), the second protein within the STIM family, is structurally homologous to STIM1. Both proteins diverge significantly in their C-terminal region after the ERM/coiled-coil domain.

**Orai domains.** All three Orai proteins, containing four predicted transmembrane segments (Fig. 1B), form Ca²⁺ selective plasma-membrane channels. Within the cytosolic strands, the N-terminus of only Orai1 includes a proline/arginine-rich region. In their C-terminus all three Orai proteins contain a putative coiled-coil domain, a common protein interaction motif (Fig. 1B), with varying predicted probabilities which are 5–6 fold higher for Orai2 and Orai3 than for Orai1. Co-expression of STIM1 with Orai1 is required to fully reconstitute CRAC currents while their expression alone results in no significant increase in or even reduced store-operated Ca²⁺ entry. Additionally co-expression of STIM1 with Orai2 as well as Orai3 resulted in Ca²⁺ current activation with biophysical characteristics similar to CRAC currents, however, with slightly different selectivity profiles and differences in feedback to intracellular Ca²⁺. The selectivity filter of Orai pores are formed by acidic residues in transmembrane domain one and three and the first loop domain. Also STIM2 is able to activate all three Orai proteins.

**Store-Operated Activation of STIM/Orai**

Store-depletion dependent STIM1 redistribution. STIM1 mediated robust Orai currents are initially stimulated by Ca²⁺ store-depletion sensed by the STIM1 luminal EF-hand. In resting cells, STIM1 is uniformly distributed within the ER, displays tubular structures and binds to the microtubule-plus-end-tracking protein EB1 at those sites where microtubule ends come in contact with the ER. Moreover, STIM1 co-localises with endogenous α-tubulin. The STIM1 EF-hand is adequate to sense a decrease in the ER Ca²⁺ level that is approximately 300–500 μM at rest. Subsequently, STIM1 forms oligomers before it redistributes with an EC₅₀ of 210 μM Ca²⁺ into punctuate clusters close to the plasma-membrane. It has recently been shown that oligomerisation of about four STIM1 proteins is the critical process transmitting ER store depletion to STIM1/Orai1 clusters thereby activating store-operated Ca²⁺ entry. TIRF microscopy in combination with electrophysiology has revealed that STIM1 translocation proceeds CRAC activation by 6–10 seconds compatible with an upstream step in CRAC channel activation. In addition, fluorescence quenching and microscopic analysis visualise that STIM1 clusters colocalise with areas of elevated Ca²⁺ concentrations from endogenous CRAC or coexpressed Orai1 channels and are located within a distance of 10–25 nm from the plasma-membrane. Moreover, upon store-depletion Orai1 proteins require STIM1 co-expression to form a clustered localisation in the plasma-membrane at sites in close proximity to STIM1 punctae. The process of store-operated STIM1 aggregation has been demonstrated by FRET microscopy to be fully reversible following store depletion. A STIM1 EF-hand mutant that is no longer able to sense ER Ca²⁺ concentration, already exhibits a punctuate pattern. Furthermore, expression of the STIM1 EF-hand mutant results in constitutive CRAC currents even when stores are full. It is under debate if store-depletion mediated translocation of STIM1 results in insertion into or accumulation underneath the plasma-membrane. A partial integration of STIM1 into the plasma-membrane has been reported by a number of laboratories employing biotinylation experiments, Gill and coworkers have proposed an interaction of endoplasmic and plasma-membrane localised STIM1, due to a constant STIM1 plasma-membrane expression independent of store-depletion. In contrast, an increase in plasma-membrane expression of STIM1 upon store-depletion has been observed with immunofluorescence, immuno-electron microscopy and biotinylation experiments. Moreover, a small hexahistidine-Zn²⁺-tag fused to the N-terminus of STIM1 has allowed demonstrating its plasma membrane insertion following store-depletion. It is of note that large N-terminal fusion proteins in contrast to C-terminal ones interfere with STIM1 plasma-membrane insertion. Functional evidence for plasma-membrane STIM1 is presented by antibodies against an N-terminal sequence of STIM1 that results in partial suppression of SOCs but could not be confirmed in another study. Reports that observe STIM1 redistribution underneath but not in the plasma-membrane have been widely based on TIRF microscopy using YFP-tagged STIM1 as well as other, even larger tags. In line, antibodies against these fluorophores fail to detect any insertion of YFP-STIM1 into the plasma-membrane. As CFP or YFP N-terminally tagged STIM1 is evenly distributed when Ca²⁺ stores are full, but STIM1 is evenly distributed when Ca²⁺ stores are filled, and following ER depletion it forms oligomers. In line, co-immunoprecipitation experiments suggest an essential role of the N-terminal part, including the SAM domain in STIM1-
The STIM/Orai coupling machinery

