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CRAC channelopathies

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Abstract

Store-operated Ca^{2+} entry (SOCE) is an important Ca^{2+} influx pathway in many non-excitable and some excitable cells. It is regulated by the filling state of intracellular Ca^{2+} stores, notably the endoplasmic reticulum (ER). Reduction in $[\text{Ca}^{2+}]_{\text{ER}}$ results in activation of plasma membrane Ca^{2+} channels that mediate sustained Ca^{2+} influx which is required for many cell functions as well as refilling of Ca^{2+} stores. The Ca^{2+} release activated Ca^{2+} (CRAC) channel is the best characterized SOC channel with well-defined electrophysiological properties. In recent years, the molecular components of the CRAC channel, long mysterious, have been defined. ORAI1 (or CRACM1) acts as the pore-forming subunit of the CRAC channel in the plasma membrane. Stromal interaction molecule (STIM) 1 is localized in the ER, senses $[\text{Ca}^{2+}]_{\text{ER}}$, and activates the CRAC channel upon store depletion by binding to ORAI1. Both proteins are widely expressed in many tissues in both human and mouse consistent with the widespread prevalence of SOCE and CRAC channel currents in many cell types. CRAC channelopathies in human patients with mutations in *STIM1* and *ORAI1* are characterized by abolished CRAC channel currents, lack of SOCE and—clinically—immunodeficiency, congenital myopathy, and anhydrotic ectodermal dysplasia. This article reviews the role of ORAI and STIM proteins for SOCE and CRAC channel function in a variety of cell types and tissues and compares the phenotypes of ORAI1 and STIM1-deficient human patients and mice with targeted deletion of *Orai* and *Stim* genes.

Keywords

ORAI1; STIM1; CRAC; SOCE; Store-operated calcium entry; Ca^{2+} ; T cells; B cells; Mast cells; Lymphocytes; Immunodeficiency; SCID; Signal transduction; Myopathy; Muscle; Ectodermal dysplasia; Amelogenesis; Platelets; Gene-targeting; Mice

Store-operated Ca^{2+} entry (SOCE) and CRAC channels

Initially known under the name “capacitative Ca^{2+} entry”, this Ca^{2+} influx pathway is now referred to mostly as store-operated Ca^{2+} entry (SOCE) because depletion of Ca^{2+} stores triggers the activation of plasma membrane Ca^{2+} channels resulting in an increase in $[\text{Ca}^{2+}]_{\text{i}}$ and refilling of endoplasmic reticulum (ER) stores [117]. SOCE has been observed in a variety of non-excitable and excitable cells including lymphocytes (reviewed in [36]), pancreatic acinar [86], vascular endothelial [1], and smooth muscle cells [4,83,112] (reviewed in [102]).

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Conflicts of interest The author is a scientific co-founder and advisor of CalciMedica, a biotechnology company that seeks to develop CRAC channel inhibitors.

SOCE is initiated by agonist binding to different types of cell surface receptors including tyrosine kinase and G protein coupled receptors that mediate activation of phospholipase C resulting in the production of inositol 1,4,5-triphosphate (InsP₃). InsP₃ binds to the InsP₃ receptor in the ER membrane resulting in the release of Ca²⁺ from ER stores. In vitro, passive depletion of intracellular Ca²⁺ stores is often used to initiate SOCE by preventing Ca²⁺ re-uptake into the ER using sarcoplasmic/endoplasmic reticulum ATPase (SERCA) pump inhibitors such as thapsigargin or cyclopiazonic acid or by facilitating Ca²⁺ efflux from the ER with Ca²⁺ ionophores.

The prototypical store-operated Ca²⁺ channel is the Ca²⁺ release activated Ca²⁺ (CRAC) channel, first characterized in greater detail in mast cells and T cells of the immune system by whole cell patch clamp recordings [53,162]. Its activity has since been identified in many other cells types as well (reviewed in [101,102]. CRAC channel currents are defined by a set of characteristic features which include an extremely high selectivity for Ca²⁺ over monovalent cations, low single-channel conductance (<1 pS), lack of significant voltage-dependent gating, inwardly rectifying current–voltage relationship, rapid Ca²⁺-dependent inactivation, blockade by submicromolar La³⁺, and modulation by 2-Aminoethoxydiphenyl borate (2-APB; reviewed in [102,113]). The molecular nature of the CRAC channel has long been a mystery, and many candidate genes have been proposed, most prominently members of the large family of transient receptor potential (TRP) channels. TRPV6 (CaT1) for instance was shown to share a number of properties such as Ca²⁺ selectivity and the sequence of divalent permeability with the CRAC channel [145,158] and was briefly considered a part of the CRAC channel complex. Other properties of TRPV6, particularly the fact that it is constitutively open and not store-operated, however, do not match those of the CRAC channel; in addition, RNAi knockdown of TRPV6 had no effect on I_{CRAC} and SOCE in mast cells [62]. Other TRP channels such as TRPC1, TRPC3, and TRPC6 were reported to be activated by store depletion under certain conditions (reviewed in [102]), although they are generally considered to be store-independent [25] and activated by second messengers generated by phospholipase C activity such as diacylglycerol [140]. Furthermore, TRPC channels are nonselective Ca²⁺ permeable ion channels, a fact that is hard to reconcile with the strong Ca²⁺ selectivity of the CRAC channel. While therefore unlikely to be involved in CRAC channel function, TRPC proteins may contribute to SOCE in some tissues as will be discussed further below. In the past years, research on CRAC channels and SOCE has focused on a new family of molecules, the recently discovered ORAI proteins. These represent tetraspanning plasma membrane proteins unrelated to other ion channels. The role of ORAI proteins for SOCE, CRAC channel function, and CRAC channelopathies will be discussed in detail in the following sections.

Equally mysterious as the molecular nature of the CRAC channel remained the gating mechanism of store-operated Ca²⁺ channels. In the past, a number of mechanisms have been proposed postulating conformational coupling, a soluble calcium influx factor [14] and secretion/fusion of channel-containing vesicles with the plasma membrane (reviewed in [102]). Dramatically new and significant insight has been gained with the discovery of stromal interaction molecule (STIM) 1 as an essential regulator of SOCE in a development paralleling that of the discovery of ORAI1 as the CRAC channel [73,121]. STIM1 controls SOCE by sensing [Ca²⁺]_{ER} and by translocating to the plasma membrane where it activates ORAI1 CRAC channels [70] as well as TRPC channels [156].

This review will focus on the consequences of impaired SOCE and CRAC channel function due to mutations in *ORAI1* and *STIM1* in human patients and gene-targeting of *Orail* and *Stim1* in mice. The phenotypes associated with ORAI1 and STIM1 deficiency (here collectively termed CRAC channelopathies) will be described. In addition, the review will discuss some of the important roles of SOCE in other tissues that are emerging from molecular and genetic studies of ORAI1 and STIM1.

ORAI1 and STIM1 as essential mediators of CRAC channel function

The field of SOCE and CRAC channel research has seen a sea change with the discovery of STIM1 and ORAI1 in 2005 and 2006, respectively [40,73,121,143,160]. Both molecules have since been investigated in great detail on the molecular level for their role in CRAC channel function and in vivo as essential components of SOCE in a variety of tissues (for detailed reviews on ORAI and STIM1, see [118,119] and other articles in this special issue of *Immunological Reviews*).

ORAI1

ORAI1 (also called CRACM1, TMEM142a) is the founding member of a new class of ion channels that is structurally unrelated to other calcium channels except its paralogues ORAI2 (or CRACM2, TMEM142b) and ORAI3 (CRACM3, TMEM142c). ORAI1 was identified as olf186-F in RNAi screens in *Drosophila* S2 cells for regulators of Ca^{2+} signaling and NFAT activation [40,143,160] and as hypothetical protein FLJ14466 by positional cloning as the gene mutated in immunodeficient patients with defects in CRAC channel function [40]. The human and *Drosophila* genes were renamed *ORAI1* and *dOrai*, respectively, after the Orai (hours)—the keepers of heaven's gate—in Greek mythology [131]. The human *ORAI1* gene consists of only two exons and encodes for a highly conserved 301 amino acid protein with a tetraspanning plasma membrane topology and intracellular N- and C-termini. A negatively charged glutamate residue, E106, in the first transmembrane domain of ORAI1 was identified by several labs as a putative Ca^{2+} binding site in the ion channel pore because charge-neutral substitution of E with D interfered with the high Ca^{2+} selectivity of the CRAC channel ($P_{\text{Ca}}/P_{\text{Na}} \approx 1,000$) without affecting the channels' expression or gating properties [114,141,153,155].

The composition stoichiometry of the CRAC channel was proposed to be a tetramer of four ORAI1 subunits based on fluorescence photobleaching experiments [60,108], biochemical analysis [108], and a study using concatenated multimers of wild-type and mutant ORAI1 [85]. In a variation of the tetramer model, Penna et al. found that ORAI1 exists predominantly as a dimer in the plasma membrane under resting conditions but forms tetramers after store depletion [108]. It is assumed that in the functional multimeric channel complex, each ORAI1 subunit contributes a glutamate residue (E106) for coordinated Ca^{2+} binding in the CRAC channel pore. Indeed, recent evidence from cysteine scanning mutagenesis suggests that the first transmembrane alpha-helix of ORAI1 containing E106 lines the CRAC channel pore [84].

Research into the properties of ORAI1 CRAC channels has been facilitated greatly by the fact that ectopic expression of ORAI1 together with STIM1 results in Ca^{2+} currents with properties almost identical to I_{CRAC} but with amplitudes ~tenfold to 100-fold larger than native CRAC currents recorded in Jurkat T cells or and HEK293 cells [107,127], depending on absolute expression levels and the ratio of ORAI1/STIM1 expression [126]. Co-expression with STIM1 has also enabled studying the function of ORAI2 and ORAI3, which—like ORAI1—are tetraspanning membrane proteins. Both form Ca^{2+} channels when ectopically expressed together with STIM1 and show electrophysiological properties similar to native I_{CRAC} and the large CRAC channel currents observed following ORAI1/STIM1 over-expression [26,74]. They differ, however, in their activation and inactivation kinetics, monovalent permeation, and responses to 2-APB, which at 50 μM inhibits native I_{CRAC} and ORAI1/STIM1-mediated Ca^{2+} currents but potentiates ORAI3/STIM1 currents [74]. Whether these differences are due to intrinsic properties of ORAI1, ORAI2, and ORAI3 channels or features resulting from ectopic expression of these genes remains unclear at this point, as the properties of endogenous ORAI2 and ORAI3 have not yet been defined. Information on the contribution of ORAI2 and ORAI3 to SOCE in vivo is likewise missing despite their apparent expression in multiple tissues and organs [37,49]. Of note is the inability of ORAI2 to restore SOCE in cells lacking ORAI1, whereas ORAI3 expression resulted in partial reconstitution [49,82].

Stromal interaction molecule (STIM) 1

STIM1 is essential for SOCE by functioning both as the sensor of $[Ca^{2+}]_{ER}$ and activator of CRAC channels in the plasma membrane. STIM1 was initially identified as a putative tumor suppressor named GOK, which is highly expressed in skeletal muscle but absent in rhabdomyosarcoma where its expression induces cell death [104,123], and as SIM, a protein that promotes proliferation of pre-B cells [97]. STIM1—and its paralogue STIM2—was characterized as a phosphorylated transmembrane protein containing multiple protein domains by Dziadek et al. (Fig. 1), but a connection between STIM1 and SOCE was not made at the time [81,147]. STIM1 was identified as an essential regulator of SOCE in two limited RNAi screens for modulators of Ca^{2+} signaling in which depletion of STIM1 robustly decreased SOCE [73,121].

STIM1 is a single-pass transmembrane protein that is localized predominantly in the ER membrane, although expression in the plasma membrane has also been reported [81,128,148]. Protein domains in STIM1 include a pair of ER luminal low-affinity EF hand calcium-binding domains required for sensing $[Ca^{2+}]_{ER}$, a sterile alpha motif (SAM), two coiled-coil (CC) domains, as well as serine/proline and lysine-rich regions at the C terminus (Fig. 1) [147]. Depletion of Ca^{2+} from the ER results in dissociation of Ca^{2+} from the EFh domains, unfolding of the adjacent EF-SAM domains, and multimerization of STIM1 (Fig. 2) [20,129,130]. Multimerization is a prerequisite for the subsequent translocation of STIM1 to ER-plasma membrane junctions where it accumulates and forms large clusters conventionally called puncta [72,77]. ORAI1—recruited into these clusters in a STIM1-dependent manner—mediates localized Ca^{2+} influx at sites of puncta formation [72,78,151]. Multimerization, puncta formation, and activation of SOCE can be initiated also by mutating the Ca^{2+} binding aspartate or glutamate residues in the EFh domain of STIM1 [73] or by functionally replacing the N terminus of STIM1 with an artificial inducible protein–protein binding domain [77]. STIM1 is likely to directly bind to ORAI1 based on biochemical and fluorescence resonance energy transfer experiments [21,88,90,155].

