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Physiological Functions of CRAC Channels

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Abstract

Store-operated Ca^{2+} entry (SOCE) is a ubiquitous Ca^{2+} signaling pathway that is evolutionarily conserved across eukaryotes. SOCE is triggered physiologically when the endoplasmic reticulum (ER) Ca^{2+} stores are emptied through activation of inositol 1,4,5-trisphosphate receptors. SOCE is mediated by the Ca^{2+} release-activated Ca^{2+} (CRAC) channels, which are highly Ca^{2+} selective. Upon store depletion, the ER Ca^{2+}-sensing STIM proteins aggregate and gain extended conformations spanning the ER–plasma membrane junctional space to bind and activate Orai, the pore-forming proteins of hexameric CRAC channels. In recent years, studies on STIM and Orai tissue-specific knockout mice and gain- and loss-of-function mutations in humans have shed light on the physiological functions of SOCE in various tissues. Here, we describe recent findings on the composition of native CRAC channels and their physiological functions in immune, muscle, secretory, and neuronal systems to draw lessons from transgenic mice and human diseases caused by altered CRAC channel activity.

INTRODUCTION

The physiological function of all eukaryotic cells is regulated by intracellular calcium (Ca^{2+}). Ca^{2+} is indispensable as a secondary messenger that regulates a diverse array of cellular processes, including fertilization, immunity, contraction, metabolism, transcription, and apoptosis (1–5). Although essential in all tissue types, cytosolic Ca^{2+} must be maintained at extremely low concentrations (~100 nM) relative to the enormous concentration in the extracellular milieu (~1–2 mM). Altered Ca^{2+} homeostasis and Ca^{2+} overload are implicated in a wide variety of inflammatory, neurological, and metabolic disorders (6–8). To maintain this steady state, cells utilize an exquisite array of ion channels, transporters, and exchangers to store Ca^{2+} within the endoplasmic reticulum (ER) (300 μM–1 mM) and pump excess Ca^{2+} out across the plasma membrane (PM) (1). The predominant mechanism of Ca^{2+} entry in nonexcitable cells is through the ubiquitous store-operated Ca^{2+} entry (SOCE) pathway, first recognized and formulated by Jim Putney (9). SOCE involves a complex choreography between PM Orai channel proteins and ER-resident Ca^{2+}-sensing stromal interaction molecules (STIMs) (4, 5, 10). Stimulation of PM receptors coupled to isoforms of the phosphoinositide-specific phospholipase C (PLC) results in production of diffusible inositol 1,4,5-trisphosphate (IP_3), which triggers release of ER Ca^{2+} through IP_3 receptors (IP_3Rs). The depletion of ER Ca^{2+} is sensed by STIM1 and its homolog STIM2, causing a conformational change of STIM proteins, which unmasks their C-terminal STIM Orai-activating region/CRAC-activating domain (SOAR/CAD) (11, 12), and their translocation to ER–PM junctions where they trap and gate PM Orai channels to drive Ca^{2+} entry into the cell (1, 4, 5, 10) (**Figure 1**). The biophysical manifestation of STIM/Orai-mediated SOCE is the Ca^{2+} release-activated Ca^{2+} (CRAC) current, I_{CRAC} (13, 14).

The tissue-specific functions of CRAC channels have largely been informed by the identification of human patients with inherited loss-of-function (LoF) mutations in *Orai1* and *STIM1* (15–18). These patients manifest with a variety of symptoms, including severe combined immunodeficiency with reoccurring viral, bacterial, and fungal infections, autoimmunity, hemolytic anemia, ectodermal dysplasia with defective dental enamel development and anhidrosis, and muscle hypotonia (19). Patients with defective CRAC channel function were identified in the 1990s, but the molecular identity of CRAC remained unknown for over a decade (20–22). Large-scale RNA interference (RNAi) screening against proteins with conserved Ca^{2+} signaling domains first led to the identification of STIM1 (23, 24). Genome-wide RNAi screening and genetic linkage analysis discovered the identity of Orai1 (17, 25, 26). These landmark findings have since led to the development of new transgenic animal models to understand the function of CRAC channels in physiology and pathophysiology (5). It is now well established that Orai1 is an essential subunit of the native CRAC channel and that Orai1 and STIM1 contribute to SOCE in most primary tissue types that have been investigated (27, 28).

Although the physiological functions of Orai1 and STIM1 have been the subject of intense study over the past 15 years, vertebrates have evolved a second STIM protein, STIM2, and two additional Orai isoforms encoded by independent genes. *STIM2* and *Orai2* genes are found in all vertebrates, whereas the *Orai3* gene is exclusive to mammals, potentially duplicating from the *Orai1* gene (29). Extensive work has demonstrated that Orai2 and Orai3 are highly homologous to Orai1 and that both homologs form functional CRAC channels when ectopically expressed with STIM1, albeit with distinct biophysical properties (30, 31). Despite Orai2 and Orai3 mRNAs being ubiquitously expressed, the role these channels play under native conditions has largely remained obscure. There are currently no reported patient mutations in either *Orai2* or *Orai3* genes that are associated with disease. Further, the lack of reliable antibodies, channel potentiators, or inhibitors that can distinguish between Orai isoforms has hampered our understanding of the

