In many animal cells, store-operated Ca\(^{2+}\) release-activated Ca\(^{2+}\) (CRAC) channels function as an essential route for Ca\(^{2+}\) entry. CRAC channels control fundamental cellular functions including gene expression, motility, and cell proliferation and are involved in the etiology of several disease processes including a severe combined immunodeficiency syndrome. With several distinguishing biophysical and molecular features, CRAC channels are constructed as a two-component system consisting of the stromal interaction molecule (STIM) proteins, which serve as the ER Ca\(^{2+}\) sensors, and the ORAI proteins, which form the channel pores. ER Ca\(^{2+}\) store depletion evokes direct interactions between the STIM sensors and the ORAI channels, driving their redistribution and accumulation into overlapping puncta at peripheral cellular sites to elicit localized elevations of [Ca\(^{2+}\)]\(_i\) at clusters of CRAC channels. This chapter examines the molecular features of the STIM and ORAI proteins that regulate the operation of CRAC channels and highlights their physiological roles in select organ systems.

### 33.1 INTRODUCTION

Ca\(^{2+}\) is a ubiquitous intracellular signaling messenger involved in a wide range of cellular functions including enzyme activation, gene expression, chemotaxis, and neurotransmitter release. Cellular Ca\(^{2+}\) signals generally arise from the opening of Ca\(^{2+}\)-permeable ion channels, a diverse family of membrane proteins that include voltage-gated Ca\(^{2+}\) channels, ligand-gated channels, and sensory transduction channels such as the TRP channels. In this large family, store-operated channels (SOCs) are recognized as a major mechanism for eliciting long-lasting Ca\(^{2+}\) signals in metazoans. Physiologically, SOCs are activated by the depletion of intracellular Ca\(^{2+}\) stores that occurs in response to stimulation of cell surface receptors coupled to the generation of inositol trisphosphate (IP3). The ensuing store-operated calcium entry (SOCE) is implicated in diverse cellular functions that include gene expression, cell motility, and differentiation. Clinical studies have revealed that patients with mutations in SOCs suffer from a devastating immunodeficiency, muscle weakness, and abnormalities in the skin and teeth \([1,2]\). Moreover, animal studies have implicated a growing list of possible diseases from allergy \([3]\), multiple sclerosis \([4,5]\), cancer \([6]\), thrombosis \([7]\), and inflammatory bowel disease \([8]\) to loss or gain of SOC activity, highlighting the potential importance of these channels for human health and disease.

Early studies of SOCE relied on cytosolic Ca\(^{2+}\) indicator dyes to examine Ca\(^{2+}\) entry through SOCs \([9,10]\). These investigations were strongly aided by the discovery of thapsigargin, a plant alkaloid that is a potent inhibitor of the SERCA pump in the ER membrane \([11,12]\). Thapsigargin allowed investigators to directly deplete intracellular Ca\(^{2+}\) stores without stimulating surface receptors.
store-operated CRAC channels

Despite early identification and characterization of CRAC currents, however, the molecular identity of CRAC channels and the mechanism linking ER Ca\(^{2+}\) store depletion to their activation remained unknown for several decades. This changed dramatically following the identification of STIM1 in 2005 as the ER Ca\(^{2+}\) sensor and ORAI1 in 2006 as a prototypic SOC subunit. These discoveries were a major catalyst for elucidation of the molecular mechanisms of CRAC channels and their physiological functions in many organ systems [16]. We now know that CRAC channels are activated through the binding of the ER Ca\(^{2+}\) sensors stromal interaction molecule 1 (STIM1) and STIM2 to the CRAC channel proteins ORAI1, ORAI2, and ORAI3 [17,18]. The STIM proteins bind to and directly activate ORAI channels, and these two families of molecules can fully reconstitute SOCE in heterologous expression systems, indicating that these proteins are both necessary and sufficient for SOCE. This chapter focuses on the molecular characteristics of STIM and ORAI proteins that regulate the activation of CRAC channels and their ion conduction mechanisms.