Figure 1. (A and B) Predicted structures of the Orai and STIM protein families. (A) Structure of STIM1 and STIM2. STIM proteins are single-transmembrane spanning proteins located in the ER, with their N-termini extending into the ER lumen. The N-termini contain a Ca\textsuperscript{2+}-sensing EF-hand and an SAM domain. The single transmembrane domain is followed by the C-terminus containing two coiled-coil motifs and an overlapping ERM domain with a subsequent glutamate-, serine/proline- and a lysine-rich region. Only STIM1 additionally comprises a glutamine- and a serine/threonine-rich region. (B) Structure of Orai1, Orai2 and Orai3, with only Orai1 containing two proline- and one arginine-rich region in its N-terminal strand. All three Orai proteins consist of four transmembrane regions and a putative coiled-coil domain in their C-terminus which is predicted with an about 5-6-fold higher probability for Orai2 and Orai3 than for Orai1. Further, Orai3 displays a much longer second extracellular loop than Orai1 and Orai2. (C) Model for STIM1/Orai1 interaction. Following ER store depletion and punctae formation, STIM1 directly or indirectly interacts with Orai1 triggering CRAC channel activation. On the one hand it is proposed that the Orai1/STIM1 coupling is mediated by intermediate steps probably involving CIF.\textsuperscript{61} Nevertheless, this might be not required for the STIM1 C-terminal fragment that activates Orai1 currents independent of store-depletion. On the other hand, the C-terminus of STIM1 may directly interact with the C-terminus of Orai1 as shown by in vitro pulldown experiments, probably via coiled-coil interactions.\textsuperscript{51} Further auxiliary proteins including calmodulin (CaM) may also regulate/modulate STIM1-Orai1 coupling.
STIM1 homomeric interaction.57 Deletion of the SAM domain (aa 122–199)23 prevents punctae formation and CRAC activation. The C-terminus of STIM1 seems particularly important for Orai1/CRAC activation, as the cytosolic C-terminal strand alone is sufficient to stimulate both endogenous CRAC24 as well as Orai1 currents.30,51 This fragment shows predominantly targeting to the plasma-membrane when co-expressed with Orai151 in HEK293 cells, but it is incapable of punctuate clustering yet closely co-localises with Orai1.16,51 A STIM1 mutant lacking the C-terminus fails to co-localise with co-expressed Orai1 upon store depletion and is diffusely distributed throughout the ER membrane.29 Deletion of the coiled-coil domain results in much larger tubular-vesicular arrangement and this mutant lacks homo-oligomerisation and punctate formation upon store-depletion as well as CRAC activation.23,24 Additionally and this mutant lacks homo-oligomerisation and punctate formation upon store-depletion as well as CRAC activation.23,24

Furthermore, the C-terminus of STIM1 has been proposed to eliminate punctae upon store-depletion as well as CRAC activation.23,24 Deletion of the coiled-coil domain results in much larger tubular-vesicular arrangement and this mutant lacks homo-oligomerisation and punctate formation upon store-depletion as well as CRAC activation.23,24