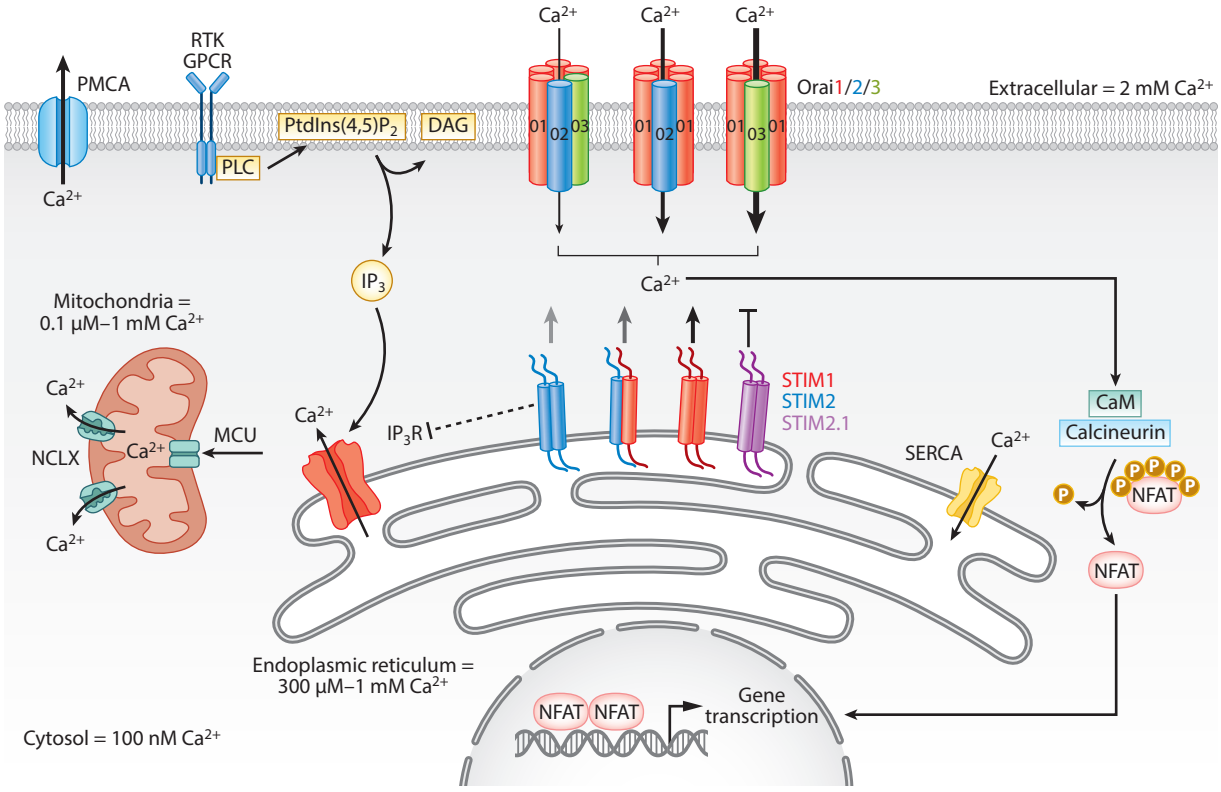

Figure 1

Activation of CRAC channels. Binding of agonists to their specific PLC-coupled receptors in the PM leads to production of IP_3 and release of Ca^{2+} from ER stores. ER store depletion activates STIM proteins, which translocate to ER–PM junctions where they trap and gate CRAC channels composed of hexameric Orai subunits. Native CRAC channels are likely heterohexamers of Orai1/2/3 isoforms with varying potencies. Different STIM homodimers or heterodimers work together to activate or inhibit (e.g., STIM2.1) CRAC channels, thus fine-tuning their activity. Resting STIMs tonically inhibit IP_3Rs. This complex choreography between STIM/Orai/IP_3Rs ensures that Ca^{2+} release, Ca^{2+} entry, and NFAT activation are coordinated and tailored to the concentration of agonist and level of store depletion. Abbreviations: CaM, calmodulin; CRAC, Ca^{2+} release-activated Ca^{2+}; DAG, diacylglycerol; ER, endoplasmic reticulum; GPCR, G protein–coupled receptor; IP_3R, inositol 1,4,5-trisphosphate receptor; MCU, mitochondrial Ca^{2+} uniporter; NCLX, $Na^+/Ca^{2+}/Li^+$ exchanger; NFAT, nuclear factor of activated T cells; PLC, phospholipase C; PM, plasma membrane; PMCA, plasma membrane Ca^{2+} ATPase; PtdIns(4,5)P_2, phosphatidylinositol 4,5-bisphosphate; RTK, receptor tyrosine kinase; SERCA, sarco-/endoplasmic reticulum Ca^{2+}-ATPase; STIM, stromal interaction molecule.

physiological functions of Orai2 and Orai3. Similarly, small interfering RNA (siRNA) knockdown approaches used in earlier studies largely failed to identify significant contributions of either Orai2 or Orai3 to native SOCE induced by maximal store depletion, except for Orai3 in subsets of cancer cells (32, 33).

Nevertheless, knockout mice and CRISPR/Cas9 gene knockout in cell lines have shed light on the role of Orai2 and Orai3 within the native cellular context. Global Orai2 knockout mice and cell lines devoid of each Orai isoform individually and in combination have provided strong evidence that Orai2 and Orai3 form heteromeric CRAC channels with Orai1 and negatively regulate native CRAC channel activity (34–37). Within the native CRAC channel hexamer, current evidence supports a model whereby Orai2 and Orai3 negatively regulate Orai1 function and expand the repertoire of receptor-stimulated Ca^{2+} signaling events (**Figure 2**). This reflects the enhanced

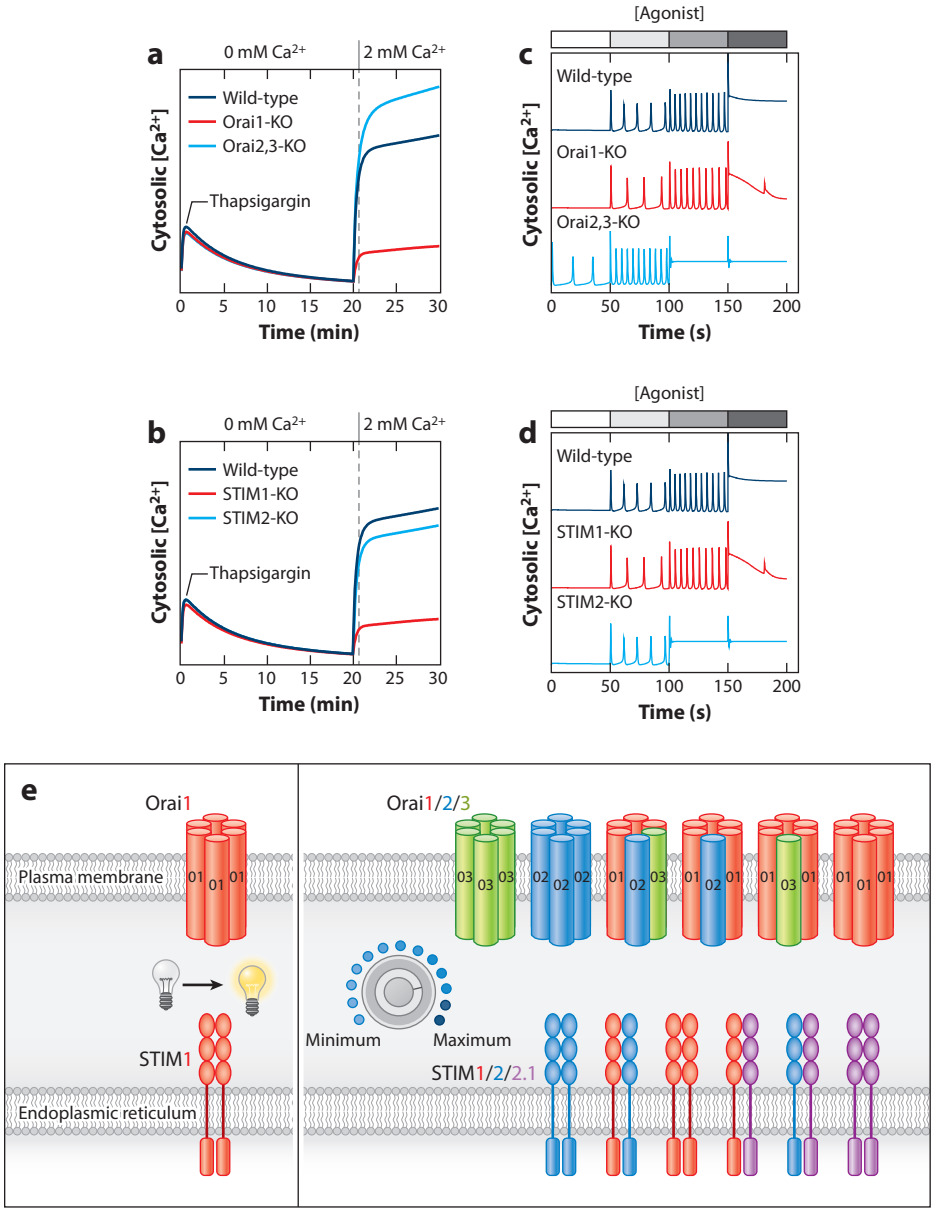

Figure 2

Multiple Orai and STIM isoforms underlie the diversity of Ca^{2+} signaling events. Orai1 (*a*) and STIM1 (*b*) mediate the majority of SOCE in response to maximal store depletion, whereas Orai2/3 are negative regulators (*a*). (*c*,*d*) In response to agonist stimulation, cells lacking either Orai1 or STIM1 cannot support SOCE plateaus, and cells lacking either Orai2/3 or STIM2 develop plateaus at lower agonist concentrations and thus have a reduced bandwidth of Ca^{2+} signaling events. (*e*) Orai1 and STIM1 are sufficient to support SOCE, but the graded diversity of Ca^{2+} oscillatory frequencies is largely reduced (on/off switch). Involvement of various STIM isoforms and splice variants and that of homo- and heterohexameric CRAC channels made of combinations of Orai isoforms support the graded diversity of mammalian Ca^{2+} signaling events (dimmer switch). Abbreviations: CRAC, Ca^{2+} release-activated Ca^{2+}; KO, knockout; SOCE, store-operated Ca^{2+} entry; STIM, stromal interaction molecule.

interaction of both Orai2 and Orai3 with STIM1 under conditions where ER Ca^{2+} stores are replete, as well as the enhanced fast Ca^{2+}-dependent inactivation of these two isoforms (36, 38). Thus, different heteromeric combinations of the Orai isoform trio provide a robust mechanism to fine-tune SOCE by matching the strength of agonist stimulation to downstream Ca^{2+} signaling events (**Figure 2**). Although the precise Orai heteromeric assembly or assemblies in any given cell type remain unknown, different CRAC heteromers are likely tailored to specific cell types and their respective functions. For instance, Orai1 is upregulated while Orai2 is downregulated in effector T cells in comparison to naïve T cells (35). Orai2-mediated inhibition of CRAC channel activity in naïve T cells likely prevents unwanted activation of those naïve T cells. Unlike Orai1 and Orai2, Orai3 is resistant to oxidant-mediated inhibition (39, 40), and some cancer cells remodel to signal through Orai3 (33, 41–43), likely as a mechanism to maintain channel activity in hypoxic or oxidant-rich tumor environments.