Under physiological conditions, STIM1 activates SOCE following ER Ca^{2+} store depletion. Intriguingly, expression of the C terminus of STIM1 alone also resulted in CRAC channel activation in the absence of puncta formation [56] suggesting that the non-ER restricted soluble C terminus of STIM1 can multimerize, translocate to the plasma membrane, and bind to and activate CRAC channels. A ~99–108 aa fragment of the STIM1 C terminus, alternatively termed CRAC channel activation domain (CAD) [103], STIM1 Orai activating region (SOAR) [157], Orai1 activating small fragment (OASF) [87], or coiled-coil fragment b9 (CCb9) [63], encompassing the second coiled-domain is sufficient to bind to ORAI1 and activate it. The CRAC channel activating fragment of STIM1 was shown to bind to ORAI1 involving an N-terminal domain just proximal of the first transmembrane alpha-helix of ORAI1 and a C-terminal CC domain (Fig. 1) [63,103,157] (reviewed in [34]). The CRAC activating STIM1 domain is flanked by a ~40 aa inhibitory domain that restricts binding of the activation domain to ORAI1 [63,87] and is required for Ca^{2+} -dependent fast inactivation of CRAC currents following channel activation and local Ca^{2+} influx [28,69,89]. Within the STIM1 inhibitory domain, a stretch of negatively charged glutamate and aspartate residues (475-DDVDDMDEE-483) is critical for fast inactivation [28,69,89] in conjunction with calmodulin binding to the N terminus of ORAI1 (Fig. 1) [89]. From the currently available data, it can be hypothesized that under resting conditions (i.e., replete Ca^{2+} stores), binding of the STIM1 activation domain to ORAI1 is restricted by the STIM1 inhibitory domain. Upon store depletion and dissociation of Ca^{2+} from the N-terminal EFh domain, STIM1 multimerization, and putative conformational changes in the C terminus of STIM1, the inhibitory domain is released allowing binding of the CRAC channel activation domain of STIM1 to ORAI1 (Fig. 2).

It is of note that STIM1 is involved not only in the regulation of ORAI1 CRAC channels but also TRP channels, as it was shown to bind to and activate TRPC1 and other Ca^{2+} permeable TRPC family members [56,71,100,159]. The cytoplasmic C terminus of STIM1 and a polybasic domain at its very end are required for activation of TRPC1 and TRPC3 [56,159]. STIM1 binding to TRP channels and its role in recruiting TRPC into lipid rafts and STIM1 puncta [5,100] may provide an explanation for the long reported but controversial [25] role of TRPC channels as store-operated Ca^{2+} channels.

While most studies exploring SOCE and CRAC channel function have focused on STIM1, it is of note that STIM2—a closely related paralogue of STIM1—shares its overall protein domain architecture and is able to hetero-multimerize with STIM1 [147]. STIM2 forms homomultimers and puncta in the absence of STIM1 and activates ORAI1 CRAC channels in the plasma membrane [15]. Interestingly, STIM2 is activated upon smaller decreases in ER Ca^{2+} concentrations than STIM1 and has been proposed to regulate basal cytosolic Ca^{2+} concentrations [15]. Lack of STIM2 expression in T cells from gene-targeted mice does not initially impair SOCE or I_{CRAC} in the first minutes after store depletion but interferes with sustained Ca^{2+} influx and activation of the transcription factor NFAT resulting in impaired cytokine gene expression [95]. STIM2 therefore is a positive regulator of SOCE and has partially overlapping functions with STIM1.

CRAC channelopathies in ORAI1 and STIM1-deficient human patients and mice

The role of SOCE and CRAC channel function in vivo is illustrated by the phenotypes of human patients lacking expression of functional ORAI1 or STIM1 and mice with targeted deletion of *Orai1*, *Stim1*, and *Stim2* genes. While extremely rare, human ORAI1 and STIM1-deficient patients offer important insight into the function of both genes in vivo.

ORAI1-deficient human patients

Patients from three unrelated families suffering from immunodeficiency due to mutations in ORAI1 have been reported [39,40,42,68,82,105]. In the first family, a defect in store-operated Ca^{2+} influx and CRAC channel function was observed in infants suffering from a rare form of immunodeficiency due to strongly impaired T cell activation but normal T cell development. SOCE was undetectable in T cell lines established from the patients in response to T cell receptor stimulation or passive store depletion with ionomycin or the SERCA inhibitor thapsigargin [39]. The defect in SOCE was not limited to T cells but was also observed in (EBV-transformed) B cells and fibroblasts of the patients. Ca^{2+} release from intracellular stores was normal in all patient cell types investigated suggesting that the defect is downstream of store depletion and either in the CRAC channel itself or mechanisms influencing Ca^{2+} influx such as K^{+} channels responsible for a negative membrane potential and providing the driving force for Ca^{2+} influx. Patch clamp measurements of patients' T cells, however, failed to detect CRAC channel activity at negative membrane potentials under whole cell recording conditions [42]. I_{CRAC} was absent in both Ca^{2+} containing and divalent free extracellular bath solution, indicating that both Ca^{2+} and Na^{+} conductance through the CRAC channel is abolished in immunodeficient patients' T cells [42]. The CRAC channel defect severely compromises T cell activation resulting in impaired T cell proliferation, cytokine production, and global gene expression [39].

Positional cloning in the patients' family led to—combined with a genome-wide RNAi screen in *Drosophila* S2 cells—the identification of ORAI1 as a CRAC channel subunit and a mutation in *ORAI1* as the cause for immunodeficiency [40]. Patients are homozygous for a missense mutation in exon1 of *ORAI1* that results in substitution of a highly conserved arginine residue

with tryptophan at position 91 (R91W) of ORAI1 protein (Fig. 1A) [40]. Located at the beginning of the first transmembrane domain of ORAI1, the R91W mutation allows for normal ORAI1 expression in the plasmamembrane but abolishes CRAC channel function and SOCE (Fig. 3B) [40]. Expression of wild-type ORAI1 in patients' T cells restores SOCE and I_{CRAC} with properties indistinguishable from native I_{CRAC} in primary wild-type control T cells confirming that ORAI1 is an essential component of the CRAC channel [40]. It is of note that heterozygous carriers of the R91W mutation showed reduced SOCE only at lower than physiological $[Ca^{2+}]_o$ (0.2–0.5 mM), whereas SOCE at 2 mM $[Ca^{2+}]_o$ was all but normal potentially explaining why heterozygous carriers do not suffer from immunodeficiency disease [39,40]. The mechanism by which the R91W mutation interferes with CRAC channel function is not entirely clear. Mutant ORAI1-R91W is expressed and can interact with STIM1 [90]. The mutation is likely to interfere with opening of ORAI1 CRAC channels by stabilizing the closed channel configuration since substitution of R91 with hydrophobic but not charged or neutral amino acid residues was shown to effectively abolish CRAC channel function (S. Feske unpublished) [27].

Defects in SOCE have been reported previously in patients from two additional, unrelated families who also suffered from severe immunodeficiency in early infancy [68,105]. In a second family, SOCE and CRAC channel function were absent in T cells isolated from peripheral blood of one patient. A complete defect in SOCE and I_{CRAC} was observed independent of the stimulus used. Neither active store depletion by crosslinking the TCR with anti-CD3 antibodies or including InsP3 in the patch pipette nor passive depletion with thapsigargin or ionomycin was able to evoke I_{CRAC} in the patient's T cells [105]. By contrast, release of Ca^{2+} from internal stores recorded in the absence of external Ca^{2+} was normal. The gene defect in these patients remained unresolved due to the unknown genetic identity of the CRAC channel at the time. We have now identified an insertion mutation in ORAI1 in these patients that causes a frame shift beginning at amino acid residue A88 and premature termination of ORAI1 at position 112 (*ORAI1-A88EfsX25*) at the end of the first transmembrane domain (Figs. 1A and 3C) [82]. The mutation results in abolished ORAI1 mRNA and protein expression consistent with absent CRAC channel function. It is of note that I_{CRAC} was abolished in both freshly isolated T cells from this patient lacking ORAI1 expression and T cell lines from patients with ORAI1-R91W mutation [40], indicating that ORAI1 is the predominant CRAC channel subunit in both naive human T cells and antigen-experienced human T cell lines.

A lack of SOCE was also reported in a patient from a third family unrelated to those described above [68]. Ca^{2+} influx in T cells from this patient was severely impaired following TCR crosslinking and passive depletion of Ca^{2+} stores with thapsigargin even under conditions when the membrane potential was clamped to -60 mV to exclude a defect in the T cells' membrane potential. In addition to T cells, SOCE was abolished in other hematopoietic cell lineages such as B cells, neutrophils, and platelets as well as fibroblasts. We have now identified two missense mutations in exon 2 of *ORAI1* in this patient that lead to the substitution of an alanine with glutamate (A103E) and a leucine with proline (L194P) in the first and third transmembrane domains of ORAI1, respectively (Figs. 1A and 3C) [82]. Both mutations interfere with stable protein expression, as no endogenous ORAI1 protein was detected in the patient's fibroblasts and in HEK293 cells ectopically expressing the ORAI1 mutants [82]. While the patient is compound heterozygous for both mutations and lacks ORAI1 expression completely, his parents, both healthy, are heterozygous carriers and have normal SOCE even at low $[Ca^{2+}]_o$ (0.2 mM) [82] indicating that monoallelic expression of ORAI1 is sufficient to sustain normal SOCE.

In summary, mutations in ORAI1 from three unrelated families have been reported that abolish ORAI1 expression or function and as a consequence CRAC channel activity. The clinical phenotype associated with ORAI1 deficiency will be discussed below.

STIM1-deficient human patients

SOCE is severely impaired in cells from patients of a fourth family who suffer from combined immunodeficiency. Store depletion with thapsigargin failed to induce Ca^{2+} influx even in the presence of 20 mM $[\text{Ca}^{2+}]_o$ and despite normal release of Ca^{2+} from ER stores. In contrast to the patients described above, no mutations in ORAI1 (or ORAI2 and ORAI3) were found. Instead, mRNA and protein expression levels of STIM1 were strongly reduced or undetectable in the patients' cells consistent with the observed pronounced defect in SOCE (Fig. 3D) [110]. Patients from this family are homozygous for an insertion mutation in *STIM1* that results in a frameshift starting at position E128 of STIM1 protein and its premature termination at position 136 (E128RfsX9) (Fig. 1B) [110]. SOCE in the patient's cells could be restored by expression of STIM1; interestingly, ectopic expression of STIM2 partially rescued SOCE suggesting that both genes have overlapping functions. Endogenous expression levels of STIM2, however, were not sufficient to compensate for the lack of STIM1 and prevent immunodeficiency. The clinical phenotype associated with STIM1 deficiency in human patients, as will be discussed in more detail below, is very similar to that of ORAI1 deficiency, suggesting that both genes act in the same pathway and are critical for SOCE in the same tissues.

Orai1- and Stim1-deficient mice

Additional and important insight in the role of ORAI and STIM family proteins in vivo comes from ORAI1, STIM1, and STIM2-deficient mice that have been generated by several labs using homologous recombination or insertional mutagenesis approaches for gene-targeting [7,17,50,95,132,138,142]. While some tissue-specific phenotypes will be discussed in the next sections, the most notable difference between human ORAI1- and STIM1-deficient patients and their animal counterparts is the severe perinatal lethality observed in mice (Table 1). Human patients lacking ORAI1 or STIM1 succumb to immunodeficiency in their first years of life, whereas ORAI1 and STIM1-deficient mice die perinatally of unknown causes [7,17,50,95,132,138,142]. Similar severe neonatal lethality was observed in ORAI1 knock-in mice expressing a nonfunctional ORAI1-R93W protein (S Feske unpublished) [11]. The penetrance of the perinatal lethality seems to depend on the genetic background as intercrossing STIM1 and ORAI1-deficient mice generated on the C57Bl/6 background to the outbred ICR strain partially rescued survival [50,95]; about one third of *Orai1*^{-/-} mice survived for more than 90 days showing a ~25–30% reduction in body-weight [50]. Partial survival was also observed in STIM1 and ORAI1-deficient mice generated by insertional mutagenesis on a mixed genetic background, with ~60% of ORAI1-deficient mice dying shortly after birth and the remaining pups being severely runted and dying ~4 weeks post partum [17]. The cause of death in STIM1 and ORAI1-deficient mice remains unknown but has been suggested to be due to defects in skeletal muscle differentiation and/or function [132] and respiratory failure [7].