Additionally disruption of the C-terminus after the ERM domain similarly impairs STIM1 redistribution and fails to activate both CRAC and Orai1.29 However, deletion of a glutamate-rich domain that overlaps with the first coiled-coil in the ERM domain, as well as the serine/proline-rich regions (aa 600–629) has been reported to be less essential for CRAC activation.24 Deletion of the lysine-rich domain at the very end of the C-terminus has been proposed to eliminate punctate formation despite preserved STIM1-STIM1 interaction. Thus the lysine-rich domain allows the separation of STIM1 homomeric interaction from punctae formation.49 Moreover, this mutant results in delayed stimulation when co-expressed with Orai1.29 Therefore both coiled-coil domain and lysine-rich region of STIM1 are essential for Orai1/CRAC activation.

The role of STIM2. Worms and flies only possess one STIM protein, while mammals include both STIM1 and STIM2.10,15 Both proteins are approximately 61% homologous and show ubiquitous tissue expression. Besides homologous STIM1-STIM1 interaction, STIM1 can form heteromers with STIM2.46,55 Co-expression of both STIM proteins favors translocation of STIM2 into punctae upon store depletion.46 Functionally Soboloff et al.46 have also revealed that STIM2 is a powerful SOC inhibitor when expressed in HEK293, PC12, A7r5 and Jurkat T cells.

In the absence of Ca2+, STIM2 does not similarly aggregate as STIM1.25 This functional difference is due to their distinct EF-hand Ca2+ affinity. While the EF-hand of STIM1 binds Ca2+ with low affinity in a range ideally adjusted to sense substantial changes in ER Ca2+ concentrations, the EF-hand of STIM2 is more sensitive to mild reduction of ER Ca2+.44 STIM2 already forms punctae with an EC50 at 406 μM ER Ca2+ that represents resting levels. Accordingly, STIM2 knock-down in Jurkat T-cells selectively lowers basal cytosolic and ER Ca2+ concentrations. Therefore STIM2 is partially active at resting ER Ca2+ levels,44 covering both store-dependent or -independent modes of Orai1 activation.38 A plausible model suggests that STIM2 is part of a feedback module that keeps basal cytosolic and ER Ca2+ concentrations within tight limits. STIM2 deficiency, however, imposes a smaller effect on Ca2+ influx than STIM1 knock-out.

STIM1 and Orai1 coupling. Following store-depletion labeled STIM1 proteins form clusters in close proximity to Orai1 leading to CRAC channel activation.29,47,48 However, it remains unresolved if the two proteins interact directly or if the signal cascade involves a third, intermediate component. While some laboratories report co-immunoprecipitation of STIM1 and Orai1,32,36 others have failed to detect an interaction.28 The distance between ER and PM estimated in a range between 10–25 nm45 is in principle small enough to allow direct STIM1/Orai1 interaction. Balla and coworkers have utilized chemically inducible bridges of different length to define the distance between plasma- and ER-membrane in an attempt to identify whether STIM1, Orai1 and STIM1/Orai1 assembles fit into distinct ER plasma-membrane space.58 Before store-depletion STIM1 localises in regions of a 4–6 nm ER-PM distance, while Orai1 requires distances in the range of about 11–14 nm. Upon store-depletion STIM1 moves into the larger regions and colocalizes with Orai1 suggesting that Orai1 channels are part of a larger macromolecular complex. An extended conformation of STIM1 probably might span the distance to one of the Orai1 strands. We and others demonstrated by FRET microscopy a distance closer than 10 nm between CFP/YFP-labels linked to STIM1 and Orai1 in store-depleted cells.51,59 Moreover a cytosolic STIM1 C-terminal fragment couples to Orai1 in in vitro as well as in vivo studies and accordingly activates constitutive Orai1 currents.51 Thus, at least in the case of STIM1 C-terminus we propose a rather direct than indirect interaction of these two proteins via their C-termini. STIM1 coupling to Orai1 as well as STIM1 homomerization is reversed when Ca2+ stores are refilled.10,51