Similarly, new findings have further clarified the physiological function of STIM2. While earlier studies characterized STIM2 as an essential regulator of basal ER and cytosolic Ca^{2+} levels (44), recent evidence suggests that STIM2 is an active participant in SOCE across the full spectrum of agonist strengths (45). STIM2 plays a critical role in STIM1 recruitment to drive efficient activation of Orai1 at low agonist intensities (46, 47). Recent studies have also revealed that STIM2 is a major contributor to SOCE in specific cell types, including neurons (48), colorectal carcinoma cells (49), and NIH T3T fibroblasts and the gonadotropic cell line αT3 (50). STIM1 and STIM2 coordinate across a range of agonist concentrations for optimal activation of nuclear factor of activated T cells (NFAT) (45, 49, 50). Emrich et al. (45) showed that while activated STIM proteins interact with Orai to stimulate Ca^{2+} entry, unactivated STIMs inhibit IP_3Rs, thus tailoring Ca^{2+} release to Ca^{2+} entry (**Figure 1**). Interestingly, a widely expressed STIM2 splice variant, STIM2.1 (also called STIM2β), was identified. STIM2.1 is a potent negative regulator of SOCE in several cell types, including $CD4^+$ T, Jurkat, HEK293, and C2C12 cell lines (51, 52). STIM2.1 inhibits Orai1 channel clustering, agonist-induced Ca^{2+} oscillations, and myogenesis (53, 54). As discussed in detail below, STIM1 has a long splice variant called STIM1L that is found in skeletal muscle (55). Recently, Ramesh et al. (56) identified a short splice variant of STIM1 that is exclusive to neurons, called STIM1B. STIM1B locates to presynaptic sites and mediates CRAC currents with slower kinetics and reduced inactivation that support short-term synaptic enhancement during high-frequency neuronal stimulation (56).

In summary, while both STIM1 and Orai1 are undoubtedly the major components of the canonical SOCE pathway, their combined function represents only a narrow window of the diverse Ca^{2+} signaling events (**Figure 2**). It is becoming apparent that all five Orai and STIM members and their splice variants, along with IP_3Rs and likely other regulatory proteins, continuously operate in a harmonious manner to shape the diversity of the physiological Ca^{2+} signal in response to the graded concentrations of agonist encountered by cells in vivo. Below, we seek to highlight new research on the role of SOCE in physiology and disease, within the context of this dynamic model of STIM/Orai isoform interactions. We discuss how SOCE function is tailored to different tissue systems and regulates diverse cellular functions, including proliferation, secretion, contraction, differentiation, development, and metabolism.

IMMUNE SYSTEM

T Cells

In T cells, Ca^{2+} influx through CRAC channels is required for the activation of the calcineurin/NFAT pathway, subsequent IL-2 secretion and clonal expansion (1). One of the major phenotypes of patients with LoF mutations in *Orai1* or *STIM1* is severe combined

immunodeficiency coupled with autoimmune disease, including hemolytic anemia, thrombocytopenia, lymphadenopathy, and hepatosplenomegaly (28). The role of SOCE in T cell function has been extensively reviewed elsewhere (1, 3, 57, 58). Below, we highlight critical progress within the field grouped into three broad categories: (*a*) molecular makeup of native CRAC channels in T cells, (*b*) Ca^{2+}-mediated regulation of T cell metabolism, and (*c*) the functions of SOCE in specific T cell subsets.

CRAC makeup in T cells. In patients with the R91W LoF mutation in *Orai1*, T cells display a complete loss of SOCE and I_{CRAC} (15–17). Of note, residual SOCE is observed in B cells from patients with LoF in *Orai1* (21). Curiously, SOCE in T cells isolated from global Orai1$^{-/-}$ mice on the mixed Institute of Cancer Research (ICR) background, CD4^{+} T cell–specific knockout (Orai1$^{fl/fl}$Cd4^{cre}) mice, or Orai1^{R93W} knock-in mice (equivalent of human R91W) is only partially attenuated, suggesting that Orai2 and/or Orai3 mediate the residual SOCE in mouse T cells (35, 59, 60). Surprisingly, SOCE in naïve, but not effector, Orai2$^{-/-}$ T cells was enhanced while Orai1$^{-/-}$ Orai2$^{-/-}$ T cells displayed a near complete loss of SOCE (35). I_{CRAC} was increased in Orai2$^{-/-}$ bone marrow–derived macrophages. Critically, ectopic expression of pore-dead mutants for either Orai1 (E106Q) or Orai2 (E80Q) blocked native SOCE in Orai1$^{-/-}$ or Orai2$^{-/-}$ T cells, suggesting that Orai1 and Orai2 form heteromeric CRAC channels. Orai1$^{fl/fl}$Cd4^{cre}/Orai2$^{-/-}$ double knockout mice show impaired regulatory T cell development and reduced germinal center (GC) formation in response to viral infection. Adoptive transfer of Orai1$^{-/-}$ Orai2$^{-/-}$ T cells into immune-deficient mice also failed to induce colitis or graft-versus-host disease (35). These data on Orai2$^{-/-}$ T cells is in agreement with results from Orai2-deficient mast cells, Orai2 knockdown human chondrocyte and neuroglioma cell lines, and Orai2 knockout HEK293 cells, where SOCE is also enhanced (34, 36, 61, 62). Cumulatively, these studies identify Orai2 as a robust negative regulator of Orai1 channel function. In addition to its role as a negative regulator of native CRAC channels, Orai3 was proposed as a component of an Orai1/Orai3 heteromeric channel activated independently of store depletion by arachidonic acid and its metabolite, leukotriene C_4, and termed arachidonate-regulated Ca^{2+} (ARC) channel (38, 63–65). In contrast to Orai1 and Orai2, the role of native Orai3 in T cell function is less clear. Earlier reports demonstrated that Orai3 is upregulated in human effector T cells as a potential mechanism to resist oxidative stress within inflammatory environments (40). A recent study showed that reduced expression of the transcriptional repressor IKAROS causes chronic inflammation through enhanced Orai3 expression and ARC activity in T cells from rheumatoid arthritis patients (66). Whether native Orai3 contributes to the miniscule amount of SOCE remaining in Orai1$^{-/-}$ Orai2$^{-/-}$ T cells remains unknown (35). Furthermore, whether the composition of native CRAC channels substantially varies between T cell subsets [T helper (Th)1, Th2, Th17, and regulatory T cells (Tregs)] and whether the Orai stoichiometry within these subsets in vivo is fixed or dynamic are important questions that remain unanswered.

CRAC and T cell metabolism. Patients and mice with SOCE-deficient T cells have largely unaltered thymic T cell development (28). Patients with LoF *Orai1* or *STIM1* display normal frequencies of conventional αβ T cell subsets, yet their T cells are severely defective in cytokine and chemokine expression and differentiation into effector subsets and fail to proliferate in response to antigenic stimulation due to the lack of SOCE (3). Furthermore, proliferation of T cells from patients with *Orai1* or *STIM1* LoF mutations is significantly impaired even in the presence of exogenous interleukin 2 (IL-2), which is a potent cytokine that signals through Ca^{2+}-independent mechanisms, suggesting that impairment of additional pathways contributes to this proliferative defect (20, 67, 68). Naïve T cells are maintained in a metabolically quiescent state with minimal

biosynthesis, and T cell receptor (TCR) stimulation triggers the rapid uptake of nutrients as T cells prepare to enter the cell cycle and clonally expand (69). This metabolic reprogramming is driven through a key switch from oxidative phosphorylation to aerobic glycolysis, as glucose is the primary carbohydrate source utilized in anabolic metabolism (69). How initiation of SOCE and its downstream signaling effectors, namely the calcineurin-NFAT pathway, contributes to this initial metabolic reprogramming and proliferation of T cells had long been obscure. Utilizing $CD4^+$ T cell–specific double STIM1/STIM2 knockout ($Stim1^{fl/fl}Stim2^{fl/fl}Cd4^{cre}$) mice, Feske and coworkers (70) showed that SOCE, through Ca^{2+}-dependent activation of NFAT and subsequent upregulation of the transcription factors cellular-Myc (c-Myc), interferon regulatory factor 4 (IRF4), and hypoxia-inducible factor 1 alpha (HIF1α), regulates the expression of the glucose transporters GLUT1 and GLUT3, along with key glycolytic and mitochondrial respiration chain enzymes. The transcriptional regulation of these transporters and metabolic enzymes, as well as the expression of the master transcription factors c-Myc, HIF1α, and IRF4, was mediated through NFAT1 and NFAT2. Ectopic expression of a constitutively active form of NFAT2 or GLUT1 in T cells from $Stim1^{fl/fl}Stim2^{fl/fl}Cd4^{cre}$ mice rescued defects in glucose uptake and c-Myc/HIF1α/IRF4 expression, while partially restoring in vivo proliferative capacity in response to lymphocytic choriomeningitis virus (LCMV) infection. Conversely, treatment of wild-type T cells with the calcineurin inhibitor FK506 phenocopied the proliferative and metabolic defects of $STIM1^{-/-}$ $STIM2^{-/-}$ T cells. SOCE and calcineurin were also proposed to post-translationally regulate members of the PI3K-AKT-mTOR (phosphatidylinositol 3-kinase-AKT-mechanistic target of rapamycin) pathway as phosphorylation of AKT, mTOR, and S6 ribosomal protein was significantly impaired in T cells from $Stim1^{fl/fl}Stim2^{fl/fl}Cd4^{cre}$ mice. How Ca^{2+} signals through SOCE regulate the PI3K-AKT-mTOR pathway in T cells is currently unclear. These data further highlight the central role of calcineurin in T cell function and the clinical efficacy of calcineurin inhibitors as potent immunosuppressants (71). Interestingly, defects in the proliferation and metabolic reprogramming of $STIM1^{-/-}$ $STIM2^{-/-}$ T cells can be partially bypassed by IL-2 and IL-7 costimulation, but not by either cytokine alone (70).