Immune dysfunction in ORAI1 and STIM1 deficiency

Immunodeficiency in both ORAI1- and STIM1-deficient patients is characterized by recurrent severe infections with viral, bacterial, mycobacterial, and fungal pathogens resulting in repeated episodes of, for instance, pneumonia, meningitis, and gastroenteritis (Table 1) [41,68,82,105,110] (reviewed in more detail in [37]). These infections limit the survival of SOCE-deficient patients beginning a few months after birth and necessitating therapy by hematopoietic stem cell transplantation (HSCT). The severity and spectrum of infections as well as the failure to thrive observed in the patients resembles that observed in patients with

severe combined immunodeficiency which is caused by a lack or severe reduction in T, B, or NK cell numbers. While CD4⁺ and CD8⁺ T cells counts were normal in all ORAI1- and STIM1-deficient patients, their function was severely impaired with defects in T cell proliferation and cytokine production reported in vitro and absent skin delayed-type hypersensitivity reactions in vivo [41,68,82,105,110,125] (reviewed in [37]). The prognosis of patients lacking functional ORAI1 or STIM1 is poor unless treated by HSCT. Two of six ORAI1-deficient patients (one ORAI1-R91W and one ORAI1-A103E/L194P patient) and one of three STIM1-deficient patients were treated successfully by HSCT. The remaining four patients with mutations in ORAI1 died of infections in their first year of life despite treatment with antibiotics and intravenous immunoglobulins [68,82,105]; two patients with STIM1 mutations died at 1.5 and 9 years from infections and complications of HSCT [110].

In addition to immunodeficiency, all STIM1 and one of the ORAI1-deficient patients showed signs of *lymphoproliferative disease and autoimmunity* [82,110]. Lymphoproliferation in patients was characterized by lymphadenopathy and splenomegaly. Autoimmunity in STIM1-deficient patients manifested through autoimmune thrombocytopenia and hemolytic anemia [110] and autoimmune neutropenia and thrombocytopenia in one patient lacking ORAI1 expression [82]. An important cause of autoimmunity in patients lacking STIM1 is the reduced number of CD4⁺ Foxp3⁺ regulatory T cells (T_{reg}). This population of T cells is required for suppression of autoreactive T cells, maintenance of immunological tolerance, and prevention of autoimmunity. A similar lymphoproliferative phenotype is observed in mice lacking STIM1 [13] or both STIM1 and STIM2 expression (*Stim1^{fl/fl} Stim2^{fl/fl} CD4-Cre*) [95]. Similar to a patient lacking STIM1 expression, STIM1/STIM2 double-deficient mice have severely reduced numbers of T_{reg} cells which, in addition, display a defect in suppressive function [95] (for a more detailed discussion, see [37,94]).

ORAI1 and STIM1-deficient mice show compromised immune functions in vitro and in vivo similar to patients with mutations in ORAI1 and STIM1, although mice do not succumb to immunodeficiency when kept under pathogen free conditions (Table 1) [7,17,50,95,142] (reviewed in more detail in [37,94]). Because of the perinatal lethality associated with lack of ORAI1 or STIM1 in mice, immune function was studied either in conditionally gene-targeted mice with T cell specific deletion (*Stim1^{fl/fl} CD4-Cre*) [95], in mice on mixed genetic backgrounds [50,142] or in fetal liver chimeric mice [17]. SOCE and CRAC channel function were found to be severely impaired in CD4⁺ and CD8⁺ T cells, B cells, mast cells, and macrophages isolated from most of the mouse strains investigated with the exception of normal SOCE in ORAI1-deficient mice in one study [142]. As a consequence, expression of cytokines interleukin (IL)-2, interferon- γ , IL-4, and IL-10 was substantially reduced in T cells from *Orai1^{-/-}* and *Stim1^{-/-}* mice [50,95] similar to the multiple cytokine expression defect found in human patients with ORAI1-R91W mutation [38]. Mast cells from ORAI1- and STIM1-deficient mice showed impaired synthesis of tumor necrosis factor- α and IL-6 along with decreased serotonin and leukotriene C₄ release consistent with attenuated passive cutaneous anaphylaxis in vivo [7,142]. While B cells from *Orai1^{-/-}* mice proliferated poorly in response to BCR stimulation [50], in vitro proliferation of T cells isolated from *Orai1^{-/-}* and *Stim1^{-/-}* mice was normal [13,50,142]. T cell-dependent antibody responses in *Stim1^{-/-}* mice and the ability of T cells from these mice to induce GvHD were normal or modestly impaired compared to wild-type controls [13], suggesting that some but not all T cell functions are attenuated in the absence of ORAI1 and STIM1 in mice. In addition, macrophages from *Stim1^{-/-}* mice showed severely compromised Fc γ RII/III-mediated Ca²⁺ influx and phagocytosis indicating that STIM1 and SOCE regulate macrophage function [16]. The defect in macrophage function resulted in the protection of *Stim1^{-/-}* mice from destruction of platelets and red blood cells by phagocytosis in animal models of autoimmune thrombocytopenia and hemolytic anemia [16]. This finding is in contrast to STIM1-deficient patients who suffered from autoimmune thrombocytopenia and hemolytic anemia [110]. The reasons for this discrepancy are unclear

but may be due to differential requirements for SOCE in mouse and human macrophage function.

In summary, SOCE mediated by STIM1 and ORAI1 has been demonstrated to be important for the function, but not the development of several types of immune cells including T cells, B cells, mast cells, and macrophages. More functions of SOCE in immunity in both health and disease will undoubtedly emerge from the study of ORAI- and STIM-deficient mice in the future.

Nonimmune phenotypes in ORAI1 and STIM1 deficiency

STIM1- and ORAI1-deficient human patients also suffer from, in addition to immunodeficiency, congenital myopathy and ectodermal dysplasia with anhydrosis (EDA). This combination of symptoms associated with defective CRAC channel function is unique and constitutes a novel disease entity. It is important to note that the clinical phenotypes of ORAI1- and STIM1-deficient patients described in this and the previous section largely overlap, indicating that ORAI1 and STIM1 are the predominant mediators of SOCE in the same tissues and that STIM1 mainly regulates ORAI1 function.

Myopathy

Myopathy in ORAI1- and STIM1-deficient patients becomes apparent soon after birth as global, nonprogressive muscular hypotonia with reduced muscle strength and endurance (Table 1) [82]. In ORAI1-deficient patients, it initially manifested through insufficient head control and general reduction in muscle tone [82,105]. In the two patients surviving after HSCT myopathy is characterized by delayed ambulation, reduced walking distance, and a positive Gowers' sign. In addition, both patients suffer from chronic pulmonary disease due to respiratory muscle insufficiency and retention of bronchial secretion resulting in a predisposition to recurrent chest infections and bronchiectasis [82]. A muscle biopsy from a patient with ORAI1-R91W mutation showed variations in muscle fiber size with a predominance of type I fibers and atrophic type II fibers suggesting a defect in fast twitch muscle fiber differentiation in the absence of functional ORAI1. Other structural abnormalities characteristic of congenital myopathies were not observed. A clinically similar nonprogressive global muscular hypotonia with partial iris hypoplasia was also observed in all three patients lacking STIM1 expression, although a muscle biopsy revealed no abnormalities [110]. Electromyograms in both ORAI1- and STIM1-deficient patients were normal. These findings suggest that SOCE mediated by ORAI1 and STIM1 is required for the differentiation and/or function of human skeletal muscle.

The myopathy in patients is consistent with a defect in skeletal muscle development and function observed in STIM1-deficient mice which was suggested to cause their perinatal lethality [132]. Morphologically, the myopathy in *Stim1*^{-/-} mice is characterized by reduced muscle cross-sectional area and mitochondriopathy. We observed a similar if less pronounced myopathy in mice transgenic for the nonfunctional ORAI1-R93W mutant (S. Feske, unpublished) [11]. The myopathy in human patients and gene-targeted mice is consistent with the robust expression of both ORAI1 and STIM1 in human and mouse skeletal muscle [50, 82,142,147] and colocalization of STIM1 with ryanodine receptor (RyR) 1 at the junction of plasma membrane T-tubules with the terminal cisternae of the sarcoplasmic reticulum (SR) [132].

Contraction of skeletal muscle fibers requires Ca²⁺ release from the (SR) through RyR, which are coupled to voltage-gated dihydropyridine receptors in the plasma membrane. Released Ca²⁺ is quickly taken back up into the SR and muscle fibers can theoretically twitch for hours in the absence of extracellular Ca²⁺ without an apparent need for refilling of SR stores with

Ca^{2+} from the outside. Nevertheless, SOCE was shown to exist in skeletal muscle and participate in refilling of depleted SR Ca^{2+} stores [67]. Impaired SOCE in mice lacking mitsugumin 29 (mg29), a protein located at the junction between SR and plasma-membrane in skeletal muscle, resulted in accelerated depletion and impaired refilling of SR Ca^{2+} stores under conditions of continuous stimulation of cells by depolarization [99]. Based on studies in mg29^{-/-} mice, mg29 and SOCE were suggested to be dispensable for short-term excitation-contraction but required for long-term Ca^{2+} homeostasis and fatigue resistance due to the role of mg29 in SR store refilling [99]. SOCE in primary cultures of murine skeletal myotubes was shown to be mediated by STIM1 and ORAI1 [80,132], and myotubes from STIM1 deficient have severely impaired SOCE [132]. Consistent with the proposed role of SOCE in store refilling and long-term Ca^{2+} homeostasis, skeletal muscle isolated from STIM1 deficient mice showed reduced tetanic force and fatigued rapidly upon repeated stimulation [132]. In addition to its role in muscle fiber function, SOCE was shown to be required for differentiation of human myoblasts from isolated satellite cells, the stem cells of adult skeletal muscle [24]. Taken together, SOCE mediated by STIM1 and ORAI1 emerges to have a somewhat unexpected but important role in skeletal muscle function and development that is consistent with the myopathy observed in human patients and mice.

Anhydrotic ectodermal dysplasia (EDA)

Patients lacking STIM1 expression and the two surviving ORAI1-deficient patients suffer from EDA characterized by a defect in dental enamel formation (Table 1) [82,110] and, in ORAI1-deficient patients, impaired sweat production or anhydrosis [82]. Ectodermal dysplasias constitute a large group of inherited disorders that are characterized by defects in ectodermal-derived tissues such as skin and skin appendages including hair, nails, teeth, and sweat glands and that can be caused by a variety of genetic defects [58,111]. In the case of ORAI1- and STIM1-deficient patients, other symptoms often found in ectodermal dysplasias such as sparse scalp hair and eyebrows or nail defects were not observed except in one patient with facial dysmorphism [82]. EDA with immunodeficiency has been observed in some patients with defective activation of the transcription factor NF- κ B due to mutations in the genes for NF- κ B essential modulator (NEMO) or I κ B α [23,31,59,161]. Myopathy is missing in these patients in contrast to ORAI1/STIM1-deficient patients, and the cellular defects underlying immunodeficiency in both patient groups differ (reviewed in [37,116]). Taken together, the EDA phenotype in patients lacking STIM1 or ORAI1 suggests that SOCE plays an important role in the development and/or function of certain ectodermal-derived tissues such as sweat glands and teeth.

Dental enamel—Dental enamel of deciduous and permanent teeth in ORAI1- and STIM1-deficient patients is severely dysplastic due to hypocalcification resulting in use-dependent loss of the soft enamel layer and exposure of the underlying dentin [82]. This condition, called hypocalcified amelogenesis imperfecta, is not unique to ORAI1 and STIM1 deficiency and has been linked to mutations in genes encoding the extracellular matrix proteins amelogenin and enamelin [54,150]. More recently, a form of autosomal recessive hypocalcified amelogenesis imperfecta was identified that is caused by mutations in a gene of unknown function. FAM83H is expressed in ameloblasts and features an N-terminal transactivation domain suggesting that it may serve as a transcription factor [65]. Dental enamel is formed by ameloblasts, specialized columnar epithelial cells that form tight junctions and generate a barrier against the enamel extracellular space enriched in enamel proteins and calcium. Ameloblasts secrete enamel-forming proteins such as amelogenin, enamelin, and ameloblastin and regulate Ca^{2+} transport into the enamel extracellular space where it is incorporated into densely packed calcium hydroxyapatite crystals [57]. The cellular and molecular mechanisms underlying this Ca^{2+} transport are poorly understood. Several mechanisms have been suggested including para-cellular Ca^{2+} transport through tight junctions between ameloblasts or

transcellular Ca^{2+} transport through the cytoplasm and the ER of ameloblasts, respectively (reviewed in [57]). The transcellular route involves Ca^{2+} uptake at the basal membrane and apical Ca^{2+} release, potentially through plasma membrane Ca^{2+} ATPases [57]. For transport via the cytoplasm, Ca^{2+} was proposed to bind to cytosolic Ca^{2+} handling proteins such as calbindin to buffer intracellular free Ca^{2+} . Alternatively, Ca^{2+} could be shuttled from the basal to the apical pole of the ameloblast through the ER given the $\sim 10^4$ higher $[\text{Ca}^{2+}]$ in the ER than the cytoplasm and its high Ca^{2+} buffering capacity. In this model, SOCE may play an important role for Ca^{2+} influx at the basal side of the cell. Direct experimental evidence for such a role of SOCE is missing. A paradigm for vectorial transport in polarized cells exists in pancreatic acinar cells in which SOCE and STIM1 puncta formation occur predominantly at the basolateral membrane [79,86,109] where they are involved in refilling of ER Ca^{2+} stores. By contrast, Ca^{2+} release from the ER and its delivery into the extracellular space via IP_3R and plasma membrane Ca^{2+} pumps, respectively, occurs at the apical pole of acinar cells [109]. The defect in dental enamel formation in ORAI1- and STIM1-deficient patients provides an important clue that SOCE may be required for directed Ca^{2+} transport by ameloblasts in the dental enamel.