Alternative to store-dependent direct coupling that may involve the putative coiled-coil domains of STIM1 and Orai1 as depicted in the model (Fig. 1C), a yet unidentified Ca2+ influx factor (CIF) might additionally be involved. STIM1 has been proposed to trigger the production of CIF,60 which could contribute to signal transmission from STIM1 to Orai1.61 CIF has been suggested to displace calmodulin (CaM) from phospholipase A2 (iPLA2), stimulating lysophospholipid generation that in turn activates CRAC.62 However, bromenolactone (BEL), an inhibitor of iPLA2, fails to prevent activation of heterologously co-expressed STIM1/Orai1 currents14 in contrast to its blocking effect onto endogenous CRAC in RBL cells and SOC in smooth muscle cells.63 Additionally Ca2+-dependent CaM binding has been detected within a peptide including residues 667–685 in STIM1 C-terminus.64 A CaM binding domain search [http://calcium.uchicago.edu/cdb/cf cdb/sequence.html] has revealed additional putative CaM binding sites both in the C-terminus of STIM1 as well as in the N-terminus of Orai1 (Fig. 1C). Since CIF has been proposed to displace CaM from PLA2, in the context with the STIM1/Orai1 system, it might be interesting to see whether CIF would also be able to displace CaM either from STIM1 or Orai1 as an additional way of modulation within the STIM1/Orai1 signaling cascade.

In recent studies, Orai1 domains that are involved in the coupling process to STIM1 and Ca2+ current activation have been identified. While the C-terminus of Orai1 is required for STIM1 coupling and subsequent current activation, Orai1 N-terminus is suggested to be essential for Orai1 gating.29,51 A highly conserved 26 amino acid long N-terminal region immediately before the first transmembrane domain is essential for channel activation.29 Moreover, the proline/arginine-rich domain in Orai1 N-terminus has a regulatory role as Orai2 chimeras containing the N-terminus of Orai1 have been reported to show enhanced Ca2+ entry.65 Orai1 C-terminal deletion mutants fail to colocalize with STIM1 clusters upon Ca2+ store-depletion29,51 and accordingly lack store-operated activation.51 Pull-down experiments provide evidence for an interaction of STIM1
C-terminus with the C-terminus rather than the N-terminus of Orai1 further underscoring the role of Orai1 C-terminus for coupling to STIM1. Especially a predicted putative coiled-coil domain within Orai1 C-terminus has been suggested as functional coupling domain, as a destabilizing single point mutation (L273S) eliminates coupling to STIM1 and Ca^{2+} current activation.\(^{51}\) Therefore, it is tempting to speculate that potential direct interaction of STIM1 and Orai1 is mainly mediated by their C-terminal coiled-coil domains—possibly together with additional auxiliary components (see Fig. 1C). Accordingly, a putative coiled-coil domain is also predicted within the C-terminus of Orai2 and Orai3, even with a 5–6 fold higher probability than that of Orai1. This may allow for a tighter or at least distinct coupling to STIM1 that has still to be evaluated.

**Channel Architecture of Orai Channels**

**Oligomerisation of Orai proteins.** Many ion channel families have been shown to require a multimeric assembly of various numbers of individual subunits.\(^{56}\) Coimmunoprecipitation\(^{28,36}\) studies have shown for Drosophila Orai as well as mammalian Orai1 that they assemble to dimers or higher order multimers. Moreover all combinations of heteromeric Orai channel assemblies are possible.\(^{28,30,33}\) A recent study\(^{67}\) has demonstrated that the functional CRAC channel pore is formed by a tetrameric assembly of Orai1 subunits. This evidence is based on maximal CRAC currents developed after expression of preassembled tandem Orai1 multimers comprising different numbers of subunits into cells stably overexpressing STIM1. Assembly of Orai1 channels is mainly mediated by their transmembrane domain\(^{29,51}\) as deletion of one of their cytosolic strands has not affected interaction of Orai1 channels. Additionally the N-terminus of Orai1 functions dominant negative on co-expressed Orai1 channels.\(^{65}\) However, discrete domains that are involved in the multimerisation remain so far elusive.