CRAC in specific T cell subsets. Following maturation in the thymus, T cells commit to various lineages based on expression of the glycoproteins CD4 (helper T cells) and CD8 (cytotoxic T cells) (72). Further differentiation of naïve $CD4^+$ T cells into effector subsets is driven by cytokines and secondary signals that induce activation of lineage-specific transcription factors and gene programs (72). SOCE is critical in driving the differentiation and unique genetic profiles of $CD4^+$ T cell subsets. In response to immunization or infection with pathogens, the humoral immune response regulates the production of antigen-specific antibodies and long-lived immunological memory. This antibody response is orchestrated through $CD4^+$ T follicular helper (Tfh) cells that traffic to secondary lymphoid organs to provide cognate help to GC B cells for efficient class-switch recombination and affinity maturation (73). Critically, antigen-specific antibody responses must be counterbalanced by the function of T follicular regulatory (Tfr) cells that dampen the GC response to prevent autoantibody production (73, 74). Vaeth et al. (75) identified a dichotomous role for SOCE in the differentiation of both Tfh and Tfr cells. By studying humoral immune responses to acute and chronic LCMV infection in $Stim1^{fl/fl}Stim2^{fl/fl}Cd4^{cre}$ mice, they showed that SOCE is essential for the expression of the key transcription factors IRF4, basic leucine zipper transcription factor, ATF-like (BATF), and B cell lymphoma 6 (Bcl6) that drive Tfh/Tfr differentiation. Accordingly, LCMV-infected $Stim1^{fl/fl}Stim2^{fl/fl}Cd4^{cre}$ mice display significantly reduced Tfh populations, fail to generate LCMV-specific IgG antibodies, and ultimately succumb to chronic infection. These effects were predominately due to impaired activation of the NFAT2αA isoform. In agreement with previous work, $Stim1^{fl/fl}Stim2^{fl/fl}Cd4^{cre}$ mice also show

reduced populations of Foxp3$^+$ central Tregs and even further reductions in differentiation to Foxp3$^+$ Tfr cells (76). The lack of Tfr populations leaves the GC response unchecked, allowing for expansion of self-reactive Tfh cells, production of autoantibodies from GC B cells, and severe autoimmunity in aging Stim1$^{fl/fl}$Stim2$^{fl/fl}$Cd4^{cre} mice (75). These studies argue that the inability to produce antigen-specific antibodies in LoF *Orai1* patients is due to defective Tfh function and differentiation and not to impaired B cell populations.

Although SOCE is dispensable for the development of immune cells, one notable exception is the development of Foxp3$^+$ Tregs. The role of SOCE in regulating immunological tolerance and preventing autoimmunity has been attributed to defects in development of Foxp3$^+$ Tregs in the thymus of SOCE-deficient mice (76). It has long been appreciated that the magnitude of SOCE is different between lymphocyte subsets and between naïve and memory T cells (77, 78). Tregs appear to have the largest SOCE response owing to a less prominent Orai2 expression in these cells (78). However, whether the magnitude of SOCE is related to its unique role in the development of Tregs is unclear. SOCE regulates the complex transcriptional programs that drive peripheral tissue-resident Treg and Tfr differentiation. Using mice lacking STIM1 and STIM2 in mature Foxp3$^+$ Tregs (Stim1$^{fl/fl}$Stim2$^{fl/fl}$Foxp3^{YFPcre}), researchers revealed that SOCE controls the expression of chemokine receptors and integrins required for tissue homing and trafficking, along with multiple metabolic pathways (IL-2 signaling and mTOR) and transcription factors required for tissue-resident Treg differentiation and function (79). Accordingly, tissue-resident Foxp3$^+$ Treg populations were largely ablated in the bone marrow, liver, and lungs, but not in the blood or secondary lymphoid organs of Stim1$^{fl/fl}$ Stim2$^{fl/fl}$ Foxp3^{YFPcre} mice. Mice with loss of STIM1 and STIM2 in all T cell populations or selectively in Foxp3$^+$ Tregs develop severe lymphoproliferative and type 2 autoimmune disease driven by robust autoantibody production (79, 80). Interestingly, depletion of B cells substantially reduces autoantibody titers in these mice but is not sufficient to prevent premature death due to systemic multiorgan inflammation, suggesting additional roles of SOCE in regulating Treg function (79).

In addition to altered Treg function, the pathology of a wide variety of autoimmune and inflammatory disorders is driven by another CD4$^+$ T cell subset, Th17 cells (81). Th17 cells are characterized by the secretion of key proinflammatory cytokines (IL-17A, IL-17F, IL-22) and expression of the master transcriptional regulator retinoic acid-related orphan receptor-γt (RORγt) (81). High-throughput screening for novel CRAC channel inhibitors demonstrated a critical role for Orai1-mediated Ca^{2+} signals in the differentiation of Th17 cells both in vitro and in vivo (82). Inhibition of SOCE reduced expression of RORγ, RORα, and IL-23R and protected mice from Th17-mediated experimental autoimmune encephalomyelitis (82, 83). It remains poorly understood why Th17 cells demonstrate increased sensitivity to CRAC channel inhibition compared to other CD4$^+$ subsets or how SOCE regulates pathogenic Th17 development and metabolism. Kaufmann et al. (84) have identified an unexpected role for SOCE in regulating mitochondrial homeostasis and oxidative phosphorylation of pathogenic Th17 cells. In mice with T cell–specific expression of hyperactive STAT3C, which drives robust expansion of Th17 populations, crossed with Stim1$^{fl/fl}$Cd4^{cre} mice to abrogate SOCE specifically in CD4$^+$ T cells, expression of many subunits of the electron transport chain was significantly downregulated. This coincided with reductions in mitochondrial respiration, alterations in mitochondrial ultrastructure, and protection from Th17 cell–mediated inflammation in vivo. RNA sequencing of Th17 cells from STAT3C Stim1$^{fl/fl}$ Cd4^{cre} mice showed reversal to a largely nonpathogenic Th17 cell signature. How SOCE controls the expression of many nuclear encoded components of the electron transport chain in Th17 cells is unresolved, but NFAT-dependent mechanisms are likely involved (84). In contrast to classic αβ T cells, which are the most abundant T cell lineage in humans and mice, the role of SOCE in the development and effector function of unconventional γδ T cells remains unexplored.

B Cells

The role of SOCE in B cells has been significantly understudied and remains largely enigmatic. A landmark study from Matsumoto et al. (85) established the importance of SOCE in regulating B cell survival and proliferation. Although STIM1 mediated the majority of SOCE in B cells, only the deletion of both STIM proteins in mice ($Stim1^{fl/fl}Stim2^{fl/fl}Mb1^{cre}$) significantly impaired B cell proliferation and survival. The relatively minor reduction in proliferation of $STIM1^{-/-}$ B cells phenocopied that of $Orai1^{-/-}$ B cells, which also retain residual SOCE, demonstrating that minute levels of Ca^{2+}, likely mediated by remaining STIM2 and Orai2/3, are sufficient to drive antiapoptotic and proproliferative gene programs in B cells (59, 60). Paradoxically, $Stim1^{fl/fl}Stim2^{fl/fl}Mb1^{cre}$ mice demonstrated normal antibody titers, GC B cell populations, and antibody affinity maturation to both T cell–dependent and –independent antigens (85). Although STIM1 and STIM2 were dispensable for antigen-specific antibody production, they were required for the efficient activation of NFAT1 and the subsequent production of IL-10 by regulatory B cells. Analogous to other immune cell populations, all B cell subsets in the bone marrow, spleen, and peritoneal cavity of $Stim1^{fl/fl}Stim2^{fl/fl}Mb1^{cre}$ mice were unaltered, suggesting that SOCE is dispensable for B cell development (85). Similarly, B cell populations are unchanged in human patients with LoF mutations in *Orai1* and *STIM1* (28). Interestingly, recent work investigating B cell–specific IP_3R triple-knockout mice revealed a substantial reduction in conventional B2 and B1 cell populations in peripheral tissues (86). Loss of all three IP_3Rs also affected acute, but not long-term, T cell–dependent and –independent humoral antibody responses as well as B cell proliferation and survival (86). These results suggest that Ca^{2+} signals mediated by IP_3Rs, but not CRAC channels, are critical for the early positive and negative selection of B cells.