Sweat glands—Lack of functional ORAI1 in human patients is associated with pronounced anhidrosis in tests of sweat gland function, dry skin, and heat intolerance with recurrent fever suggesting that ORAI1 plays an important role for SOCE in eccrine sweat glands [82]. Skin biopsies to distinguish between a developmental and functional defect were not available, but ORAI1 expression was observed in eccrine sweat gland cells from healthy individuals [82]. Ca^{2+} influx in eccrine sweat glands has long ago been recognized to be required for secretion [115,124]. SOCE was described to occur at the basolateral membrane of equine epithelial sweat gland cells, and its inhibition resulted in attenuated anion secretion [66]. A defect in thermoregulation found in anhydrotic ORAI1-deficient patients has not been observed in *Orai1*^{-/-} or *Stim1*^{-/-} mice, most likely because in rodents, eccrine sweat glands are localized in the footpads of the paws only [64] and are not significantly involved in thermoregulation [146]. Taken together, the EDA phenotype in human patients points to a nonredundant role for ORAI1 and STIM1 in SOCE in ectodermal-derived tissues including teeth and eccrine sweat glands.

Platelet function

Ca^{2+} influx is required for activation of platelets and their function in hemostasis and thrombosis [139]. Ca^{2+} influx occurs in response to agonists such as subendothelial collagen, thrombin, or ADP released from activated platelets [139]. Several Ca^{2+} signaling mechanisms are present in platelets and involve receptor operated Ca^{2+} entry (ROCE) and SOCE. ROCE in platelets is mediated by TRPC6 and the purinergic receptor P2X1 [51,52,98]. The contribution of SOCE to platelet activation and the nature of the store-operated Ca^{2+} channel have remained controversial. Recent studies in mice show that SOCE in platelets is mediated by STIM1 and ORAI1 and that this pathway is essential for platelet activation in vitro and thrombus formation in vivo [11,17,46,138]. Gene-targeted mice lacking STIM1 or ORAI1 [17,138] and those homozygous for the mutant *Orai1*-R93W gene [11] showed greatly reduced platelet Ca^{2+} influx in response to physiological agonists such as thrombin or collagen. While platelet numbers were normal in ORAI1- and STIM1-deficient mice, platelet activation was severely impaired resulting in compromised thrombus formation in vitro [11,17,138]. Importantly, platelet aggregation was also impaired in *Orai1*^{-/-} and *Stim1*^{-/-} mice in vivo in several models of pathological thrombus formation. Mice with attenuated SOCE in platelets were protected from severe ischemic brain infarction compared to wild-type controls in the middle cerebral artery occlusion (MCAO) model [17,138]. Bleeding times in ORAI1 and STIM1-deficient mice [11,17,138] and human patients [82,110], by contrast, were only moderately prolonged or normal, and patients lacked signs of an enhanced bleeding diathesis.

The thrombocytopenia observed in all STIM1- and one ORAI1-deficient patient is caused, as discussed above, by autoantibodies directed against platelet surface antigens and is not an intrinsic defect in platelet development or viability. Collectively, these findings indicate that ORAI1 and STIM1 are essential mediators of SOCE in platelets and that they are required for platelet function in vivo.

ORAI1 and STIM1 function in tissues not apparently affected in patients and mice

SOCE and CRAC channel function have been observed in many non-excitabile cells outside the immune system as well as some excitable cell types (reviewed in [102]). These include epidermoid A431 cells [76], epithelial tumor cell lines from colon and prostate [92,137], hepatocytes [9,61,122], pancreatic acinar cells [86], vascular endothelial [1,35,136] and smooth muscle cells [3,6,75,112,135] (reviewed in [47]), endocrine cells from the adrenal [33,43,120] and pituitary [144] gland, and certain cell types in the central nervous system (CNS) such as microglia [93] or cultured hippocampal pyramidal neurons [8]. This widespread prevalence of SOCE and CRAC channel currents is paralleled by the almost ubiquitous expression of both STIM1 and ORAI1 in a large variety of cell types and tissues [37,49,82, 97,142,147]. Abundant levels of STIM1 mRNA were found in skeletal muscle, heart, platelets, and the CNS [32,46,132]. ORAI1 mRNA is detected in spleen, thymus, lung, liver, kidney, heart, pancreas, and skeletal muscle of human and mice [49,55]. Immunohistochemical analysis of ORAI1 protein expression at the cellular level reveals a similar near ubiquitous distribution of the CRAC channel subunit in many cell types including keratinocytes, eccrine sweat glands, skeletal muscle, hepatocytes, lung alveolar pneumocytes, pancreatic acinar, and islets cells [82], as well as vascular endothelium, kidney tubules, and secretory epithelia of several exocrine glands such as pancreas, salivary gland, breast, and prostate (Fig. 4 and Table 2). Interestingly, no significant ORAI1 expression was detected in cardiomyocytes and the CNS.

Given the widespread prevalence of SOCE and expression of ORAI1 and STIM1 in many cell types, it is surprising to find only a limited spectrum of disease in human patients with mutations in either gene. The lack of other obvious symptoms does not preclude a role for SOCE or even ORAI1 and STIM1 in these tissues but suggests that other store-operated or non-store-operated Ca^{2+} channels function in these tissues that compensate for a lack of ORAI1 and STIM1. A role for both molecules may only emerge under pathophysiological conditions under which lack of SOCE exerts either a protective effect or exacerbates the disease phenotype. A good example is the important function of STIM1 and ORAI1 in mouse platelets during thrombus formation [11,17,138]. Additional roles for ORAI1 and STIM1 in SOCE and CRAC channel function have recently been demonstrated in cell types that are not obviously affected in human patients or mice.

Vascular endothelial cells

ORAI1 protein is readily detectable in the vascular endothelium of arteries and venules (Fig. 4a, b and Table 2). This finding is consistent with the description of SOCE in endothelial cells isolated from human umbilical cord (HUVEC) [96], bovine pulmonary artery [106], or bovine aorta [136] and the demonstration of I_{CRAC} in bovine pulmonary artery endothelial cells [35]. A recent report demonstrates that I_{CRAC} in HUVEC cells depends on STIM1 and ORAI1 expression because RNAi-mediated knockdown of either gene abolished I_{CRAC} and SOCE [1]. Previous studies by several labs had shown that TRPC1 [2, 18] and TRPC4 [44, 134] mediate SOCE and Ca^{2+} channel currents in endothelial cells. In particular, aortic and lung vascular endothelial cells from *Trpc4*^{-/-} mice showed a severe reduction in SOCE, store-operated Ca^{2+} currents [44, 134], and impaired agonist induced Ca^{2+} influx and vasorelaxation

[44]. By contrast, siRNA-mediated silencing of TRPC1 or TRPC4 had no effect on SOCE in HUVEC cells [1]. The arguments for a role of ORAI1 and TRPC proteins in SOCE in endothelial cells are intriguing, and it is possible that both contribute to SOCE in these cells [10], potentially explaining the lack of a vascular phenotype in human patients lacking STIM1 or ORAI1 function.

Vascular smooth muscle cells

SOCE has also been demonstrated in vascular smooth muscle cells (VSMC), where it is involved in vascular tone, VSMC proliferation, and blood vessel integrity [22,45]. In these cells, SOCE co-exists with voltage-dependent and voltage-independent Ca^{2+} influx pathways (reviewed in [47]). Recent studies suggest that SOCE in VSMCs from rat aorta and pulmonary artery is mediated by STIM1 and ORAI1 [75,112]. RNAi silencing of either gene effectively reduced SOCE and CRAC channel currents in response to store depletion or hypoxia and impaired proliferation and migration of VSMC. By contrast, knockdown of TRPC1, TRPC4, or TRPC6 had no effect on SOCE [112]. Similar observations were made in VSMC isolated from aorta and cerebral arteries of *Trpc1*^{-/-} mice in which SOCE was normal, whereas RNAi-mediated knockdown of STIM1 inhibited thapsigargin-induced Ca^{2+} influx [30]. These findings are in contrast however to a number of studies which showed that TRPC1 is important for SOCE in VSMC [19,91,133,152].

An important role for STIM1 in VSMC function, namely, proliferation and neointima formation in vivo, was demonstrated in a rat carotid artery balloon injury model in which knockdown of STIM1 significantly suppressed neointimal hyperplasia [6,48]. In one of the studies, the effect of STIM1 silencing on VSMC function was attributed, however, to inhibition of TRP channels rather than ORAI1 [6]. In fact, STIM1 was shown to be involved not only in the regulation of ORAI1 but also to bind to and activate TRPC1 and other TRPC channels following ectopic expression [56,71,100,149,156,159]. The relative contributions of TRPC and ORAI channels for SOCE in vascular endothelial and smooth muscle cells still need to be worked out. Collectively, the recent data discussed above illustrate an emerging role for STIM1 and ORAI1 in blood vessel function.

SOCE, ORAI1, and STIM in disease

As demonstrated by the role of ORAI1 and STIM1 in platelets discussed earlier, the importance of SOCE in some tissues may become apparent only under pathological conditions. Lack of STIM1 or ORAI1 in gene-targeted mice prevented pathological thrombus formation and limited the extent of thromboischemia in several models of thrombosis [17,138]. The role of ORAI1 and STIM1 in platelets together with that in vascular endothelial cells and smooth muscle discussed above portends a potentially important role of SOCE in the pathophysiology of cardiovascular diseases. In addition, lack of STIM2, recently demonstrated to be required for SOCE in murine cortical neurons, protected mice from neuronal damage after cerebral ischemia [12]. Another example for the role of STIM1 and ORAI1 under pathological conditions is the reported role of both molecules—and by extension SOCE and CRAC channel function—for the migration and metastasis of breast cancer cells in mice [154]. Suppression of ORAI1 or STIM1 expression by RNAi or SOCE with a pharmacological inhibitor (SKF96365) decreased tumor metastasis. This finding is in contrast, however, to the initial identification of STIM1 as a putative tumor suppressor linked to rhabdomyosarcoma [104, 123]. While the role of ORAI1 and STIM1 in colitis has not been addressed directly, a recent study using pharmacological suppression of CRAC channel function showed reduced inflammatory cytokine expression in lamina propria mononuclear cells isolated from patients with inflammatory bowel disease, suggesting that SOCE is required for activation of auto-reactive T cells [29]. Additional roles for SOCE, ORAI, and STIM in the pathophysiology of

disease are likely to emerge from the study of animal models and tissue samples from human patients.

Concluding remarks

CRAC channels, long defined solely by their functional, i.e., electrophysiological, properties are now being redefined through their molecular components, namely, proteins of the ORAI and STIM families. Information on the molecular function of these proteins in ectopic expression systems and under native conditions in specific cell types with regard to SOCE, CRAC channel function, and cellular physiology is rapidly emerging. In parallel, the role of ORAI1, STIM1, and CRAC channels in vivo is becoming more clear with the identification of the first human patients with CRAC channelopathy due to mutations in *ORAI1* and *STIM1* and studies in mice with targeted deletion of *Orai1*, *Stim1*, and *Stim2*. CRAC channelopathy in human patients is restricted to defects in immunity, skeletal muscle, and ectodermal-derived tissues despite the observation of SOCE in many tissues and cell types and the near ubiquitous expression of ORAI1 and STIM1. Their absence may be compensated for by ORAI2, ORAI3, and STIM2, respectively, in those tissues and cell types in which these paralogues are expressed. In addition, other Ca^{2+} permeable channels such as TRPCs that were shown to function in a store-operated manner under certain conditions and some of which are gated by STIM1, may compensate for the lack of STIM1 and ORAI1 as well. The phenotypes of ORAI1- and STIM1-deficient patients, however, are remarkably similar suggesting that STIM1 is predominantly responsible for the gating of ORAI1 CRAC channels.

Studies in ORAI1- and STIM1-deficient mice have revealed additional roles for SOCE mediated by ORAI1/STIM1 in vivo that may become clear only under pathological conditions, for instance, during thrombus formation [17,138] or tumor cell metastasis [154]. Despite the limitations of animal models in predicting human physiology (given for instance the severe perinatal lethality in ORAI1- and STIM1-deficient mice but not humans), they will provide invaluable information about the role of CRAC channels in vivo.