**Orai pore architecture.** Further evidence that Orai channels form the pore of CRAC channels is provided by point mutations in the putative selectivity filter of Orai1. The narrowest region of native CRAC, Orai1 or Orai3 pore is only -3.9 Å wide.\(^{37,68,69}\) Amino acids that form part of the selectivity filter in Orai1 include E106 in the first transmembrane domain, E190 in the third transmembrane domain and D110, D112 as well as D114 in the first loop domain.\(^{27,32,36}\) While the glutamates within the transmembrane domains are fully conserved in Orai2 and Orai3, the three aspartates (D110, D112, D114) in the loop domain of Orai1 are either glutamic acids or glutamines in Orai2 (E84, Q86, Q88) and Orai3 (E85, D87, E89). The glutamates and aspartates play an essential role for the Orai channel’s high Ca^{2+} selectivity, its Ca^{2+} block, regulation by Ca^{2+} and determine its pore geometry.\(^{36}\) Mutation of the respective glutamates in Orai1-E106 and -E190 in the first and third transmembrane region as well as corresponding E81 and E165 in Orai3, alters ion selectivity, which underlines that Orai1 is a pore subunit of the CRAC channel. Orai1-E106D\(^{36}\) and an analog Orai3-E81D\(^{37}\) show reduced Ca^{2+} selectivity in comparison to wild-type. In contrast Orai1-E106Q acts in a dominant negative manner on all three STIM1 mediated Orai Ca^{2+} currents\(^{33,36}\) as well as endogenous CRAC currents in T-cells.\(^{27,28}\) Yet this E106Q mutant is able to couple to STIM1.\(^{70}\) The mutation E190Q or E190A in the third transmembrane region of Orai1, as well as Orai3-E165Q leads to a leftward shift in the reversal potential resulting in less Ca^{2+} selective channels.\(^{28,37,71}\) The more conservative Orai1-E190D\(^{27}\) as well as Orai3-E165D\(^{70}\) mutations show similar pore characteristics like wild-type Orai channels in both a Ca^{2+} and a monovalent bath solution. The aspartates D110/112/114 in the first extracellular loop additionally contribute to the increased Ca^{2+} selectivity of Orai1 as their mutation to alanine (D110/112/114A) enhanced monovalent cation permeation.\(^{36,68}\) Structurally these mutations of the pore-relevant amino acids lower Ca^{2+} selectivity and result in a striking increase in Cs\({ }^{+}\) permeation, which suggests an enlargement of the unusually narrow pore of the CRAC channel, relieving steric hindrance for Cs\({ }^{+}\) permeation. In addition, the mutations diminish Ca^{2+}-mediated fast inactivation, a key mode of CRAC channel regulation.\(^{68}\) Pore mutants that modify Orai1 channel voltage selectivity include point mutation V102I as well as V105I close to the selectivity filter.\(^{72}\) In conclusion these studies reveal that structural elements contributing to ion permeation are located close to those involved in the voltage gating of Orai1 channels.

**Comparison of Orai1, Orai2 and Orai3.** The three highly homologous Orai1, Orai2 and Orai3 proteins are widely expressed at the mRNA level and display similar plasma-membrane expression.\(^{28,73}\) All three channels activate in a store-dependent manner when co-expressed with STIM1, while the extent of their current amplitudes is 2–3 fold smaller for Orai2 and Orai3 compared to Orai1. These reduced currents may reflect differences in their expression levels, efficiency in coupling to STIM1 and distinct single channel properties.\(^{35}\) Moreover all combinations of heteromeric Orai channel assemblies are possible.\(^{28,30,33}\) A recent study\(^{67}\) has demonstrated that the functional CRAC channel pore is formed by a tetrameric assembly of Orai1 subunits. This evidence is based on maximal CRAC currents developed after expression of preassembled tandem Orai1 multimers comprising different numbers of subunits into cells stably overexpressing STIM1. Assembly of Orai1 channels is mainly mediated by their transmembrane domains\(^{29,51}\) as deletion of one of their cytosolic strands has not affected interaction of Orai1 channels. Additionally the N-terminus of Orai1 functions dominant negative on co-expressed Orai1 channels.\(^{65}\) However, discrete domains that are involved in the multimerisation remain so far elusive.