33.2 THE BIOPHYSICAL FINGERPRINT OF THE CRAC CHANNEL

Long before the identification of the STIM and ORAI proteins, several decades of electrophysiological studies had already revealed many important biophysical and pharmacological features of the CRAC channel. Overall, CRAC channels are widely noted for their exquisite Ca\(^{2+}\) selectivity (P\(_{Ca}\)/P\(_{Na}\) > 1000), which places them in a unique category of highly Ca\(^{2+}\)-selective channels together with voltage-gated Ca\(^{2+}\) (Ca\(_v\)) channels [19]. However, its unitary conductance is >100-fold smaller than that of Ca\(_v\) channels; too small to measure from single-channel openings. It has been estimated from noise analysis to be 10–30 fS for Ca\(^{2+}\) and ~1 pS for Na\(^{+}\) in the absence of extracellular divalent cations [14,20,21]. Interestingly, high Ca\(^{2+}\) selectivity is only manifested in Ca\(^{2+}\)-containing solutions, CRAC channels readily conduct a variety of small monovalent ions (Na\(^{+}\), Li\(^{+}\), and K\(^{+}\)) in divalent-free solutions [20,22,23], indicating that high Ca\(^{2+}\) selectivity is not an intrinsic feature of the CRAC channel pore but arises due to ion–ion and ion–pore interactions. This is clearly revealed by the blockade of monovalent currents by micromolar concentrations of Ca\(^{2+}\) (K\(^{+}\), ~20 μM at ~100 mV) [19–24]. Occupancy by a single Ca\(^{2+}\) ion appears sufficient to block the large monovalent conductance, and, as expected for a binding site within the pore, Ca\(^{2+}\) block is voltage-dependent [21,25]. These characteristics are qualitatively reminiscent of the properties of L-type Ca\(_v\) channels, in which Ca\(^{2+}\) ions similarly bind tightly to a high-affinity binding site within the pore to occlude Na\(^{+}\) flux [26,27]. In contrast to Ca\(_v\) channels, however, CRAC channels are virtually impermeable to the large monovalent cation, Ca\(^{2+}\) (P\(_{Ca}\)/P\(_{Na}\) < 0.1). This feature has been traced to a relatively narrow CRAC channel pore that likely limits the electrodiffusion of Ca\(^{2+}\) [21,25]. The channels are not sensitive to many of the common inhibitors of Ca\(_v\) channels, but are inhibited by imidazole antimycotics, submicromolar concentrations of lanthanides, and 2-aminoethylphenyl borate (2-APB), among others [28]. Overall, the high Ca\(^{2+}\) selectivity, low conductance, and high sensitivity to lanthanides emerged as stringent criteria by which to screen candidates for the CRAC channel gene, resulting in the exclusion of many candidates belonging to the TRP gene family, as reviewed in [29,30].