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Abbreviations

CRAC	Calcium release activated calcium (channel)
EDA	Anhydrotic ectodermal dysplasia
SCID	Severe combined immunodeficiency
SERCA	Sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase
SOCE	Store-operated calcium entry
STIM1	Stromal interaction molecule 1
VSMC	Vascular smooth muscle cells

References

1. Abdullaev I, Bisailon J, Potier M, Gonzalez J, Motiani R, Trebak M. Stim1 and orai1 mediate CRAC currents and store-operated calcium entry important for endothelial cell proliferation. *Circ Res* 2008;103:1289–1299. [PubMed: 18845811]

2. Ahmmed GU, Mehta D, Vogel S, Holinstat M, Paria BC, Tiruppathi C, Malik AB. Protein kinase Calpha phosphorylates the TRPC1 channel and regulates store-operated Ca^{2+} entry in endothelial cells. *J Biol Chem* 2004;279:20941–20949. [PubMed: 15016832]
3. Albert AP, Large WA. A Ca^{2+} -permeable non-selective cation channel activated by depletion of internal Ca^{2+} stores in single rabbit portal vein myocytes. *J Physiol* 2002;538:717–728. [PubMed: 11826160]
4. Albert AP, Large WA. Store-operated Ca^{2+} -permeable non-selective cation channels in smooth muscle cells. *Cell Calcium* 2003;33:345–356. [PubMed: 12765681]
5. Alicia S, Angelica Z, Carlos S, Alfonso S, Vaca L. STIM1 converts TRPC1 from a receptor-operated to a store-operated channel: moving TRPC1 in and out of lipid rafts. *Cell Calcium* 2008;44:479–491. [PubMed: 18420269]
6. Aubart FC, Sassi Y, Coulombe A, Mougnot N, Vrignaud C, Leprince P, Lechat P, Lompre AM, Hulot JS. RNA interference targeting STIM1 suppresses vascular smooth muscle cell proliferation and neointima formation in the rat. *Mol Ther* 2009;17:455–462. [PubMed: 19107116]
7. Baba Y, Nishida K, Fujii Y, Hirano T, Hikida M, Kurosaki T. Essential function for the calcium sensor STIM1 in mast cell activation and anaphylactic responses. *Nat Immunol* 2008;9:81–88. [PubMed: 18059272]
8. Baba A, Yasui T, Fujisawa S, Yamada RX, Yamada MK, Nishiyama N, Matsuki N, Ikegaya Y. Activity-evoked capacitative Ca^{2+} entry: implications in synaptic plasticity. *J Neurosci* 2003;23:7737–7741. [PubMed: 12944501]
9. Barritt GJ, Litjens TL, Castro J, Aromataris E, Rychkov GY. Store-operated Ca^{2+} channels and microdomains of Ca^{2+} in liver cells. *Clin Exp Pharmacol Physiol* 2009;36:77–83. [PubMed: 19196257]
10. Beech DJ. Harmony and discord in endothelial calcium entry. *Circ Res* 2009;104:e22–e23. [PubMed: 19179662]
11. Bergmeier W, Oh-Hora M, McCarl CA, Roden RC, Bray PF, Feske S. R93W mutation in Orai1 causes impaired calcium influx in platelets. *Blood* 2009;113:675–678. [PubMed: 18952890]
12. Berna-Erro A, Braun A, Kraft R, Kleinschnitz C, Schuhmann MK, Stegner D, Wultsch T, Eilers J, Meuth SG, Stoll G, Nieswandt B. STIM2 regulates capacitative Ca^{2+} entry in neurons and plays a key role in hypoxic neuronal cell death. *Sci Signal* 2009;2:ra67. [PubMed: 19843959]
13. Beyersdorf N, Braun A, Vogtle T, Varga-Szabo D, Galdos R, Kissler S, Kerkau T, Nieswandt B. STIM1-independent T cell development and effector function in vivo. *J Immunol* 2009;182:3390–3397. [PubMed: 19265116]
14. Bolotina VM, Csutora P. CIF and other mysteries of the store-operated Ca^{2+} -entry pathway. *Trends Biochem Sci* 2005;30:378–387. [PubMed: 15951181]
15. Brandman O, Liou J, Park WS, Meyer T. STIM2 is a feedback regulator that stabilizes basal cytosolic and endoplasmic reticulum Ca^{2+} levels. *Cell* 2007;131:1327–1339. [PubMed: 18160041]
16. Braun A, Gessner J, Varga-Szabo D, Syed S, Konrad S, Stegner D, Vogtle T, Schmidt R, Nieswandt B. STIM1 is essential for Fc receptor activation and autoimmune inflammation. *Blood* 2008;113:1097–1104. [PubMed: 18941110]
17. Braun A, Varga-Szabo D, Kleinschnitz C, Pleines I, Bender M, Austinat M, Bosl M, Stoll G, Nieswandt B. Orai1 (CRACM1) is the platelet SOC channel and essential for pathological thrombus formation. *Blood* 2009;113:2056–2063. [PubMed: 18832659]
18. Brough GH, Wu S, Cioffi D, Moore TM, Li M, Dean N, Stevens T. Contribution of endogenously expressed Trp1 to a Ca^{2+} -selective, store-operated Ca^{2+} entry pathway. *FASEB J* 2001;15:1727–1738. [PubMed: 11481220]
19. Brueggemann LI, Markun DR, Henderson KK, Cribbs LL, Byron KL. Pharmacological and electrophysiological characterization of store-operated currents and capacitative Ca^{2+} entry in vascular smooth muscle cells. *J Pharmacol Exp Ther* 2006;317:488–499. [PubMed: 16415091]
20. Cahalan MD. STIMulating store-operated Ca^{2+} entry. *Nat Cell Biol* 2009;11:669–677. [PubMed: 19488056]
21. Calloway N, Vig M, Kinet JP, Holowka D, Baird B. Molecular clustering of STIM1 with Orai1/CRACM1 at the plasma membrane depends dynamically on depletion of Ca^{2+} stores and on electrostatic interactions. *Mol Biol Cell* 2009;20:389–399. [PubMed: 18987344]

22. Casteels R, Raeymaekers L, Suzuki H, Van Eldere J. Tension response and ^{45}Ca release in vascular smooth muscle incubated in Ca-free solution. *Pflugers Arch* 1981;392:139–145. [PubMed: 6798548]
23. Courtois G, Smahi A, Reichenbach J, Doffinger R, Cancrini C, Bonnet M, Puel A, Chable-Bessia C, Yamaoka S, Feinberg J, Dupuis-Girod S, Bodemer C, Livadiotti S, Novelli F, Rossi P, Fischer A, Israel A, Munnich A, Le Deist F, Casanova JL. A hypermorphic $\text{IkappaB}\alpha$ mutation is associated with autosomal dominant anhidrotic ectodermal dysplasia and T cell immunodeficiency. *J Clin Invest* 2003;112:1108–1115. [PubMed: 14523047]
24. Darbellay B, Arnaudeau S, Konig S, Jousset H, Bader C, Demaurex N, Bernheim L. STIM1- and orai1-dependent store-operated calcium entry regulates human myoblast differentiation. *J Biol Chem* 2008;284:5370–5380. [PubMed: 19088073]
25. DeHaven WI, Jones BF, Petranks JG, Smyth JT, Tomita T, Bird GS, Putney JW Jr. TRPC channels function independently of STIM1 and Orai1. *J Physiol* 2009;587:2275–2298. [PubMed: 19332491]
26. DeHaven WI, Smyth JT, Boyles RR, Putney JW. Calcium inhibition and calcium potentiation of Orai1, Orai2, and Orai3 calcium release-activated calcium channels. *J Biol Chem* 2007;282:17548–17556. [PubMed: 17452328]
27. Derler I, Fahrner M, Carugo O, Muik M, Bergsmann J, Schindl R, Frischauf I, Eshaghi S, Romanin C. Increased hydrophobicity at the N-terminus/membrane interface impairs gating of the SCID-related ORAI1 mutant. *J Biol Chem* 2009;284:15903–15915. [PubMed: 19366689]
28. Derler I, Fahrner M, Muik M, Lackner B, Schindl R, Groschner K, Romanin C. A Ca^{2+} release-activated Ca^{2+} (CRAC) modulatory domain (CMD) within STIM1 mediates fast Ca^{2+} -dependent inactivation of ORAI1 channels. *J Biol Chem* 2009;284:24933–24938. [PubMed: 19622747]
29. Di Sabatino A, Rovedatti L, Kaur R, Spencer JP, Brown JT, Morisset VD, Biancheri P, Leakey NA, Wilde JJ, Scott L, Corazza GR, Lee K, Sengupta N, Knowles CH, Gunthorpe MJ, McLean PG, MacDonald TT, Kruidenier L. Targeting gut T cell Ca^{2+} release-activated Ca^{2+} channels inhibits T cell cytokine production and T-box transcription factor T-bet in inflammatory bowel disease. *J Immunol* 2009;183:3454–3462. [PubMed: 19648266]
30. Dietrich A, Kalwa H, Storch U, Mederos y Schnitzler M, Salanova B, Pinkenburg O, Dubrovskaya G, Essin K, Gollasch M, Birnbaumer L, Gudermann T. Pressure-induced and store-operated cation influx in vascular smooth muscle cells is independent of TRPC1. *Pflugers Arch* 2007;455:465–477. [PubMed: 17647013]
31. Doffinger R, Smahi A, Bessia C, Geissmann F, Feinberg J, Durandy A, Bodemer C, Kenwrick S, Dupuis-Girod S, Blanche S, Wood P, Rabia SH, Headon DJ, Overbeek PA, Le Deist F, Holland SM, Belani K, Kumararatne DS, Fischer A, Shapiro R, Conley ME, Reimund E, Kalhoff H, Abinun M, Munnich A, Israel A, Courtois G, Casanova JL. X-linked anhidrotic ectodermal dysplasia with immunodeficiency is caused by impaired NF-kappaB signaling. *Nat Genet* 2001;27:277–285. [PubMed: 11242109]
32. Dziadek MA, Johnstone LS. Biochemical properties and cellular localisation of STIM proteins. *Cell Calcium* 2007;42:123–132. [PubMed: 17382385]
33. Ely JA, Ambroz C, Baukal AJ, Christensen SB, Balla T, Catt KJ. Relationship between agonist- and thapsigargin-sensitive calcium pools in adrenal glomerulosa cells. Thapsigargin-induced Ca^{2+} mobilization and entry. *J Biol Chem* 1991;266:18635–18641. [PubMed: 1917986]
34. Fahrner M, Muik M, Derler I, Schindl R, Fritsch R, Frischauf I, Romanin C. Mechanistic view on domains mediating STIM1-Orai coupling. *Immunol Rev* 2009;231:99–112. [PubMed: 19754892]
35. Fasolato C, Nilius B. Store depletion triggers the calcium release-activated calcium current I_{CRAC} in macrovascular endothelial cells: a comparison with Jurkat and embryonic kidney cell lines. *Pflugers Arch* 1998;436:69–74. [PubMed: 9560448]
36. Feske S. Calcium signalling in lymphocyte activation and disease. *Nat Rev Immunol* 2007;7:690–702. [PubMed: 17703229]
37. Feske S. ORAI1 and STIM1 deficiency in human and mice: roles of store-operated Ca^{2+} entry in the immune system and beyond. *Immunol Rev* 2009;231:189–209. [PubMed: 19754898]
38. Feske S, Draeger R, Peter HH, Eichmann K, Rao A. The duration of nuclear residence of NFAT determines the pattern of cytokine expression in human SCID T cells. *J Immunol* 2000;165:297–305. [PubMed: 10861065]