**Physiological role of STIM and Orai channels.** CRAC channels are key mediators of sustained Ca^{2+} signaling in T-cells mediating...
The STIM/Orai coupling machinery

stable formation of immunological synapses. When a T-cell gets in contact with an antigen-presenting dendritic cell, both STIM1 and Orai1 redistribute to the immunological synapse, leading to Ca\(^{2+}\) influx.\(^{70}\) These elevated calcium levels induced by CRAC channels prolong dephosphorylation of NFAT (nuclear factor of activated T-cells) and accordingly its accumulation into the nucleus where it acts as a transcription factor in the early immune response of T-cells.\(^{75,76}\) Moreover long-term function of CRAC channel activation includes lymphocyte proliferation, effector functions as well as differentiation of naïve T-cells.\(^{77}\) The importance of Ca\(^{2+}\) influx through CRAC channels in T-cells is further highlighted by the existence of at least three families of patients with SCID resulting in severe defects in function of store-operated Ca\(^{2+}\) entry, impaired cytokine expression and lymphocyte function.\(^{17,77,78}\) In addition CRAC channels play an important role in mast cell activation, degranulation as well as secretion of proinflammatory lipid mediators.\(^{79,80}\)

Knock-down of either STIM or Orai proteins has been shown to impair SOCE/CRAC entry in a variety of cell types.\(^{10,15-18,21,31,46,77,79-82}\) STIM1 as well as STIM2 are detectable in various primary lymphocytes such as T\(_p\), T\(_C\), and B-cells.\(^{83}\) Furthermore both are expressed at a low, uniform level in heart, brain, kidney, thymus, lung, spleen, skeletal muscle, small intestine as well as in primary aortic endothelial cells.\(^{83,84}\) High expression levels of STIM1 are found in certain cell types and cellular regions such as the central and the peripheral nervous systems as well as platelets.\(^{85}\) Northern blot analysis of heart, brain, kidney, thymus, lung, spleen, skeletal muscle, small intestine, placenta, primary aortic endothelial and mast cells suggests an ubiquitous expression of Orai1 and Orai3 proteins, while Orai2 is predominantly expressed in brain and to a lower extent in the lung, spleen and small intestine.\(^{28,73,84}\) Moreover all three Orai proteins are expressed in primary lymphocytes. With the exception of a lower expression of Orai3 in skeletal muscle and placenta, it seems to be expressed in the same tissues as Orai1.

In mice the regulatory role of STIM as well as Orai proteins has been recently examined in particular by knock-out and knock-down studies. Mouse T-cells and fibroblasts lacking STIM1 (either by knock-out or siRNA) exhibit impaired SOCE, while it was only moderately diminished in STIM2-deficient T-cells.\(^{77}\) STIM1, as well as STIM2-knock-out T-cells, show less cytokine production, nuclear translocation of NFAT and additionally the number of regulatory T-cells is decreased. Thus, these STIM proteins are required as a prerequisite for T-cell development.\(^{77,79}\)

Moreover STIM1 has been identified as critical to mast cell function, since STIM1-deficient mast cells of mice display much less degranulation and cytokine production after FcεRI stimulation. Hence, STIM1 acts as a key for mast cell activation and anaphylaxis.\(^{79}\) Besides the requirement of STIM1 in non-excitable cells, there is further evidence that STIM1 has a regulatory role in excitable cells, such as skeletal muscle and myotubes. STIM1 has been demonstrated to be expressed in both tissues. Mice lacking STIM1 die perinatally from a skeletal myopathy and their myotubes fail to show SOC and fatigue rapidly.\(^{85}\) Further STIM1 is abundantly expressed in platelets. STIM1-deficient platelets display a marked defect in agonist-induced Ca\(^{2+}\) response, impaired activation and thrombus formation under flow suggesting STIM1 as an important mediator of ischemic cardio- and cerebrovascular events.\(^{86}\) A mouse cell line expressing a constitutively CRAC current activating EF-hand mutant of STIM1 revealed macrothrombocytopenia and a bleeding disorder. A preactivation state of platelets has been observed as a result of an increase in basal intracellular calcium levels leading to platelet consumption.\(^{87}\) Hence, STIM1 displays an essential role in the regulation and function of platelets.