33.3 STIM1 IS THE ER Ca\(^{2+}\) SENSOR FOR SOCE

STIM (stromal interaction molecule) proteins were first discovered as cell surface proteins that can bind to pre-B lymphocytes and mature B lymphocytes [31]. Initial studies suggested a role for these proteins in tumor growth but subsequent studies using RNAi screens to inhibit SOCE found an unmistakable role for these proteins in regulating CRAC channel function. These screens used thapsigargin to deplete ER Ca\(^{2+}\) stores and examined suppression of SOCE in either Drosophila S2 [32] and mammalian cells [33]. The mammalian homologue identified from these studies, STIM1, is a 77 kDa single-pass ER membrane protein, with a luminal N-terminal domain containing the signal peptide and a large C-terminal domain in the cytosol [18]. Both regions exhibit several domains critical to its function including a sterile alpha motif (SAM) and two EF hands in the N-terminus (Figure 33.1a) and three coiled–coiled domains, a Ser/Pro-rich region, and a Lys-rich region in the C-terminus. Whereas Drosophila has a single STIM gene, mammals have two closely related genes, STIM1 and STIM2, which differ significantly in their C-terminal region. In resting cells, STIM1 is largely localized in the bulk ER [33–38]. ER Ca\(^{2+}\) store depletion triggers unbinding of Ca\(^{2+}\) from the luminal EF-hand, which ultimately results in the redistribution of STIM1 from the bulk ER into puncta located in close apposition to the plasma membrane [33–38]. The EF-hand and SAM domains (EF-SAM) mediate critical roles in this process. EF-hand STIM1 mutants with impaired Ca\(^{2+}\) binding form puncta and activate CRAC channels independently of ER Ca\(^{2+}\) store depletion [33,34]; the absence of Ca\(^{2+}\) binding in these mutants essentially tricks the molecules into responding as if stores are depleted. Moreover, deletion of the SAM domain abrogates oligomerization and puncta formation in response to store depletion [35], indicating that this domain mediates a critical role in initiating the STIM1 conformational response to store depletion. The cytosolic C-terminal portion is essential for the redistribution of STIM1 oligomers to ER-PM junctions and subsequent CRAC channel activation occurs through a critical channel interaction domain encompassing the second coiled–coiled domain [35,39–42]. In this manner, changes in Ca\(^{2+}\)-binding at the N-terminus are coupled to SOCE initiation through protein–protein interactions in the STIM1 C-terminus. Thus, STIM1 fulfills two critical roles in the activation process of CRAC channels: sensing the depletion of ER Ca\(^{2+}\) stores, and communicating store depletion to CRAC channels located in the plasma membrane.
33.4 THE DISCOVERY OF ORAI PROTEINS

Over a two-decade period from 1990, large scale efforts were undertaken to identify the molecular basis of the CRAC channel and how it is turned on by ER Ca\(^{2+}\) store depletion. These efforts culminated in 2006 with the identification of ORAI1 as the canonical CRAC channel protein [43–46]. In the intervening period, several candidate molecules including several TRP channels and voltage-gated Ca\(^{2+}\) channels were presented as possible candidates as CRAC channel pore [29,30], only to be discarded due to inconsistencies in their pore properties with those of native CRAC channels. Ultimately, efforts that led to the identification of the CRAC channel protein harnessed the power of high-throughput screening, linkage analysis, and the human genome sequencing project, tools that became available only in the new millennium. An important step in this discovery was the identification of human patients with a severe combined immunodeficiency lacking CRAC channel function in T-cells [47–49]. These patients exhibited a devastating immunodeficiency characterized by impaired T cell activation and effector gene expression [50], which confirmed earlier pharmacological and genetic evidence that CRAC channels orchestrate many aspects of lymphocyte development and function [51,52]. Feske et al., took advantage of a partial reduction in Ca\(^{2+}\) entry in the heterozygotes in the patient pedigree to localize the source of the defect to a small region in chromosome 12 with ~70 genes [43].

Simultaneously, genome-wide RNAi screens for genes involved in SOCE in Drosophila S2 cells carried out by three groups identified a novel gene as a critical mediator of Drosophila SOCE [43,53,54]. A human ortholog of this protein was mapped to the same region on chromosome 12 identified by linkage analysis [43]. This molecule, ORAI1, is a widely expressed 33 kDa cell surface protein with four predicted transmembrane domains, intracellular N- and C-termini (Figure 33.1b) and no significant sequence homology to other previously identified ion channels. The human SCID defect was found to arise from a missense mutation in ORAI1 (R91W) that abrogated CRAC channel activity [43].