B cell receptor (BCR) stimulation of naïve B cells rapidly upregulates aerobic glycolysis and oxidative phosphorylation to meet the metabolic demands required for efficient B cell differentiation and clonal expansion (87). Berry et al. (88) provided evidence for the critical role of SOCE in regulating B cell survival, cell-cycle entry, and proliferation. They demonstrated that BCR stimulation of $STIM1^{-/-}$ $STIM2^{-/-}$ B cells results in substantial cell death, as SOCE is critical for the transcription and upregulation of the antiapoptotic protein B cell lymphoma-extra large (Bcl-xL). BCR-mediated Ca^{2+} entry activated both NFAT and nuclear factor-kappa B (NF-κB) pathways, ultimately converging on mammalian target of rapamycin complex 1 (mTORC1) activation and induction of c-Myc to drive B cell proliferation (88). The survival and proliferation of $STIM1^{-/-}$ $STIM2^{-/-}$ B cells can be partially rescued by costimulation with CD40 or Toll-like receptor (TLR) ligands [e.g., lipopolysaccharides (LPS) and cytosine-phosphate-guanine (CpG)] (85, 88). In fact, costimulation with either CD40 or TLR ligands robustly activated the canonical NF-κB and mTORC1 pathways, bypassing the need for BCR-mediated Ca^{2+} signaling to promote B cell survival and cell-cycle entry and prevent mitochondrial dysfunction (88, 89). These findings may partially explain the normal antibody responses of $Stim1^{fl/fl}Stim2^{fl/fl}Mb1^{cre}$ mice, where secondary CD40 and cytokine signals from T cells in vivo might compensate for SOCE deficiency in GC B cells. Indeed, GC B cells display attenuated BCR signaling and require robust costimulation with CD40 to induce c-Myc expression and phosphorylation of S6 protein (90). Interestingly, GC B cells also demonstrate unique metabolic demands and preferentially oxidize fatty acids to fuel oxidative phosphorylation (91). The metabolic requirement of SOCE is not restricted to lymphocytes. Analysis of SOCE-deficient fibroblasts and hepatocytes from both humans and mice showed diminished fatty acid oxidation, resulting from impaired activation of master transcription factors peroxisome proliferator-activated receptor-gamma coactivator-1 alpha (PGC-1α) and peroxisome proliferator-activated receptor alpha (PPARα) that facilitate lipid metabolism (92). These data warrant further work to determine whether SOCE regulates similar metabolic programs in GC

B cells or other B cell subsets. Although the work of Berry et al. (88) has reaffirmed the importance of STIM molecules in orchestrating B cell survival and expansion, the precise Orai isoforms that make up CRAC channels in various B cell populations and how the expression of these isoforms is modulated by physiological and pathological stimuli await further investigations.

Innate Immune Cells

Current evidence suggests a critical role for SOCE in regulating neutrophil nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activation and subsequent reactive oxygen species (ROS) production along with the activation, degranulation, and cytokine secretion of mast cells (93, 94). Using mice with hematopoietic deletion of *Orai1* and/or *Orai2*, researchers recently suggested that Orai1 and Orai2 cooperatively regulate SOCE in mouse neutrophils (95). However, unlike T cells and mast cells, SOCE was decreased in Orai2$^{-/-}$ neutrophils, although I_{CRAC} measurements were not performed on isolated neutrophils from these mice (95). If I_{CRAC} indeed turns out to be decreased in Orai2$^{-/-}$ neutrophils, these results would suggest that Orai2 function varies between innate and adaptive immune cells. Surprisingly, STIM1$^{-/-}$ STIM2$^{-/-}$ bone marrow–derived macrophages, dendritic cells, and natural killer cells demonstrate relatively normal effector function and cytolytic activity (96, 97). Whether other Ca^{2+}-conducting channels [e.g., transient receptor potential, purinergic receptors, voltage-gated Ca^{2+} channels (1)] are more functionally relevant in these innate populations is unclear. Gwack and coworkers (98) identified a Ca^{2+}-independent mechanism by which STIM1 may contribute to autoimmune pathology. They characterized fibroblasts and bone marrow–derived macrophages from STIM1$^{-/-}$ mice along with peripheral blood mononuclear cells from a human patient with a nonsense mutation in *STIM1* (E136X) that ablated STIM1 protein expression (98). Unexpectedly, STIM1$^{-/-}$, but not STIM2$^{-/-}$, in macrophages increased the expression of genes regulating the type I interferon response. Furthermore, elevated levels of proinflammatory cytokines were found in serum from the *STIM1* E136X patient. This enhanced type I interferon response was absent in Orai1$^{-/-}$ cells, suggesting that this phenotype is independent of the lack of SOCE. STIM1 was shown to physically interact with the ER-resident adapter protein stimulator of interferon genes (STING) and resting STIM1 retains STING in the ER, reducing its trafficking to the ER-Golgi intermediate compartment, which is required for activation of interferon regulatory factor 3 (IRF3), an interferon-inducing transcription factor. STIM1$^{-/-}$ cells demonstrated increased resistance to infection with DNA viruses such as herpes simplex virus-1 (HSV-1) and murine γ-herpesvirus. Stim1$^{fl/fl}$Lyz2^{cre} mice, which lack STIM1 specifically in myeloid cell populations, were protected from HSV-1-induced death (98). Finally, Demaurex and colleagues (99) reported a critical function of STIM1 in antigen-presenting dendritic cells, whereby STIM1-mediated Ca^{2+} signals supported the delivery of endolysosomal enzymes to phagosomes to promote antigen cross-presentation.

Given the dynamic structure of their N and C termini, it is not surprising that STIM proteins possess SOCE-independent functions and likely bind a host of ER and cytosolic proteins, the physiological importance of which is poorly understood. For instance, STIM1 regulates actin stress fiber formation and endothelial permeability in response to agonists independently of SOCE and Orai1 (100). STIM2, but not STIM1, was identified as a novel binding protein for AMPK, regulating its activation through a Ca^{2+} mechanism, but not an energy stress-mediated mechanism (101). Furthermore, conflicting reports of STIM proteins directly interacting with IP_3Rs and regulating their activity necessitate further work to understand potential cross talk between these molecules within the ER network (102, 103). Defining the STIM interactome within various cell types and ER Ca^{2+} depletion conditions will be critical to parse out novel Ca^{2+}-independent physiological functions for STIM1 and STIM2.

Platelets

The activation of platelets and thrombus formation at sites of vascular injury critically depend on increases in cytosolic Ca^{2+} concentrations through SOCE (104). Earlier studies have established that Orai1 and STIM1 are the key regulators of SOCE and procoagulant activity in human and murine platelets with no apparent role for STIM2 (105–107). Patients with LoF mutations in *Orai1* and *STIM1* show relatively normal numbers of platelets with no increase in bleeding diathesis. However, intermittent periods of thrombocytopenia occur owing to autoantibodies against platelet glycoproteins (28). Conversely, patients with gain-of-function (GoF) mutations in *Orai1* or *STIM1* generating constitutively active CRAC channels manifest with a host of platelet defects and the development of York platelet and Stormorken syndromes (108–111). Both disorders are characterized by thrombocytopenia, increased bleeding, and skeletal myopathy and are caused by the same activating R304W mutation located in the STIM1 C terminus (28). York platelet syndrome is also caused by the additional I115F mutation located within the EF2 motif of STIM1 (108). Platelets from these patients are preactivated with plasma membrane phosphatidylserine exposure, impaired ATP secretion, and altered ultrastructure (108–110). Heterologous expression of the STIM1-R304W mutant results in preclustered STIM1 puncta, constitutive activation of I_{CRAC}, and enhanced SOCE compared to wild-type STIM1 (111, 112). Romanin and colleagues (112) have shown that the R304W mutation promotes STIM1 C terminus unfolding by increasing the homomerization and helical elongation of its coiled-coil 1 domain (CC1), thus exposing the STIM1 SOAR/CAD, which is normally shielded by CC1 under store replete conditions. Interestingly, patients with constitutively active CRAC channel activity due to mutations in the STIM1 EF-hand (e.g., D84G) that lead to preclustering of STIM1 manifest with nonsyndromic tubular-aggregate myopathy but no obvious platelet defects or bleeding disorders (113). The more severe phenotype of the STIM1-R304W mutation may result from the lack of fast Ca^{2+}-dependent inactivation of CRAC channels, which is unaffected in the STIM1 EF-hand mutants (111). Nevertheless, heterozygous *Stim1*^{Sax} mice with the STIM1 D84G mutation found in patients, present with thrombocytopenia, increased bleeding times and preactivated platelets (114). The discrepancy in bleeding diathesis between patients harboring this mutation and *Stim1*^{Sax} mice is currently unclear. Recently, two new STIM1 GoF mouse models have been developed by multiple groups and carry the human equivalent of the York platelet I115F mutation or the Stormorken R304W mutation (115–117). Homozygosity for the R304W mutation is embryonic lethal, while the outcome of homozygous I115F mice was not reported. Thrombocytopenia, prolonged bleeding times, and impaired platelet function were observed in both heterozygous R304W and I115F STIM1 GoF mice (115–117).