39. Feske S, Giltane J, Dolmetsch R, Staudt LM, Rao A. Gene regulation mediated by calcium signals in T lymphocytes. *Nat Immunol* 2001;2:316–324. [PubMed: 11276202]
40. Feske S, Gwack Y, Prakriya M, Srikanth S, Puppel SH, Tanasa B, Hogan PG, Lewis RS, Daly M, Rao A. A mutation in *Orai1* causes immune deficiency by abrogating CRAC channel function. *Nature* 2006;441:179–185. [PubMed: 16582901]
41. Feske S, Muller JM, Graf D, Kroczeck RA, Drager R, Niemeyer C, Baeuerle PA, Peter HH, Schlesier M. Severe combined immunodeficiency due to defective binding of the nuclear factor of activated T cells in T lymphocytes of two male siblings. *Eur J Immunol* 1996;26:2119–2126. [PubMed: 8814256]
42. Feske S, Prakriya M, Rao A, Lewis RS. A severe defect in CRAC Ca^{2+} channel activation and altered K^{+} -channel gating in T cells from immunodeficient patients. *J Exp Med* 2005;202:651–662. [PubMed: 16147976]
43. Fomina AF, Nowycky MC. A current activated on depletion of intracellular Ca^{2+} stores can regulate exocytosis in adrenal chromaffin cells. *J Neurosci* 1999;19:3711–3722. [PubMed: 10234003]
44. Freichel M, Suh SH, Pfeifer A, Schweig U, Trost C, Weissgerber P, Biel M, Philipp S, Freise D, Droogmans G, Hofmann F, Flockerzi V, Nilius B. Lack of an endothelial store-operated Ca^{2+} current impairs agonist-dependent vasorelaxation in *TRP4*^{-/-} mice. *Nat Cell Biol* 2001;3:121–127. [PubMed: 11175743]
45. Gibson A, McFadzean I, Wallace P, Wayman CP. Capacitative Ca^{2+} entry and the regulation of smooth muscle tone. *Trends Pharmacol Sci* 1998;19:266–269. [PubMed: 9703759]
46. Grosse J, Braun A, Varga-Szabo D, Beyersdorf N, Schneider B, Zeitlmann L, Hanke P, Schropp P, Muhlstedt S, Zorn C, Huber M, Schmittwolf C, Jagla W, Yu P, Kerkau T, Schulze H, Nehls M, Nieswandt B. An EF hand mutation in *Stim1* causes premature platelet activation and bleeding in mice. *J Clin Invest* 2007;117:3540–3550. [PubMed: 17965774]
47. Guibert C, Ducret T, Savineau JP. Voltage-independent calcium influx in smooth muscle. *Prog Biophys Mol Biol* 2008;98:10–23. [PubMed: 18614208]
48. Guo RW, Wang H, Gao P, Li MQ, Zeng CY, Yu Y, Chen JF, Song MB, Shi YK, Huang L. An essential role for stromal interaction molecule 1 in neointima formation following arterial injury. *Cardiovasc Res* 2009;81:660–668. [PubMed: 19052075]
49. Gwack Y, Srikanth S, Feske S, Cruz-Guilloty F, Oh-hora M, Neems DS, Hogan PG, Rao A. Biochemical and functional characterization of *Orai* proteins. *J Biol Chem* 2007;282:16232–16243. [PubMed: 17293345]
50. Gwack Y, Srikanth S, Oh-Hora M, Hogan PG, Lamperti ED, Yamashita M, Gelinias C, Neems DS, Sasaki Y, Feske S, Prakriya M, Rajewsky K, Rao A. Hair loss and defective T- and B-cell function in mice lacking *ORAI1*. *Mol Cell Biol* 2008;28:5209–5222. [PubMed: 18591248]
51. Hassock SR, Zhu MX, Trost C, Flockerzi V, Authi KS. Expression and role of TRPC proteins in human platelets: evidence that TRPC6 forms the store-independent calcium entry channel. *Blood* 2002;100:2801–2811. [PubMed: 12351388]
52. Hechler B, Lenain N, Marchese P, Vial C, Heim V, Freund M, Cazenave JP, Cattaneo M, Ruggeri ZM, Evans R, Gachet C. A role of the fast ATP-gated P2X1 cation channel in thrombosis of small arteries in vivo. *J Exp Med* 2003;198:661–667. [PubMed: 12913094]
53. Hoth M, Penner R. Depletion of intracellular calcium stores activates a calcium current in mast cells. *Nature* 1992;355:353–356. [PubMed: 1309940]
54. Hu JC, Yamakoshi Y. Enamelin and autosomal-dominant amelogenesis imperfecta. *Crit Rev Oral Biol Med* 2003;14:387–398. [PubMed: 14656895]
55. Huang YH, Hoebe K, Sauer K. New therapeutic targets in immune disorders: *ItpkB*, *Orai1* and *UNC93B*. *Expert Opin Ther Targets* 2008;12:391–413. [PubMed: 18348677]
56. Huang GN, Zeng W, Kim JY, Yuan JP, Han L, Muallem S, Worley PF. *STIM1* carboxyl-terminus activates native SOC, *I(crac)* and *TRPC1* channels. *Nat Cell Biol* 2006;8:1003–1010. [PubMed: 16906149]
57. Hubbard. Calcium transport across the dental enamel epithelium. *Crit Rev Oral Biol Med* 2000;11:437–466. [PubMed: 11132765]
58. Itin PH, Fistarol SK. Ectodermal dysplasias. *Am J Med Genet C Semin Med Genet* 2004;131C:45–51. [PubMed: 15468153]

59. Jain A, Ma CA, Liu S, Brown M, Cohen J, Strober W. Specific missense mutations in NEMO result in hyper-IgM syndrome with hypohydrotic ectodermal dysplasia. *Nat Immunol* 2001;2:223–228. [PubMed: 11224521]
60. Ji W, Xu P, Li Z, Lu J, Liu L, Zhan Y, Chen Y, Hille B, Xu T, Chen L. Functional stoichiometry of the unitary calcium-release-activated calcium channel. *Proc Natl Acad Sci U S A* 2008;105:13668–13673. [PubMed: 18757751]
61. Jones BF, Boyles RR, Hwang SY, Bird GS, Putney JW. Calcium influx mechanisms underlying calcium oscillations in rat hepatocytes. *Hepatology* 2008;48:1273–1281. [PubMed: 18802964]
62. Kahr H, Schindl R, Fritsch R, Heinze B, Hofbauer M, Hack ME, Mortelmaier MA, Groschner K, Peng JB, Takanaga H, Hediger MA, Romanin C. CaT1 knock-down strategies fail to affect CRAC channels in mucosal-type mast cells. *J Physiol* 2004;557:121–132. [PubMed: 15020691]
63. Kawasaki T, Lange I, Feske S. A minimal regulatory domain in the C terminus of STIM1 binds to and activates ORAI1 CRAC channels. *Biochem Biophys Res Commun* 2009;385:49–54. [PubMed: 19433061]
64. Kennedy WR, Sakuta M, Quick DC. Rodent eccrine sweat glands: a case of multiple efferent innervation. *Neuroscience* 1984;11:741–749. [PubMed: 6717807]
65. Kim JW, Lee SK, Lee ZH, Park JC, Lee KE, Lee MH, Park JT, Seo BM, Hu JC, Simmer JP. FAM83H mutations in families with autosomal-dominant hypocalcified amelogenesis imperfecta. *Am J Hum Genet* 2008;82:489–494. [PubMed: 18252228]
66. Ko WH, Chan HC, Wong PY. Anion secretion induced by capacitative Ca^{2+} entry through apical and basolateral membranes of cultured equine sweat gland epithelium. *J Physiol* 1996;497 (Pt 1):19–29. [PubMed: 8951708]
67. Kurebayashi N, Ogawa Y. Depletion of Ca^{2+} in the sarcoplasmic reticulum stimulates Ca^{2+} entry into mouse skeletal muscle fibres. *J Physiol* 2001;533:185–199. [PubMed: 11351027]
68. Le Deist F, Hivroz C, Partiseti M, Thomas C, Buc HA, Oleastro M, Belohradsky B, Choquet D, Fischer A. A primary T-cell immunodeficiency associated with defective transmembrane calcium influx. *Blood* 1995;85:1053–1062. [PubMed: 7531512]
69. Lee KP, Yuan JP, Zeng W, So I, Worley PF, Muallem S. Molecular determinants of fast Ca^{2+} -dependent inactivation and gating of the Orai channels. *Proc Natl Acad Sci U S A* 2009;106:14687–14692. [PubMed: 19706554]
70. Lewis RS. The molecular choreography of a store-operated calcium channel. *Nature* 2007;446:284–287. [PubMed: 17361175]
71. Li J, Sukumar P, Milligan CJ, Kumar B, Ma ZY, Munsch CM, Jiang LH, Porter KE, Beech DJ. Interactions, functions, and independence of plasma membrane STIM1 and TRPC1 in vascular smooth muscle cells. *Circ Res* 2008;103:e97–e104. [PubMed: 18802022]
72. Liou J, Fivaz M, Inoue T, Meyer T. Live-cell imaging reveals sequential oligomerization and local plasma membrane targeting of stromal interaction molecule 1 after Ca^{2+} store depletion. *Proc Natl Acad Sci U S A* 2007;104:9301–9306. [PubMed: 17517596]
73. Liou J, Kim M, Heo WD, Jones JT, Myers JW, Ferrell JE, Meyer T. STIM is a Ca^{2+} sensor essential for Ca^{2+} -store-depletion-triggered Ca^{2+} influx. *Curr Biol* 2005;15:1235–1241. [PubMed: 16005298]
74. Lis A, Peinelt C, Beck A, Parvez S, Monteilh-Zoller M, Fleig A, Penner R. CRACM1, CRACM2, and CRACM3 are store-operated Ca^{2+} channels with distinct functional properties. *Curr Biol* 2007;17:794–800. [PubMed: 17442569]
75. Lu W, Wang J, Peng G, Shimoda LA, Sylvester JT. Knockdown of stromal interaction molecule 1 attenuates store-operated Ca^{2+} entry and Ca^{2+} responses to acute hypoxia in pulmonary arterial smooth muscle. *Am J Physiol Lung Cell Mol Physiol* 2009;297:L17–L25. [PubMed: 19395668]
76. Luckhoff A, Clapham DE. Calcium channels activated by depletion of internal calcium stores in A431 cells. *Biophys J* 1994;67:177–182. [PubMed: 7918985]
77. Luik RM, Wang B, Prakriya M, Wu MM, Lewis RS. Oligomerization of STIM1 couples ER calcium depletion to CRAC channel activation. *Nature* 2008;454:538–542. [PubMed: 18596693]
78. Luik RM, Wu MM, Buchanan J, Lewis RS. The elementary unit of store-operated Ca^{2+} entry: local activation of CRAC channels by STIM1 at ER-plasma membrane junctions. *J Cell Biol* 2006;174:815–825. [PubMed: 16966423]

79. Lur G, Haynes LP, Prior IA, Gerasimenko OV, Feske S, Petersen OH, Burgoyne RD, Tepikin AV. Ribosome-free terminals of rough ER allow formation of STIM1 puncta and segregation of STIM1 from IP(3) receptors. *Curr Biol* 2009;19:1648–1653. [PubMed: 19765991]
80. Lyfenko AD, Dirksen RT. Differential dependence of store-operated and excitation-coupled Ca^{2+} entry in skeletal muscle on STIM1 and Orai1. *J Physiol* 2008;586:4815–4824. [PubMed: 18772199]
81. Manji SS, Parker NJ, Williams RS, Van Stekelenburg L, Pearson RB, Dziadek MA, Smith PJ. STIM1: a novel phospho-protein located at the cell surface. *Biochim Biophys Acta* 2000;1481:147–155. [PubMed: 11004585]
82. McCarl CA, Picard C, Khalil S, Kawasaki T, Röther J, Papolos A, Kutok J, Hivroz C, LeDeist F, Plogmann K, Ehl S, Notheis G, Albert MH, Belohradsky BH, Kirschner J, Rao A, Fischer A, Feske S. ORAI1 deficiency and lack of store-operated Ca^{2+} entry cause immunodeficiency, myopathy and ectodermal dysplasia. *J Allergy Clin Immunol* 2009;124:1311–18e7. [PubMed: 20004786]
83. McFadzean I, Gibson A. The developing relationship between receptor-operated and store-operated calcium channels in smooth muscle. *Br J Pharmacol* 2002;135:1–13. [PubMed: 11786473]
84. McNally B, Yamashita M, Engh A, Prakriya M. Structural determinants of ion permeation in CRAC channels. *Proc Natl Acad Sci U S A* 2009;106:22516–22521. [PubMed: 20018736]
85. Mignen O, Thompson JL, Shuttleworth TJ. Orai1 subunit stoichiometry of the mammalian CRAC channel pore. *J Physiol* 2008;586:419–425. [PubMed: 18006576]
86. Mogami H, Nakano K, Tepikin AV, Petersen OH. Ca^{2+} flow via tunnels in polarized cells: recharging of apical Ca^{2+} stores by focal Ca^{2+} entry through basal membrane patch. *Cell* 1997;88:49–55. [PubMed: 9019404]
87. Muik M, Fahrner M, Derler I, Schindl R, Bergsmann J, Frischauf I, Groschner K, Romanin C. A cytosolic homomerization and a modulatory domain within STIM1 C terminus determine coupling to ORAI1 channels. *J Biol Chem* 2009;284:8421–8426. [PubMed: 19189966]
88. Muik M, Frischauf I, Derler I, Fahrner M, Bergsmann J, Eder P, Schindl R, Hesch C, Polzinger B, Fritsch R, Kahr H, Madl J, Gruber H, Groschner K, Romanin C. Dynamic coupling of the putative coiled-coil domain of ORAI1 with STIM1 mediates ORAI1 channel activation. *J Biol Chem* 2008;283:8014–8022. [PubMed: 18187424]
89. Mullins FM, Park CY, Dolmetsch RE, Lewis RS. STIM1 and calmodulin interact with Orai1 to induce Ca^{2+} -dependent inactivation of CRAC channels. *Proc Natl Acad Sci U S A* 2009;106:15495–15500. [PubMed: 19706428]
90. Navarro-Borelly L, Somasundaram A, Yamashita M, Ren D, Miller RJ, Prakriya M. STIM1-Orai1 interactions and Orai1 conformational changes revealed by live-cell FRET microscopy. *J Physiol* 2008;586:5383–5401. [PubMed: 18832420]
91. Ng LC, McCormack MD, Airey JA, Singer CA, Keller PS, Shen XM, Hume JR. TRPC1 and STIM1 mediate capacitative Ca^{2+} entry in mouse pulmonary arterial smooth muscle cells. *J Physiol* 2009;587:2429–2442. [PubMed: 19332490]
92. Nunez L, Valero RA, Senovilla L, Sanz-Blasco S, Garcia-Sancho J, Villalobos C. Cell proliferation depends on mitochondrial Ca^{2+} uptake: inhibition by salicylate. *J Physiol* 2006;571:57–73. [PubMed: 16339178]
93. Ohana L, Newell EW, Stanley EF, Schlichter LC. The Ca^{2+} release-activated Ca^{2+} current ($I(\text{crac})$) mediates store-operated Ca^{2+} entry in rat microglia. *Channels (Austin)* 2009;3:129–139. [PubMed: 19411837]
94. Oh-hora M. Calcium signaling in the development and function of T-lineage cells. *Immunol Rev* 2009;231:210–224. [PubMed: 19754899]
95. Oh-Hora M, Yamashita M, Hogan P, Sharma S, Lamperti E, Chung W, Prakriya M, Feske S, Rao A. Dual functions for the endoplasmic reticulum calcium sensors STIM1 and STIM2 in T cell activation and tolerance. *Nat Immunol* 2008;9:432–443. [PubMed: 18327260]
96. Oike M, Gericke M, Droogmans G, Nilius B. Calcium entry activated by store depletion in human umbilical vein endothelial cells. *Cell Calcium* 1994;16:367–376. [PubMed: 7859251]
97. Oritani K, Kincade PW. Identification of stromal cell products that interact with pre-B cells. *J Cell Biol* 1996;134:771–782. [PubMed: 8707854]
98. Oury C, Kuijpers MJ, Toth-Zsamboki E, Bonnefoy A, Danloy S, Vreys I, Feijge MA, De Vos R, Vermeylen J, Heemskerk JW, Hoylaerts MF. Overexpression of the platelet P2X1 ion channel in