33.5 ORAI1: A PROTOTYPIC CRAC CHANNEL PROTEIN

The conclusion that ORAI1 is a canonical CRAC channel pore-forming protein came quickly following its identification. One line of evidence came from studies showing that overexpression of ORAI1 together with STIM1 in HEK293 cells produced large currents [55–57] with characteristics consistent with native CRAC channels including high Ca\(^{2+}\) selectivity, low Cs\(^{+}\) permeability, and a narrow pore [25,55,56]. Other properties including Ca\(^{2+}\) block, the voltage-dependence of Ca\(^{2+}\) blockade, and Cs\(^{+}\) permeation [44–46]. In the ensuing years, cysteine-scanning studies that identified the pore-lining residues of the CRAC channels and altered a wide range of properties intimately associated with the pore, including La\(^{3+}\) block, the Ca\(^{2+}\) entry when co-expressed with STIM1 in HEK293 cells [60–62] and are widely expressed in most tissues [60,62,63]. However, ORAI1 remains the best-studied CRAC channel protein and appears to be the predominant isoform mediating SOCE in most cells. By contrast, there is no direct genetic evidence for a role of ORAI2 or ORAI3 channels in any cell type yet.
33.6 PHYSIOLOGICAL FUNCTIONS OF CRAC CHANNELS

Knockout studies and human patients with mutated STIM/ORAI genes have revealed many specific roles for CRAC channels in different organs systems. One well characterized physiological role is the generation of long-lasting \([\text{Ca}^{2+}]_i\) elevations essential for \(\text{Ca}^{2+}\)-dependent gene transcription and cytokine production in lymphocytes. Evidence for this role has come from several lines of study. For example, SKF96365, an imidazole compound that inhibits CRAC channels was also found to block IL-2 production in T-cells with similar efficacy [64]. Likewise, nanomolar concentrations of \(\text{La}^{3+}\) were found to block \(I_{\text{CRAC}}\), the \([\text{Ca}^{2+}]_i\), rise, and the induction of T-cell activation markers such as CD25 and CD69 in response to CD3- or thapsigargin-stimulation [65]. Subsequent genetic studies provided compelling evidence that these pharmacological effects occur through the blockade of CRAC channels. For example, mutant Jurkat T cells lacking \(\text{Ca}^{2+}\)-release-activated \(\text{Ca}^{2+}\) current, \(I_{\text{CRAC}}\), displayed severely attenuated production of cytokines such as IL-2 [51]. More recently, several studies of human patients have shown that severe immunodeficiencies arise from mutations in CRAC channels that render them inactive [2,43,47–49,66]. The abrogation of CRAC channel function in these cells results in the elimination of \(\text{Ca}^{2+}\) elevations necessary to drive nuclear translocation of NFAT [48], an important and widely expressed transcription factor involved in cytokine gene expression [67].

In addition to T-cells, CRAC channels serve as the primary \(\text{Ca}^{2+}\) influx route for agonist-evoked \(\text{Ca}^{2+}\) entry in platelets. STIM1-deficient mice exhibit impaired agonist stimulated platelet SOCE and impaired thrombus formation resulting in a mild increase in bleeding time following injury [68,69]. STIM1−/− mice were significantly protected from arterial thrombosis and ischemic brain infarction. Likewise, ORAI1 deficient mice exhibited defective SOCE in platelets, and impaired thrombus formation and resistance to various measures of thrombus formation including pulmonary thromboembolism, arterial thrombosis, and ischemic brain infarction [70]. These findings indicate that CRAC channels in platelets are crucial mediators of cerebrovascular thrombus formation, raising the possibility that blockade of channel function might be beneficial for the treatment of this condition.

In addition to the immune phenotypes, genetic studies in humans and mice reveal defects in skeletal muscle function and development [71,72]. Mice lacking STIM1 show no SOCE in skeletal muscle and this phenotype is accompanied by marked propensity for rapid muscle fatigue during repeated stimulation [71]. Interestingly, unlike in nonexcitable cells, STIM1 and ORAI1 proteins appear to be pre-localized in proximity to each other within the triad junction in skeletal muscle under resting conditions, thus permitting extremely fast and efficient trans-sarcolemmal \(\text{Ca}^{2+}\) influx during store depletion [72].