ECTODERMAL-DERIVED AND OTHER SECRETORY TISSUES

Enamel Mineralization

In patients afflicted with LoF mutations in *Orai1* and *STIM1*, some of the most frequently reported symptoms are severe defects in dental enamel formation (118). The process of enamel formation, termed amelogenesis, is driven by polarized ameloblast cells, which are derived from the ectodermal epithelium (119, 120). Amelogenesis is initiated in a secretory stage whereby ameloblasts apically secrete a multitude of enamel matrix proteins to structurally support enamel crystal growth (119, 120). During the final maturation or mineralization stage, ameloblasts differentiate into a ruffled phenotype, transporting Ca^{2+} from the bloodstream to be deposited extracellularly in the enamel matrix to drive de novo hydroxyapatite crystal formation (118). Supporting a transcytosis model where Ca^{2+} is transported across ameloblasts through the ER network, early genome-wide

transcript profiling studies identified upregulation of *STIM1* and *STIM2* in developing rat enamel tissue (121). All five components of the SOCE machinery and IP_3Rs are expressed in the maturation stage of enamel tissue, and Ca^{2+} entry is potently blocked by the SOCE inhibitor Synta-66, suggesting functional CRAC channels in enamel cells (122). Lacruz and colleagues (123) generated $Stim1^{fl/fl}Stim2^{fl/fl}$ $K14^{cre}$ mice to ablate SOCE in keratin-expressing ectodermally derived tissues such as dental enamel. The enamel of $Stim1^{fl/fl}Stim2^{fl/fl}$ $K14^{cre}$ mice was severely hypomineralized, reduced in thickness and volume, and mechanically weak compared to enamel from wild-type incisors. Critically, the key $Na^+/Ca^{2+}/K^+$ exchanger NCKX4, which mediates Ca^{2+} extrusion out of the apical end of ameloblasts, was mislocalized, likely contributing to the mineralization defect in these mice. Ameloblasts from $Stim1^{fl/fl}Stim2^{fl/fl}$ $K14^{cre}$ mice have altered morphology and defects in formation of a ruffled border, along with high proportions of mislocalized mitochondria with damaged cristae formation and elevated levels of ROS. Alterations in the glutathione system in $STIM1^{-/-}$ $STIM2^{-/-}$ ameloblasts impairs the *S*-glutathionylation of actin, likely contributing to impaired formation of the ruffled border. This phenotype appears largely driven by STIM1-mediated SOCE, as similar work showed that enamel defects are found in $Stim1^{fl/fl}$ $K14^{cre}$ and $Stim1^{fl/fl}Stim2^{fl/fl}$ $K14^{cre}$ mice, but not $Stim2^{fl/fl}$ $K14^{cre}$ mice (124). In $Orai1^{fl/fl}$ $K14^{cre}$ and global $Orai2^{-/-}$ mice, SOCE was reduced by ~50% in $Orai1^{-/-}$ enamel organ cells, whereas SOCE increased in $Orai2^{-/-}$ enamel organ cells (37), consistent with a function for Orai2 as a negative regulator of CRAC channels in enamel cells. Enamel defects were observed in $Orai1^{fl/fl}$ $K14^{cre}$ mice, but not $Orai2^{-/-}$ mice, suggesting that increased SOCE does not substantially alter ameloblast function and enamel formation (37). The mouse enamel cell line LS8 with stable short hairpin RNA (shRNA) knockdown for each Orai isoform demonstrated a substantial reduction in SOCE with Orai1 knockdown, and an increase with either Orai2 or Orai3 knockdown, with a less dramatic increase in SOCE with Orai3 knockdown (37). Thus, all members of the Orai trio contribute to the CRAC channel in enamel cells, with Orai2 and Orai3 acting as robust and moderate negative regulators of CRAC channel activity, respectively, in agreement with studies in HEK293 cells (27, 36).

Other Secretory Tissues

Studies on global and tissue-specific $STIM1^{-/-}$ and $Orai1^{-/-}$ mice revealed crucial roles for SOCE in sweat, tears, milk, and gastric fluid secretion. Patients with LoF in *Orai1* and *STIM1* display ectodermal dysplasia characterized by anhidrosis (28), the inability to sweat. Increases in intracellular Ca^{2+} concentrations within eccrine sweat glands result in the opening of Ca^{2+}-activated chloride channels (125), which provide the driving force for water release and sweat production. Concepcion et al. (126) studied sweat glands from $Stim1^{fl/fl}Stim2^{fl/fl}$ $K14^{cre}$ and $Orai1^{fl/fl}$ $K14^{cre}$ mice along with skin biopsies from patients with LoF mutations in *Orai1* and *STIM1*. Patient samples demonstrated the presence of eccrine sweat glands with relatively normal morphology, suggesting that anhidrosis in these patients is not due to defects in sweat gland development. In response to cholinergic stimulation, however, the footpads of $Stim1^{fl/fl}Stim2^{fl/fl}$ $K14^{cre}$ and $Orai1^{fl/fl}$ $K14^{cre}$ mice showed minimal sweat secretion. Similarly, Cl^- secretion of isolated sweat glands from $Orai1^{fl/fl}$ $K14^{cre}$ mice and in the human sweat gland cell line NCL-SG3 subjected to Orai1 or STIM1 knockdown was significantly reduced in response to acetylcholine stimulation. Ca^{2+}-activated chloride channel–mediated Cl^- currents activated by acetylcholine were mediated by the TMEM16A protein and were largely attenuated upon Orai1 knockdown (126).

Work by Putney and colleagues (127) showed that lacrimal gland cells isolated from global $Orai1^{-/-}$ mice lack SOCE. Although lacrimal glands from these $Orai1^{-/-}$ mice showed

normal morphology, Ca^{2+}-dependent exocytotic secretion of peroxidase was abrogated and tear production was severely reduced in response to injection with the muscarinic receptor agonist pilocarpine (127). The same group showed that, although global female Orai1$^{-/-}$ mice were fertile, their produced pups failed to survive and thrive unless they were fostered by a wild-type surrogate mother. Female Orai1$^{-/-}$ mice present with a reduction of Ca^{2+} transport into milk by approximately 50% and altered milk ejection from mammary glands. The oxytocin-mediated pulsatile contractions of the alveolar units of mammary glands from Orai1$^{-/-}$ mice were infrequent and poorly coordinated compared to those of control mice (128). The oxytocin-activated cytosolic Ca^{2+} oscillations in mammary myoepithelial cells from Orai1$^{-/-}$ mice were also severely reduced in number, amplitude, and frequency, arguing for a critical role of these Orai1-mediated repetitive Ca^{2+} oscillations in milk ejection and pup survival. This study revealed a dual role for Orai1 in concentrating milk with Ca^{2+} and in contractile signaling of mammary myoepithelial cells required for milk ejection. Quite intriguingly, the same group revealed that male Orai1$^{-/-}$ mice were sterile due to defects in spermatogenesis, with defects in spermatid maturation and degeneration of the seminiferous tubules within the testes (129). Interestingly, activation of the G protein–coupled tastant receptor in mouse spermatids couples to the G protein gustducin, PLC, and Ca^{2+} signaling and mice with deletions in these taste genes present with defects in spermatogenesis (130). While the precise mechanisms by which SOCE controls spermatogenesis await further investigations, Orai1 might mediate the Ca^{2+} entry pathway that is activated by tastant receptors and required for spermatid development (129).