- transgenic mice generates a novel prothrombotic phenotype. *Blood* 2003;101:3969–3976. [PubMed: 12521992]
99. Pan Z, Yang D, Nagaraj RY, Nosek TA, Nishi M, Takeshima H, Cheng H, Ma J. Dysfunction of store-operated calcium channel in muscle cells lacking *mg29*. *Nat Cell Biol* 2002;4:379–383. [PubMed: 11988740]
 100. Pani B, Ong HL, Brazer SC, Liu X, Rauser K, Singh BB, Ambudkar IS. Activation of TRPC1 by STIM1 in ER-PM microdomains involves release of the channel from its scaffold caveolin-1. *Proc Natl Acad Sci U S A* 2009;106:20087–20092. [PubMed: 19897728]
 101. Parekh AB, Penner R. Store depletion and calcium influx. *Physiol Rev* 1997;77:901–930. [PubMed: 9354808]
 102. Parekh AB, Putney JW. Store-operated calcium channels. *Physiol Rev* 2005;85:757–810. [PubMed: 15788710]
 103. Park CY, Hoover PJ, Mullins FM, Bachhawat P, Covington ED, Raunser S, Walz T, Garcia KC, Dolmetsch RE, Lewis RS. STIM1 clusters and activates CRAC channels via direct binding of a cytosolic domain to Orai1. *Cell* 2009;136:876–890. [PubMed: 19249086]
 104. Parker. Molecular cloning of a novel human gene (D11S4896E) at chromosomal region 11p15.5. *Genomics* 1996;37:253–256. [PubMed: 8921403]
 105. Partiseti M, Le Deist F, Hivroz C, Fischer A, Korn H, Choquet D. The calcium current activated by T cell receptor and store depletion in human lymphocytes is absent in a primary immunodeficiency. *J Biol Chem* 1994;269:32327–32335. [PubMed: 7798233]
 106. Pasyk E, Inazu M, Daniel EE. CPA enhances Ca^{2+} entry in cultured bovine pulmonary arterial endothelial cells in an IP3-independent manner. *Am J Physiol* 1995;268:H138–H146. [PubMed: 7530915]
 107. Peinelt C, Vig M, Koomoa DL, Beck A, Nadler MJ, Koblan-Huberson M, Lis A, Fleig A, Penner R, Kinet JP. Amplification of CRAC current by STIM1 and CRACM1 (Orai1). *Nat Cell Biol* 2006;8:771–773. [PubMed: 16733527]
 108. Penna A, Demuro A, Yeromin A, Zhang S, Safrina O, Parker I, Cahalan M. The CRAC channel consists of a tetramer formed by Stim-induced dimerization of Orai dimers. *Nature* 2008;456:116–120. [PubMed: 18820677]
 109. Petersen OH. Localization and regulation of Ca^{2+} entry and exit pathways in exocrine gland cells. *Cell Calcium* 2003;33:337–344. [PubMed: 12765680]
 110. Picard C, McCarl CA, Papolos A, Khalil S, Luthy K, Hivroz C, LeDeist F, Rieux-Laucat F, Rechavi G, Rao A, Fischer A, Feske S. STIM1 mutation associated with a syndrome of immunodeficiency and autoimmunity. *N Engl J Med* 2009;360:1971–1980. [PubMed: 19420366]
 111. Pinheiro M, Freire-Maia N. Ectodermal dysplasias: a clinical classification and a causal review. *Am J Med Genet* 1994;53:153–162. [PubMed: 7856640]
 112. Potier M, Gonzalez J, Motiani R, Abdullaev I, Bisailon J, Singer H, Trebak M. Evidence for STIM1- and Orai1-dependent store-operated calcium influx through Icrac in vascular smooth muscle cells: role in proliferation and migration. *FASEB J* 2009;23:2425–2437. [PubMed: 19364762]
 113. Prakriya M. The molecular physiology of CRAC channels. *Immunol Rev* 2009;231:88–98. [PubMed: 19754891]
 114. Prakriya M, Feske S, Gwack Y, Srikanth S, Rao A, Hogan PG. Orai1 is an essential pore subunit of the CRAC channel. *Nature* 2006;443:230–233. [PubMed: 16921383]
 115. Prompt. Functions of calcium in sweat secretion. *Nature* 1978;272:171–172. [PubMed: 628444]
 116. Puel A, Picard C, Ku CL, Smahi A, Casanova JL. Inherited disorders of NF-kappaB-mediated immunity in man. *Curr Opin Immunol* 2004;16:34–41. [PubMed: 14734108]
 117. Putney JW. A model for receptor-regulated calcium entry. *Cell Calcium* 1986;7:1–12. [PubMed: 2420465]
 118. Putney JW. Capacitative calcium entry: from concept to molecules. *Immunol Rev* 2009;231:10–22. [PubMed: 19754887]
 119. Rao A, Hogan PG. Calcium signaling in cells of the immune and hematopoietic systems. *Immunol Rev* 2009;231:5–9. [PubMed: 19754886]

120. Rohacs T, Bago A, Deak F, Hunyady L, Spat A. Capacitative Ca^{2+} influx in adrenal glomerulosa cells: possible role in angiotensin II response. *Am J Physiol* 1994;267:C1246–C1252. [PubMed: 7977688]
121. Roos J, DiGregorio PJ, Yeromin AV, Ohlsen K, Lioudyno M, Zhang S, Safrina O, Kozak JA, Wagner SL, Cahalan MD, Velicelebi G, Stauderman KA. STIM1, an essential and conserved component of store-operated Ca^{2+} channel function. *J Cell Biol* 2005;169:435–445. [PubMed: 15866891]
122. Rychkov G, Brereton HM, Harland ML, Barritt GJ. Plasma membrane Ca^{2+} release-activated Ca^{2+} channels with a high selectivity for Ca^{2+} identified by patch-clamp recording in rat liver cells. *Hepatology* 2001;33:938–947. [PubMed: 11283858]
123. Sabbioni S, Barbanti-Brodano G, Croce CM, Negrini M. GOK: a gene at 11p15 involved in rhabdomyosarcoma and rhabdoid tumor development. *Cancer Res* 1997;57:4493–4497. [PubMed: 9377559]
124. Sato K, Sato F. Relationship between quin-2 determined cytosolic $[\text{Ca}^{2+}]$ and sweat secretion. *Am J Phys* 1988;254:C310–C317.
125. Schlesier M, Niemeyer C, Duffner U, Henschen M, Tanzi-Fetta R, Wolff-Vorbeck G, Drager R, Brandis M, Peter HH. Primary severe immunodeficiency due to impaired signal transduction in T cells. *Immunodeficiency* 1993;4:133–136. [PubMed: 8167687]
126. Scrimgeour. Properties of orai1 mediated store-operated current depend on the expression levels of STIM1 and orai1 proteins. *J Physiology* 2009;587:2903–2918.
127. Soboloff J, Spassova MA, Tang XD, Hewavitharana T, Xu W, Gill DL. Orai1 and STIM reconstitute store-operated calcium channel function. *J Biol Chem* 2006;281:20661–20665. [PubMed: 16766533]
128. Spassova MA, Soboloff J, He LP, Xu W, Dziadek MA, Gill DL. STIM1 has a plasma membrane role in the activation of store-operated Ca^{2+} channels. *Proc Natl Acad Sci U S A* 2006;103:4040–4045. [PubMed: 16537481]
129. Stathopulos PB, Zheng L, Ikura M. Stromal interaction molecule (STIM) 1 and STIM2 calcium sensing regions exhibit distinct unfolding and oligomerization kinetics. *J Biol Chem* 2009;284:728–732. [PubMed: 19019825]
130. Stathopulos PB, Zheng L, Li GY, Plevin MJ, Ikura M. Structural and mechanistic insights into STIM1-mediated initiation of store-operated calcium entry. *Cell* 2008;135:110–122. [PubMed: 18854159]
131. Stewart, M. “The Hours”, Greek Mythology: From the Iliad to the Fall of the Last Tyrant. 2005. <http://messagenet.com/myths/bios/hours.html>
132. Stiber J, Hawkins A, Zhang ZS, Wang SW, Burch J, Graham V, Ward CC, Seth M, Finch E, Malouf N, Williams RS, Eu JP, Rosenberg P. STIM1 signalling controls store-operated calcium entry required for development and contractile function in skeletal muscle. *Nat Cell Biol* 2008;10:688–697. [PubMed: 18488020]
133. Sweeney M, McDaniel SS, Platoshyn O, Zhang S, Yu Y, Lapp BR, Zhao Y, Thistlethwaite PA, Yuan JX. Role of capacitative Ca^{2+} entry in bronchial contraction and remodeling. *J Appl Physiol* 2002;92:1594–1602. [PubMed: 11896026]
134. Tirupathi C, Freichel M, Vogel SM, Paria BC, Mehta D, Flockerzi V, Malik AB. Impairment of store-operated Ca^{2+} entry in TRPC4^{-/-} mice interferes with increase in lung microvascular permeability. *Circ Res* 2002;91:70–76. [PubMed: 12114324]
135. Trepakova ES, Gericke M, Hirakawa Y, Weisbrod RM, Cohen RA, Bolotina VM. Properties of a native cation channel activated by Ca^{2+} store depletion in vascular smooth muscle cells. *J Biol Chem* 2001;276:7782–7790. [PubMed: 11113149]
136. Vaca L, Kunze DL. Depletion of intracellular Ca^{2+} stores activates a Ca^{2+} -selective channel in vascular endothelium. *Am J Physiol* 1994;267:C920–C925. [PubMed: 7943286]
137. Vanden Abeele F, Shuba Y, Roudbaraki M, Lemonnier L, Vanoverberghe K, Mariot P, Skryma R, Prevarskaya N. Store-operated Ca^{2+} channels in prostate cancer epithelial cells: function, regulation, and role in carcinogenesis. *Cell Calcium* 2003;33:357–373. [PubMed: 12765682]
138. Varga-Szabo D, Braun A, Kleinschnitz C, Bender M, Pleines I, Pham M, Renne T, Stoll G, Nieswandt B. The calcium sensor STIM1 is an essential mediator of arterial thrombosis and ischemic brain infarction. *J Exp Med* 2008;205:1583–1591. [PubMed: 18559454]