STIM and ORAI proteins have also emerged as important regulators of several types of cancers, including breast, cervical, and glioblastomas [73]. One recent study found that ORAI1 and STIM1 were essential for breast tumor cell migration in vitro and tumor metastasis in mice [74]. Pharmacological inhibition of SOCE using SKF96365 or knockdown of ORAI1 or STIM1 RNA interference resulted in decreased tumor metastasis in mice. Another study showed that human breast cancer lines displayed increased levels of ORAI1, and microarray analysis of 295 breast cancers found that women with a transcriptional profile characterized by STIM1high/STIM2low ratios had the poorest prognosis [75]. A different study has reported that breast cancer cell lines expressing the estrogen receptors exhibit SOCE that is mediated by ORAI3, in contrast to estrogen-receptor negative lines that reportedly depend on ORAI1 [73], raising the possibility that a switch in the machinery of ORAI channels mediates a causal role in the phenotypic switch to tumorogenesis [76]. The mechanisms by which \(\text{Ca}^{2+}\) signaling is altered in breast cancers remains uncertain, although one report indicates that ORAI1 in breast cancer cells functions independently of STIM1, through a mechanism that involves activation by the Golgi 

33.7 OLIGOMERIZATION AND REDISTRIBUTION OF STIM1 TO THE ER–PLASMA MEMBRANE JUNCTIONS

The STIM proteins respond to alterations in ER \(\text{Ca}^{2+}\) store content through \(\text{Ca}^{2+}\) unbinding–binding from an EF-hand in the luminal domain. Studies of the isolated STIM1 EF-hand domain in solution indicate that the dissociation constant of \(\text{Ca}^{2+}\) binding is -500 \(\mu\text{M}\) [78,79], which is consistent with the range of \(\text{Ca}^{2+}\) concentrations known to exist in ER lumen [80,81]. Similar measurements for STIM2 indicate a lower affinity \(\text{Ca}^{2+}\) binding affinity [79], which is in agreement with the ability of STIM2 to form puncta more readily in resting cells [82]. Measurements of the dependence of \([\text{Ca}^{2+}]_\text{ER}\) on puncta formation indicate an apparent \(K_{1/2}\) of 210 \(\mu\text{M}\) for STIM1 and 406 \(\mu\text{M}\) for STIM2 [82], which implies that STIM2 needs significantly lower levels of store depletion than STIM1 for puncta formation [37]. This difference likely explains why STIM2, but not STIM1, promotes constitutive activation of SOCE when overexpressed with the ORAI proteins [82,83].

Structural studies of the isolated luminal domain fragments indicated that \(\text{Ca}^{2+}\) unbinding from the N-terminal EF-hand triggers the unfolding and aggregation of the luminal domain, resulting in the appearance of dimers and higher-order multimers [78]. This finding led to the idea that \(\text{Ca}^{2+}\) store depletion results in the formation of higher order oligomers of STIM1 [78], a hypothesis that was confirmed in follow-up studies using FRET with full-length STIM1 [39,84]. STIM1 oligomerization is an early step in the channel activation process, occurring well before STIM1 redistribution to the plasma membrane [39,80].
The critical role of STIM1 oligomerization for CRAC channel activation is underscored by the finding that artificially oligomerizing engineered STIM1 in which the luminal domain is deleted and replaced with the FRB-FKBP dimerizer leads to puncta formation and activation of SOCE independently of ER store depletion [80]. STIM1 oligomerization thus serves as a critical upstream activation switch that unfolds all subsequent steps of the channel activation process.

Perhaps the most striking feature of STIM1 behavior is its redistribution from the bulk ER in resting cells with full stores, to the plasma membrane where it accumulates into discrete puncta [33–35,85]. Accumulation of STIM1 near the plasma membrane causes ER tubules to move toward the plasma membrane and STIM1 appears to facilitate this process [36], indicating that store depletion rearranges the ER, with STIM1 facilitating this change. Consistent with the idea that changes in \( \text{Ca}^{2+} \) (and not cytoplasmic \( \text{Ca}^{2+} \)) directly regulate this process, depletion of ER \( \text{Ca}^{2+} \) stores with TPEN, a low affinity membrane permeant \( \text{Ca}^{2+} \) buffer, which is not expected to affect cytoplasmic \( \text{Ca}^{2+} \), also causes STIM1 and ORAI1 puncta formation [86]. Luik and colleagues further showed that the accumulation of Cherry-STIM1 at the plasma membrane exhibits the same dependence on ER \( \text{Ca}^{2+} \) concentration as activation of \( I_{\text{CRAC}} \) [80]. This is consistent with the notion that channel activation requires a local interaction between STIM1 and ORAI subunits, which can only occur following the redistribution of STIM1 from the bulk ER to the periphery. Moreover, the appearance of STIM1 near the surface of the cell precedes the development of \( I_{\text{CRAC}} \) by ~10 s [36], indicating that STIM1 translocation is required not only for CRAC activation, but that channel activation likely also requires additional steps.