Ahuja et al. (131) generated mice with tamoxifen-inducible tissue-specific deletion of Orai1 in pancreatic acini using the ErTCre line, which is driven by the elastase promoter. About 60–70% of adult Orai1$^{fl/fl}$ErT^{cre} mice die within three weeks of tamoxifen treatment. Although Orai1$^{fl/fl}$ErT^{cre} mice had largely preserved gut innate immunity, they showed reduced pancreatic levels of cathelicidin-related antimicrobial peptide with enhanced bacterial outgrowth in intestines, systemic infection, and dysbiosis (131). Survival of Orai1$^{fl/fl}$ErT^{cre} mice was rescued by strategies that curbed bacterial outgrowth, such as antibiotics or a purified liquid diet, but not with digestive enzyme supplementation, suggesting a crucial role of Orai1-mediated Ca^{2+} entry in antimicrobial secretions by pancreatic acini that prevent disruption of the gut microbiome (131).

SKELETAL, CARDIAC, AND SMOOTH MUSCLE

Skeletal Muscle

One tissue where both the short-term sarcoplasmic reticulum (SR) store refilling and long-term Ca^{2+}-dependent signaling functions of SOCE are readily apparent is skeletal muscle. SOCE prevents skeletal muscle fatigue during periods of prolonged, high-frequency contraction and is crucial to muscle fiber development (132, 133). Although SOCE in nonexcitable cells is a slow process that takes seconds to develop and minutes to be maximal, in skeletal muscle, store depletion leads to a more rapid SOCE activation (<1 s), likely due to the sizeable portion of STIM1 that is permanently prelocalized with Orai1 under store replete conditions within the SR-transverse tubule junctions (132). This precoupling is mediated by a long splice variant of STIM1 that contains 106 additional amino acids (called STIM1L) and binds to actin, allowing constitutive interactions with Orai1 even when stores are full (55). Most studies have focused on STIM1 and Orai1, which are highly expressed in both myotubes and adult muscle fibers. Although myotubes express abundant mRNA for STIM2, Orai2, and Orai3, the contribution of these isoforms to fine-tuning skeletal muscle SOCE and contractile activity or to long-term differentiation and growth through Ca^{2+}-dependent transcription remains unknown. Patients with both LoF and GoF mutations in *STIM1*

and *Orai1* display a diverse range of muscle disorders, including atrophy, hypotonia, and tubular aggregate myopathy (28).

Patients bearing the archetypical R91W homozygous LoF mutation in *Orai1* present with enhanced numbers of type I muscle fibers and atrophic type II fibers (15). Most global STIM1$^{-/-}$ and Orai1$^{-/-}$ mice die, but those that survive on mixed backgrounds are smaller in size and exhibit reduced muscle mass, indicating Orai1's important role in long-term skeletal muscle fiber differentiation, growth, and development (134). Several Ca^{2+}-dependent transcription factors were proposed to mediate the effects of SOCE on skeletal muscle development, including NFAT, AKT, mitogen-activated protein kinase, and extracellular signal-regulated kinase 1/2 (132). Several patients with GoF mutations in *STIM1* and *Orai1*, causing constitutively active SOCE, have been identified and were associated with a collection of syndromes affecting skeletal muscle function, including tubular aggregate myopathy. They also exhibited other pathologies affecting muscle and other organ systems such as Stormorken and York platelet syndromes, which were briefly discussed above and in detail elsewhere (132). Altered SOCE activity was also proposed to contribute to the exacerbation of other skeletal muscle pathologies with different etiologies such as Duchenne muscular dystrophy in patients and in the *mdx* mice model, malignant hyperthermia caused by mutations in type I ryanodine receptor, and age-related skeletal muscle decline (132).

Cardiac and Smooth Muscle

While healthy acutely isolated cardiomyocytes and smooth muscle cells express STIM1 proteins, the expression of Orai1 is either low (vascular smooth muscle) (135–137) or undetectable (ventricular cardiomyocytes) (138) with undetectable SOCE activity and I_{CRAC} in both cell types (137, 138). In rat ventricular myocytes, STIM1 enhances SR Ca^{2+} content by binding to phospholamban (138), suggesting the existence of SOCE-independent functions of STIM1, as reported in macrophages and endothelial cells (98, 100). Several groups showed a homeostatic role of cardiomyocyte STIM1 in the adult heart, but whether or not this function of STIM1 is mediated through Orai channels remains unclear. Inducible cardiomyocyte-specific STIM1 knockdown mice were prone to malignant ventricular arrhythmias and death (139). Noninducible cardiomyocyte-specific STIM1 knockdown mice showed increased ER stress and mitochondrial dysfunction, and they displayed cardiac inflammation, fibrosis, and decline in cardiac function with a subsequent onset of left ventricular dilatation by 9 months of age (140). The reduction of cardiac STIM1 expression by siRNA in vivo led to heart failure driven by abrogated AKT/mTORC2 signaling and enhanced activity of glycogen synthase kinase-3 beta (GSK-3β), an antihypertrophic and proapoptotic kinase (141). The role of STIM1 in the cellular homeostasis of quiescent healthy smooth muscle remains largely unknown. Vascular and intestinal smooth muscle tissues from smooth muscle–specific STIM1 knockout (Stim1$^{fl/fl}$SM22α^{cre}) mice were distended and thin, suggesting altered development (142). Mesenteric arteries and aortas from these mice had reduced α1-adrenergic-dependent contraction by ~25–50% with preserved thromboxane- and depolarization-induced contraction (142–144). Finally, the use of a peptide that inhibits STIM1-Orai1 interactions suggested a critical role of CRAC channels in the pacemaker activity by the interstitial cells of Cajal within the gastrointestinal tract (145).

Unlike the situation of the healthy cardiovasculature, the expression of STIM1 and Orai1 and the magnitude of I_{CRAC} and NFAT activity are greatly enhanced in cardiomyocytes and smooth muscle cells under conditions of pathological remodeling of the heart and vessels (146). Genetic abrogation of STIM1 or Orai1 expression in vivo in hearts and vessels protected rats from pressure overload–induced cardiac hypertrophy and neointima formation, respectively (137, 147, 148). Consistent with these findings, transgenic mice overexpressing STIM1 die suddenly at 6 weeks

of age, and mice that survive past 12 weeks of age develop hypertrophy and heart failure and show enhanced NFAT and CaMKII activity in their cardiomyocytes (149). STIM1, Orai1, and SOCE are also upregulated in vascular smooth muscle from hypertensive rats (150), and their upregulation is also a hallmark of airway smooth muscle remodeling in mouse models of asthma (151). As mentioned earlier, Orai3 is also a component of store-independent ARC channels composed of Orai1/Orai3 heteromers (38, 63–65). Evolutionarily speaking, Orai proteins appeared before STIM, suggesting that this store-independent gating mechanism of Orai channels predates SOCE (152). Intriguingly, specific deletion of cardiomyocyte Orai3 in mice caused mitochondrial dysfunction with enhanced mitochondrial fission and increased activity of calcineurin, with mice showing impaired left ventricular contractility with progression to dilated cardiomyopathy and heart failure (153), suggesting a homeostatic role for Orai3 in cardiac function. In contrast, Orai3 expression and ARC currents were increased in hypertrophied cardiomyocytes (154) and in neointimal carotid artery smooth muscle from mice subjected to balloon angioplasty (155, 156), and the knockdown of Orai3 in vivo in injured carotids inhibited neointima formation (155). These results suggest a complex role of Orai3 in both cardiovascular physiology and pathophysiology, but additional studies are needed to determine the relative contributions of Orai3 to I_{CRAC}- and I_{ARC}-mediated signaling in hearts and vessels.

NERVOUS SYSTEM

Neurons

Dysregulation of neuronal Ca^{2+} homeostasis is linked to a variety of neurodegenerative disorders (8). Although voltage-gated Ca^{2+} channels and ionotropic neurotransmitter receptors are classically ascribed as the primary channels regulating synaptic transmission, neurotransmitter release, and dendritic spine formation, a growing body of literature has implicated STIM and Orai proteins in the function of neurons from various regions of the brain and spinal cord (157). Nevertheless, the function of CRAC channels in the brain remains highly contentious, with several conflicting reports on the expression and contribution of specific STIM and Orai isoforms to Ca^{2+} signals in various neuron subsets. These issues are compounded by the vast reliance on single-wavelength Ca^{2+} dyes for Ca^{2+} measurements in neurons and the inherent difficulties in reliably dissecting and measuring I_{CRAC} in primary neurons.