In hepatocytes bile acids lead to punctae formation of STIM1 and activation of Ca\(^{2+}\)-selective SOC currents. Further knock-down of STIM1 has caused inhibited calcium entry activated by bile acids.\(^{88}\)

Human T-cells that contain a point mutation in Orai1 R91W, which has recently been identified in SCID patients result in defective T-cell signalling.\(^{11,17,89}\) It causes a complete loss of store-operated Ca\(^{2+}\) entry into T-cells, however, the detailed mechanism for this impairment of Ca\(^{2+}\) flux across Orai1 R91W is so far elusive. In contrast to human T-cells that require Orai1 for CRAC channel formation, mice may utilize Orai2, as their thymocytes and T-cells express much more Orai2 than Orai1 or Orai3. Orai1 plays a major role in mice mast cells, as mice deficient in Orai1 exhibit defective mast cell degranulation and cytokine secretion, lack allergic reactions and are smaller in size.\(^{78,80}\)

In contrast, store-operated calcium influx of naïve T cells, their development and proliferation are unaffected in Orai1-deficient mice.\(^{78,80}\) However, differentiated T-cells have been identified to express higher amounts of Orai1 than of Orai2 and Orai3 consistent with a severe but incomplete loss of store-operated Ca\(^{2+}\) entry. B-cells express besides Orai1 and Orai2, Orai3 transcripts at highest amounts among all lymphocytes. Orai1 deficient splenic B-cells exhibit also a decrease in store-operated Ca\(^{2+}\) entry. Moreover, Orai1-/- mice suffer from eyelid irritation as well as sporadic hair loss and display much thinner skin with fewer, more elongated keratinocytes.\(^{78}\)

Conclusions and perspectives. It has taken 20 years of exhaustive studies on store-operated calcium entry before Orai1 and STIM1 could be identified as the major components of I\(_{\text{CRAC}}\) in human T-cells. SOCs have been intensively examined over these past years, revealing an involvement in a wide range of cellular processes together with their pharmacological, physiological and pathophysiological roles.\(^{9}\) In the focus of autoimmune and inflammatory immune disorders an involvement of dysregulated Ca\(^{2+}\) signaling is out of question. The recent identification of STIM1 and Orai1-3 and their role in calcium signaling through SOCR/CRAC channels opens the repertoire for targeting autoimmune diseases, e.g., rheumatoid arthritis, inflammatory disorders or allograft rejection.\(^{14}\) Orai1 seems to be of primary interest as in SCID patients the point mutation Orai1 R91W abolishes CRAC currents only in T-cells, whereas currents from B-cells and fibroblasts are only reduced.\(^{17}\) Yet there are no specific inhibitors of the STIM1/Orai1 pathway available to test the effect of blocking this pathway in vivo.\(^{14}\) With the discovery of STIM1 and Orai1 as the key components of CRAC channels, a solid basis is reached to study their molecular composition, stoichiometry and activation mechanism. STIM1 as well as Orai1 have been shown to interact with members of the canonical transient receptor potential channels, a family of phospholipase C regulated channels.\(^{24,53,90-95}\) Further studies are required to reveal the nature of SOCE channels in various tissues, possibly manifested by a combination of various Orai and TRP channels. This will clarify the contribution of STIM1 and Orai1 to SOCE in different tissues and will provide a more widespread view of store-operated channels and their physiological roles in health and disease.
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