Although STIM1 redistribution is triggered by the same initial conformational change that causes oligomerization—with both steps requiring unbinding of \( \text{Ca}^{2+} \) from its N-terminal EF-hand, these processes are distinct and fully separable. Redistribution occurs with a lag of tens of seconds following STIM1 oligomerization [37,39], indicating that the two steps are kinetically separable. Additionally, truncation of a basic region at the extreme C-terminus of STIM1 attenuates redistribution of the truncated STIM1 to peripheral sites without affecting STIM1 oligomerization [39], indicating that the molecular determinants of these processes are distinct. Interestingly, the deleterious effect of removing the K-rich C-terminal region on STIM1 redistribution is only seen in cells overexpressing STIM1 alone: when co-expressed with ORAI1, STIM1ΔK accumulates into puncta to the same extent as that seen in full-length STIM1 and supports the normal extent of puncta formation [40], though activation of \( I_{\text{CRAC}} \) is still delayed compared with full-length STIM1 [87]. Because the polybasic tail is not found in Caenorhabditis elegans or Drosophila STIM proteins, these results suggest that the polybasic domain is a vertebrate adaption that facilitates STIM1 migration to the plasma membrane, but is not essential for ORAI channel activation. It is also tempting to postulate in this context that the accumulation and stability of STIM1 at the ER-PM junctions is influenced by the strength of the STIM1 plasma membrane binding interactions, with weaker interactions diminishing the efficacy of STIM1 migration and binding to the plasma membrane.

In contrast to STIM1 oligomerization, relatively little is known about the mechanisms controlling STIM1 redistribution to peripheral puncta. Studies with fluorescently labeled STIM1 (CFP- or YFP-) indicate that STIM1 is at least partially associated with microtubules (MTs) and moves rapidly along tubulovesicular structures that overlap with MTs in resting cells with replete stores [35,88,89]. STIM1 also co-IPs with the MT-associated proteins, EB1 and EB3, indicating that STIM1 is closely associated with microtubules [88]. The functional relevance of this association, however, is nebulous. Nocodazole, which depolymerizes microtubules and therefore would be expected to severely impair STIM1 association with microtubules, does not affect puncta formation or even SOCE [35,88], although it does eliminate the tubulovesicular STIM1 movements. Likewise, depletion of cellular ATP eliminates the tubulovesicular movement, but does not impact puncta formation [90]. Thus, the relationship between MT association and CRAC channel activation currently remains mysterious. One possibility is that STIM1 exhibits two forms of movement, one along MTs that is powered by motors, and second diffusive mode of migration that is MT-independent. The available data suggests that SOCE is driven solely by the diffusive mode of STIM1 mobility, but this would be predicted to limit its movement to relatively short distances, a prediction that appears borne out by limited diffusional mobility of STIM1 [39,40].