139. Varga-Szabo D, Braun A, Nieswandt B. Calcium signaling in platelets. *J Thromb Haemost* 2009;7:1057–1066. [PubMed: 19422456]
140. Venkatachalam K, Montell C. TRP channels. *Annu Rev Biochem* 2007;76:387–417. [PubMed: 17579562]
141. Vig M, Beck A, Billingsley JM, Lis A, Parvez S, Peinelt C, Koomoa DL, Soboloff J, Gill DL, Fleig A, Kinet JP, Penner R. CRACM1 multimers form the ion-selective pore of the CRAC channel. *Curr Biol* 2006;16:2073–2079. [PubMed: 16978865]
142. Vig M, DeHaven WI, Bird GS, Billingsley JM, Wang H, Rao PE, Hutchings AB, Jouvin MH, Putney JW, Kinet JP. Defective mast cell effector functions in mice lacking the CRACM1 pore subunit of store-operated calcium release-activated calcium channels. *Nat Immunol* 2008;9:89–96. [PubMed: 18059270]
143. Vig M, Peinelt C, Beck A, Koomoa DL, Rabah D, Koblan-Huberson M, Kraft S, Turner H, Fleig A, Penner R, Kinet JP. CRACM1 is a plasma membrane protein essential for store-operated Ca^{2+} entry. *Science* 2006;312:1220–1223. [PubMed: 16645049]
144. Villalobos C, Garcia-Sancho J. Capacitative Ca^{2+} entry contributes to the Ca^{2+} influx induced by thyrotropin-releasing hormone (TRH) in GH3 pituitary cells. *Pflugers Arch* 1995;430:923–935. [PubMed: 8594545]
145. Voets T, Prenen J, Fleig A, Vennekens R, Watanabe H, Hoenderop JG, Bindels RJ, Droogmans G, Penner R, Nilius B. CaT1 and the calcium release-activated calcium channel manifest distinct pore properties. *J Biol Chem* 2001;276:47767–47770. [PubMed: 11687570]
146. Wechsler HL, Fisher ER. Eccrine glands of the rat. Response to induced sweating, hypertension, uremia, and alterations of sodium state. *Arch Dermatol* 1968;97:189–201. [PubMed: 4295124]
147. Williams RT, Manji SS, Parker NJ, Hancock MS, Van Stekelenburg L, Eid JP, Senior PV, Kazenwadel JS, Shandala T, Saint R, Smith PJ, Dziadek MA. Identification and characterization of the STIM (stromal interaction molecule) gene family: coding for a novel class of transmembrane proteins. *Biochem J* 2001;357:673–685. [PubMed: 11463338]
148. Williams RT, Senior PV, Van Stekelenburg L, Layton JE, Smith PJ, Dziadek MA. Stromal interaction molecule 1 (STIM1), a transmembrane protein with growth suppressor activity, contains an extracellular SAM domain modified by N-linked glycosylation. *Biochim Biophys Acta* 2002;1596:131–137. [PubMed: 11983428]
149. Worley PF, Zeng W, Huang GN, Yuan JP, Kim JY, Lee MG, Muallem S. TRPC channels as STIM1-regulated store-operated channels. *Cell Calcium* 2007;42:205–211. [PubMed: 17517433]
150. Wright JT, Hart PS, Aldred MJ, Seow K, Crawford PJ, Hong SP, Gibson CW, Hart TC. Relationship of phenotype and genotype in X-linked amelogenesis imperfecta. *Connect Tissue Res* 2003;44 (Suppl 1):72–78. [PubMed: 12952177]
151. Wu MM, Buchanan J, Luik RM, Lewis RS. Ca^{2+} store depletion causes STIM1 to accumulate in ER regions closely associated with the plasma membrane. *J Cell Biol* 2006;174:803–813. [PubMed: 16966422]
152. Xu SZ, Beech DJ. TrpC1 is a membrane-spanning subunit of store-operated Ca^{2+} channels in native vascular smooth muscle cells. *Circ Res* 2001;88:84–87. [PubMed: 11139478]
153. Yamashita M, Navarro-Borelly L, McNally BA, Prakriya M. Orai1 mutations alter ion permeation and Ca^{2+} -dependent fast inactivation of CRAC channels: evidence for coupling of permeation and gating. *J Gen Physiol* 2007;130:525–540. [PubMed: 17968026]
154. Yang S, Zhang J, Huang X. Orai1 and STIM1 are critical for breast tumor cell migration and metastasis. *Cancer Cell* 2009;15:124–134. [PubMed: 19185847]
155. Yeromin AV, Zhang S, Jiang W, Yu Y, Safrina O, Cahalan MD. Molecular identification of the CRAC channel by altered ion selectivity in a mutant of Orai. *Nature* 2006;443:226–229. [PubMed: 16921385]
156. Yuan JP, Kim MS, Zeng W, Shin DM, Huang G, Worley PF, Muallem S. TRPC channels as STIM1-regulated SOCs. *Channels (Austin)* 2009;3:221–225. [PubMed: 19574740]
157. Yuan J, Zeng W, Dorwart MR, Choi Y, Worley P, Muallem S. SOAR and the polybasic STIM1 domains gate and regulate Orai channels. *Nat Cell Biol* 2009;11:337–343. [PubMed: 19182790]
158. Yue L, Peng J, Hediger M, Clapham D. CaT1 manifests the pore properties of the calcium-release-activated calcium channel. *Nature* 2001;410:705–709. [PubMed: 11287959]

159. Zeng W, Yuan J, Kim M, Choi Y, Huang G, Worley P, Muallem S. STIM1 Gates TRPC channels, but not Orai1, by electrostatic interaction. *Mol Cell* 2008;32:439–448. [PubMed: 18995841]
160. Zhang SL, Yeromin AV, Zhang XH, Yu Y, Safrina O, Penna A, Roos J, Stauderman KA, Cahalan MD. Genome-wide RNAi screen of Ca^{2+} influx identifies genes that regulate Ca^{2+} release-activated Ca^{2+} channel activity. *Proc Natl Acad Sci U S A* 2006;103:9357–9362. [PubMed: 16751269]
161. Zonana J, Elder ME, Schneider LC, Orlow SJ, Moss C, Golabi M, Shapira SK, Farndon PA, Wara DW, Emmal SA, Ferguson BM. A novel X-linked disorder of immune deficiency and hypohidrotic ectodermal dysplasia is allelic to incontinentia pigmenti and due to mutations in $\text{IKK}\gamma$ (NEMO). *Am J Hum Genet* 2000;67:1555–1562. [PubMed: 11047757]
162. Zweifach A, Lewis RS. Mitogen-regulated Ca^{2+} current of T lymphocytes is activated by depletion of intracellular Ca^{2+} stores. *Proc Natl Acad Sci U S A* 1993;90:6295–6299. [PubMed: 8392195]

Fig. 1.

Functional domains and human mutations in ORAI1 and STIM1. **a** ORAI1 contains four transmembrane domains (M1–4) with the alpha-helical M1 lining the calcium release activated calcium (CRAC) channel pore [84]. Putative Ca²⁺-binding glutamate and as-partate residues (E106, D110, and D112) [114,141,155], a calmodulin binding domain (CBD) [89], a coiled-coil domain (CC) containing a leucine residue (L273) critical for STIM1 binding [88], and a glycosylation site in the second extracellular loop (N223) [49] are shown. Mutations affecting ORAI1 function or protein expression in human patients are indicated in *red* (for details, see text) [40,82]. **b** STIM1 contains an N-terminal Ca²⁺ binding EF hand domain and a sterile alpha motif (SAM) both of which are localized in the ER; the cytoplasmic C terminus comprises

two coiled-coil (CC), a serine/proline rich (S/P), and a polybasic lysine-rich (K) domain. A minimal CRAC channel binding and activation domain (alternatively termed CRAC channel activation domain (CAD), STIM1 Orai activating region (SOAR), Orai1 activating small fragment (OASF), and coiled-coil fragment b9 (CCb9) [63,87,103,157] and an adjacent CRAC modulatory domain (CMD) [28,69,89] are shown. The position of a frameshift nonsense mutation abolishing STIM1 expression in human patients is indicated in *red* (for details see text) [110]

Fig. 2.

STIM1-mediated activation of ORAI1 during store-operated Ca^{2+} entry. In cells with replete ER stores, STIM1 is localized in an inactive configuration in the membrane of the ER away from the plasma membrane and ORAI1 (*left*). Depletion of Ca^{2+} stores by—for instance—antigen binding to immunoreceptors (such as the T cell or B cell receptor) [36] or engagement of G protein coupled receptors (GPCR) triggers Ca^{2+} release from the ER through InsP_3R . The reduction in $[\text{Ca}^{2+}]_{\text{ER}}$ results in dissociation of Ca^{2+} from the N-terminal EF hand of STIM1 and unfolding of the EFh-SAM domain followed by multimerization of STIM1 (*right*) [20, 130]. STIM1 translocates to sections of the ER juxtaposed to the plasma membrane and into large STIM1 clusters (puncta). It binds directly to ORAI1 via a minimal calcium release activated calcium (CRAC) channel binding and activation domain (green box) [63,87,103, 157] that interacts with binding sites in the N and C terminus of ORAI1 (*green and yellow ovals*; see Fig. 1). STIM1 binding opens ORAI1 CRAC channels—here shown as a tetramer [60,85,108]—and recruits ORAI1 into puncta that coincide with localized Ca^{2+} influx [70]

Fig. 3.
ORAI1 and STIM1 deficiency in human patients and mice. **a** Situation in cells expressing both ORAI1 and STIM1. The minimal calcium release activated calcium (CRAC) channel binding and activation domain in STIM1 (green box) is shown to activate the ORAI1 CRAC channel. **b** Situation in patients and mice with ORAI1-R91W [40] and R93W [11] mutation, respectively. Mutant ORAI1 is expressed and binds STIM1 but cannot be activated. **c** Situation in patients [82] and mice [17,50,142] lacking ORAI1 expression. ORAI2 and ORAI3 potentially compensate for the absence of ORAI1 in tissues in which they are expressed. **d** Situation in patients [110] and mice [7,95,132,138] lacking STIM1 expression. STIM2 potentially compensates for the absence of STIM1 in tissues in which it is expressed

Fig. 4.

ORAI1 tissue expression. ORAI1 protein expression in human tissues isolated from healthy donors incubated with an antibody directed against the C terminus of ORAI1 (for details, see [82]). ORAI1 is detected (*arrows*) in vascular endothelial cells of blood vessels in thymus (**a**), spleen (**b**), and heart (**c**); cardiac fibroblasts (**c**); ductal epithelial cells in the exocrine pancreas (**d**); tubular epithelial cells in the kidney (**e**); epithelial cells in salivary gland (**f**), breast (**g**), and prostate (**h**); seminiferous tubule cells in the testis (**i**); and stratified squamous nonkeratinized epithelial cells in the cervix (**j**). *A* artery, *Lu* lumen, *V* vein

Table 1

Comparison of calcium release activated calcium (CRAC) channelopathies in human and mice

	Orai1		STIM1	
	Human	Mouse	Human	Mouse
Perinatal lethality	No	Yes	No	Yes
Survival limited by	Immunodeficiency [40,41,68,82,105]	Unknown (myopathy?)	Immunodeficiency	Unknown (myopathy?) [132]
Immune dysfunction	Infections T cell activation defect [40,41,68,82,105]	T cell defect [50] Mast cell defect [142]	Infections T cell activation defect Treg numbers ↓	T cell defect [13,95] Mast cell defect [7] Macrophage defect [16]
Autoimmunity	(Neutropenia, thrombocytopenia ^a)[82]	No	Splenomegaly, lymphadenopathy AIHA, thrombocytopenia	No [95] (lymphoproliferation [13] ^b)
Myopathy	Congenital, global Respiratory muscle insufficiency Atrophy of type II muscle fibers [82]	Myopathy? [11,142]	Congenital, global	Myotube dysfunction Mitochondriopathy [132]
Ectodermal dysplasia	Dental enamel defect (amelogenesis imperfecta type III) [82] Anhydrosis [82]	Alopecia [50]	Dental enamel defect	
Thrombocyte function	No bleeding diathesis	Platelet activation ↓ [11,17] Thrombus formation ↓ [17]	No bleeding diathesis Thrombocytopenia (autoimmune)	Platelet activation ↓[138] Thrombus formation ↓[138]
References	[40,41,68,82,105]	[11,17,50,142]	[110]	[7,13,16,95,132,138]

AIHA autoimmune hemolytic anemia^a Observed in one of six patients^b Observed in some but not all Stim1^{-/-} strains

Table 2**Tissue distribution of human ORAI1**

Lymphoid tissues	
Spleen	Periarteriolar lymphoid sheath (PALS); ND in lymphatic nodules and germinal center
Tonsils	Lymphoid follicles and paracortical region (minority of cells)
Thymus	Hassall corpuscles; medulla and cortex (minority of cells)
Endocrine system	
Adrenal gland	Medulla: chromaffine cells; Cortex: ND
Thyroid and parathyroid	Parathyroid gland: chief cells; thyroid follicular cells (weak)
Hypophysis	Pars anterior (minority of cells)
Glands	
Prostate	Columnar epithelium
Salivary gland	Ductal epithelium; weak in acinar cells
Mammary	Ductal epithelium, alveolar buds, and alveoli
Pancreas	Exocrine: acinar cells, ductal epithelium. Endocrine: Islet (alpha?) cells
Integument	
Epidermis	Stratum basale and spinosum, hair follicles, eccrine, and sebaceous glands
Stratified squamous nonkeratinized epithelium	Esophagus and cervix (predominantly in basal layers)
Cardiovascular and respiratory system	
Heart	Cardiomyocytes: ND
Blood vessels	Vascular endothelium of arterioles, venules, capillaries
Lung	Pneumocytes, bronchial epithelium, alveolar macrophages
Gastrointestinal tract and kidney	
Stomach (fundus)	Epithelium, parietal cells
Small/large intestine	Epithelium, goblet cells (colon)
Liver	Hepatocytes
Kidney	Bowman capsule, tubules (strongly positive in minority, ND in majority of tubules)
Nervous system	
Cerebrum, cerebellum	Cortex, medulla: ND
Muscle	
Skeletal muscle	Sarcolemma of muscle fibers

Tissue expression of ORAI1 was determined by immunohistochemical analysis of tissue samples from healthy donors using an anti-ORAI1 antibody (for details, see [82]). The table is a compilation of data from several tissue microarrays and tissue samples including those shown in Fig. 4

ND not detected