Berna-Erro et al. (48) reported that STIM2 and Orai2 messenger RNA are predominantly expressed in mouse brain. The same group showed that SOCE in cortical and hippocampal neurons from STIM2$^{-/-}$ mice is significantly inhibited, whereas SOCE is largely unaffected in both STIM1- and Orai1-deficient neurons (48). Global STIM2$^{-/-}$ mice showed protection from ischemic stroke, reduced neuronal Ca^{2+} overload, and apoptosis (48). Similarly, Sun et al. (158) reported a critical role of STIM2-mediated SOCE in the maintenance of mushroom postsynaptic spines through constitutive CaMKII activation. SOCE was substantially reduced in cortical neurons, but not in astrocytes or microglia, from Orai2$^{-/-}$ mice. Like STIM2$^{-/-}$ mice, Orai2$^{-/-}$ mice were largely protected from neuronal damage after cerebral ischemia, presumably by preventing ischemia-induced Ca^{2+} accumulation and hypoxia-mediated neuronal apoptosis (159). Of note, Orai2$^{-/-}$ mice display no defects in cognitive function, but global loss of STIM2 results in more robust cognitive impairment (48, 159). Studies by Hartmann et al. (160) on mice lacking STIM1 in cerebellar Purkinje neurons showed a requirement for STIM1 in IP_3-mediated Ca^{2+} release from ER stores, metabotropic glutamate receptor (mGluR)-mediated synaptic transmission, and motor coordination. A subsequent report by the same group showed that Orai2 mRNA is most expressed (compared to Orai1 and Orai3 mRNA) and is required for IP_3R-mediated Ca^{2+} release from internal stores of hippocampal CA1 pyramidal neurons in response to mGluR stimulation

and suggested that store refilling in these neurons is mediated by Orai2 (161). Thus, while it appears that SOCE in neurons might be mediated through a privileged interaction between Orai2 and STIM2, studies discussed above did not offer direct measurements of ER Ca^{2+} content or that of I_{CRAC} in neurons. As such, the exact contribution of Orai2 to the makeup of CRAC channels in neurons warrants further investigations.

A thorough new study by Prakriya and colleagues (162) documented Orai1 protein expression in mouse hippocampus. This study utilized three neuron-specific Orai1 knockout mouse models, combined with Orai1 rescue and the use of CRAC channel pharmacological blockers to show that Orai1 mediates SOCE, ER refilling, and the amplification of neurotransmitter-induced Ca^{2+} signals in dendritic spines of hippocampal neurons (162). Stimulation of N-methyl-D-aspartate (NMDA) glutamate receptors and subsequent Orai1-mediated Ca^{2+} signals was critical for long-term potentiation of CA1 neurons, CaMKII activation, insertion of new glutamate receptor 1 (Glu1A) subunits of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors into postsynaptic densities, spine morphogenesis, and maintenance of synaptic plasticity. Critically, the loss of Orai1 specifically in excitatory neurons using $Orai1^{fl/fl}CaMKIIa^{cre}$ mice was sufficient to cause substantial impairment of spatial working and associative memory (162). Notably, Orai1 knockout mice showed no deficits in general locomotor and sensorimotor activity. These data agree with those from other recent studies suggesting that Orai1 contributes to nociceptive and learning behaviors (163, 164). Although studies reporting that either one STIM/Orai duo or another mediates neuronal SOCE might appear inconsistent, CRAC channels are likely heteromers of two or three Orai isoforms (27, 36), and it is conceivable that the molecular composition of neuronal SOCE would involve dynamic choreographies between all five STIM/Orai isoforms, as recently shown in nonexcitable cells (45). For instance, double STIM1/STIM2 forebrain-deficient mice showed significant defects in spatial learning and memory with enhanced long-term potentiation of CA3-CA1 hippocampal synapses, whereas single STIM forebrain-deficient mice showed only mild (STIM1) or no (STIM2) defects (165), arguing that both STIM proteins are important for neuronal function. One interesting observation is the implicit requirement of STIM/Orai for the refilling of ER stores in neurons, as they are largely dispensable for long-term ER refilling in nonexcitable cells (27). The picture is complicated further by the SOCE-independent functions of STIM1 in neurons. Indeed, Garcia-Alvarez et al. (166) reported that STIM2 is required for cAMP/PKA-mediated phosphorylation and trafficking of the AMPA receptor subunit GluA1 to the cell surface of hippocampal excitatory neurons in a SOCE-independent manner. Dittmer et al. (167) reported that glutamate-mediated STIM1 aggregation inhibits voltage-gated Ca^{2+} channels and NFATc3 nuclear translocation in hippocampal neurons. Clearly, additional experiments are needed with combinations of STIM- and Orai-deficient neurons to determine the contribution of each isoform to amplifying neurotransmitter-induced Ca^{2+} signals, synaptic plasticity, memory formation, and motor coordination.

Astrocytes

Although electrically nonexcitable, astrocytes display dynamic Ca^{2+} oscillatory behavior that regulates the release of gliotransmitters (e.g., ATP and glutamate), which modulate the function of surrounding glia and neurons (168). Early work by Motiani et al. (43) showed that SOCE is mediated by Orai1 and STIM1 in primary human astrocytes and a variety of glioblastoma cell lines. Two recent studies confirmed that SOCE in primary cultured murine astrocytes is mostly mediated by Orai1 and STIM1 (169, 170). Similarly, SOCE in microglia is predominately regulated by Orai1 and STIM1, with minor contributions from STIM2 (171, 172). Toth et al. (173) generated astrocyte-specific Orai1 knockout and brain-specific STIM1 knockout mice and showed

that SOCE activated by metabotropic, purinergic, and protease-activated receptors (PARs) in hippocampal astrocytes is mediated by STIM1 and Orai1 and is critical for astrocyte-mediated release of gliotransmitters, such as ATP. Critically, PAR-dependent activation of Orai1 in astrocytes increased inhibitory postsynaptic currents of CA1 pyramidal neurons (173).

SUMMARY AND FUTURE DIRECTIONS

Recent studies have established CRAC channels as central players in the regulation of Ca^{2+} signaling and physiological functions in many organ systems and have shown that either GoF or LoF mutations in STIM/Orai proteins are associated with disease. The expression of all five isoforms of STIM and Orai is ubiquitous, and relative expression of these isoforms in different tissues is incredibly diverse and depends on the specific cell type and its differentiation state. Although Orai1 and STIM1 are clearly the major components of CRAC channels, STIM2, Orai2, and Orai3 are emerging as important regulators of SOCE and cellular function. It should be noted that most protocols used by investigators to interrogate SOCE or CRAC channel activity rely on maximal store depletion and often reveal clear roles for Orai1 and STIM1. However, these protocols are not always suitable for detecting the more subtle regulatory functions of other STIM/Orai isoforms, whose functions become apparent under relatively low to moderate physiological concentrations of agonist. CRISPR/Cas9 knockout in cell lines and transgenic and tissue-specific knockout mouse models have revealed unexpected roles for all Orai isoforms and suggested that they function as heteromeric channels under native conditions. Yet, the exact oligomeric states of CRAC channels remain unclear. CRAC channel stoichiometries are likely driven by diverse mechanisms, including the relative protein abundance of each STIM and Orai isoform in a specific cell. Future studies will likely reveal that the Orai subunit composition of CRAC channels varies between different tissues and differentiation/activation states, and this knowledge will be critical in the specific targeting of CRAC channels by small-molecule drugs for the purpose of disease therapy. Novel mouse models with a specifically altered function of either one STIM or one Orai isoform or combinations of STIM/Orai isoforms will likely shed more light on the composition and physiological function of CRAC channels in different tissues and different cell types. Knockout studies in mice and cells have their shortcomings. For instance, based on previous studies it seems highly likely that if two Orai isoforms form native heterohexameric CRAC channels in a specific cell type and one of them is deleted, the remaining Orai isoform would continue to be functional, albeit as a homohexamer. Mouse knock-in models with different Orai isoforms tagged with fluorescent proteins combined with total internal reflection fluorescence (TIRF) and superresolution imaging in primary cells might overcome this challenge and help inform the native CRAC channel composition. The advancement in in vivo Ca^{2+} imaging in whole animals using tissue-specific genetically encoded indicators targeted to subcellular compartments combined with physiological and behavioral studies will enhance our knowledge of the signaling mechanisms of native CRAC channels, their physiological function, and their contribution to disease.

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