### 33.8 CONFORMATIONAL CHANGES IN STIM1

STIM1 contains a variety of functional domains in its luminal and cytoplasmic regions (Figure 33.1). Current models of STIM1 activation indicate that interactions between these domains both within the same molecule as well as between neighboring STIM1 molecules regulate the activation state of STIM1. Some early reports suggested that internal electrostatic interactions between different regions of STIM1 are critical for the transition from resting STIM1 oligomers to their active state [91,92]. An acidic region in the CC1 domain was found to interact with a basic region in the CC2 domain to mask the active site of STIM1 that interacts with ORAI1 (termed the CAD domain). Oligomerization appears to favor the removal of this internal autoinhibition to reveal the CAD and polybasic domains, thereby permitting productive interactions between CAD and ORAI proteins leading to channel activation [91,92]. Similar conclusions were drawn by Muik et al., who made use of an intramolecular STIM1 FRET sensor to show that the cytoplasmic region of STIM1 that interacts with ORAI1 switches from a closed to an open configuration upon interaction with ORAI1 [93]. They suggested that the closed confirmation of STIM1 is stabilized by coiled-coil interactions within the C-terminal region of STIM1 and interaction with ORAI1 opens up STIM1 to expose its active ORAI1 binding site [93]. These findings, however, have recently been called into question by more recent evidence indicating that mutations that would be expected to disrupt the electrostatic interactions have no effects on STIM1 function [94]. Moreover, the recent structures of the CAD domain are not readily compatible with interactions between acidic and basic STIM1 regions [95]. Thus, the molecular mechanisms underlying
dimer formation and stimulus-induced conformational changes in STIM1 remain to be clarified. In this context, a recent study employing lanthanide-acceptor energy transfer (LRET) to probe conformational changes in STIM1 following activation found that the CC1 domain of STIM1 directly interacts with the distal STIM1 region including CC3 in the resting state [96]. This interaction is thought to maintain STIM1 in the closed conformation wherein the CAD domain is hidden. Conversely, interactions between neighboring CC1 domains of the STIM1 dimer were suggested to extend the cytoplasmic STIM1 region, producing a conformational change that exposes CAD for association with its targets. Overall, this model is harmonious with previous findings indicating that the cytoplasmic region of STIM1 is folded back to hide CAD in the resting state [93].

33.9 STIM1 BINDS DIRECTLY TO ORAI1

CRAC channels are operationally defined as store-operated channels. The mechanistic basis for store-dependent activation of CRAC channel activation involves direct binding of STIM1 and ORAI1. Direct binding of the two proteins has been demonstrated by several methods using co-immunoprecipitation [45,46,97,98], FRET microscopy [37,38], and pull-downs [40]. ORAI1 and STIM1 associate even in a system of only purified components in solution [40,99], indicating that the interaction between these proteins is strong enough to persist in a variety of chemical environments and can occur even in detergent-solubilized extracts.

Deletion and serial truncations enabled identification of the region of STIM1 required for ORAI1 activation. The C-terminal domain consists of three putative coiled coils, but the rest of the sequence bears little similarity to any known motifs (Figure 33.1a). Huang et al., found that expressing just the cytoplasmic portion of STIM1 (STIM1-ct) was sufficient to activate SOCE, though not to the same extent as full-length STIM1 [41]. They also found that deletion of a large stretch of this domain that includes the coiled-coil motifs (amino acids 231–535) eliminated constitutive activity of this fragment [41]. Baba et al., found that deleting either 249–390 or 391-end in full-length STIM1 reduced SOCE, suggesting that these regions likely contribute to ORAI1 activation [35]. Subsequently, several groups identified a minimal region in STIM1 encompassing the second and the third CC domains as the critical element required for binding and activating ORAI1 [40,42,100,101]. This region is variously called the CRAC activation domain (CAD) [40], STIM1–ORAI1 activating region (SOAR) [42], or Ccβ9 [101] and includes the amino acids 342–444. The structure of this domain has recently been solved, revealing a R-shaped dimeric module with the main functional domains (CC2 and CC3), forming a hair-pin motif [95]. Whether this dimeric module represents the active structural underpinnings of STIM1 function.

Interestingly, the regions involved in STIM1–ORAI1 binding overlap significantly with the domains found to be important for induced STIM1–STIM1 oligomerization. Muik et al. tested a series of STIM1 C-terminal fragments and found that ORai activating STIM fragment (OASF) fragments homomerized in situ and fragments shorter than OASF lacking key elements of the CAD/SOAR domain are monomeric on native gels, don’t self-associate in vivo, and fail to activate ORAI1 [100]. Covington et al. directly investigated the regions of the STIM1 C-terminus important for store-depletion induced oligomerization and found that the critical region for oligomerization and puncta formation overlaps with the CAD/SOAR region [84]. One critical mutation identified by their analysis, A369K, exhibited enhanced resting-state oligomerization, co-localization with ORAI, and constitutive CRAC-channel activity, while at the same time nearly eliminating enhancement of oligomerization upon store-depletion [84]. A second mutation, A376K, caused STIM1 to constitutively self-associate and form puncta, but eliminated co-localization with ORAI and CRAC channel activity before or after store depletion. Their results predicted that both residues lie on a hydrophobic face of the alpha helix in CC2 [84], a prediction borne out in the crystal structure of the CAD domain. Collectively, these results suggest that residues important for ORAI1 binding are also important for STIM1 oligomerization.

33.10 ORAI1 DOMAINS INVOLVED IN STIM1 BINDING

It is now known that STIM1 interacts with both the intracellular C- and N-termini of ORAI1 subunits. Early studies found that both CRAC channel activation and STIM1–ORAI1 FRET following store-depletion was eliminated by deletions of the C-terminus, suggesting the presence of an essential STIM1 binding site in this region [37,40,87]. In vitro pull-down assays with isolated fragments of ORAI1 subsequently indicated that the ORAI1 C-terminal domain fragments encompassing the region between residues 254–301 associate with the cytosolic domains of STIM1 [40,99,100], demonstrating that the STIM1 C-terminus directly interacts with the ORAI1 C-terminus. As in STIM1, the critical binding motif in the ORAI1 C-terminus is a coiled-coil domain, and two point mutations in this region (L273S and L276D) completely disrupt the ORAI1–STIM1 interaction [37,38]. Although it has been suggested that the binding defects caused by these mutations may be traced to disruption of the tertiary structure of the ORAI1 CC domain [102], it is also plausible that the residues are located directly at the STIM1 binding interface itself; hence the mutations may affect STIM1 binding directly [103]. Both L273 and L276 are highly conserved and mutations in other ORAI isoforms at equivalent positions also impede STIM–ORAI1 binding [102,104]. Interestingly, the recent crystal structure of ORAI1 reveals that the C-terminal helices of neighboring subunits form an anti-parallel coiled-coil domain and directly interface with each other, with residues L273 and L276 of one subunit contacting L276 and L273 residues of the conjugate subunit [105]. The structural study suggested that the hydrophobic interface formed by the anti-parallel CC domains likely open to interact with the CAD domain during STIM1 binding [105–107]. However, an alternate possibility is that the anti-parallel domains stay together even when bound to STIM1, and that the STIM1 binding site on the CRAC channel is formed collectively.
by the combined surface of the anti-parallel coiled-coil domain. Indeed, a recent NMR study of a ORAI1 C-terminal fragment bound to a portion of the CC1–CC2 STIM1 domain suggests that the conformation alteration in the ORAI1 C-terminus required for STIM1 binding may be quite small [103], raising the possibility that the self-associated ORAI1 C-termini may not completely unravel. These differing conclusions highlight the need to better define the exact binding interfaces between STIM1 and ORAI1, which still remains unclear. Moreover, many prevailing models are based on results from small fragments of STIM1 and ORAI1. Whether results from protein fragments can be readily extrapolated to full-length molecules is unclear. More studies using full-length molecules are needed to test the validity of the proposed binding models.

In addition to the well-described interaction at the C-terminus of ORAI1, studies using systems of purified components have revealed a second interaction site on ORAI1, located at the N-terminus [40,99]. A purified peptide consisting of the cytosolic N-terminal region corresponding to the region 68–91 interacts with CAD in co-immunoprecipitation and split-ubiquitin assays [40]. Further, GST-pulldown assays have shown direct interaction of a purified n-terminal fragment 65–87 with purified STIM11-ct and the STIM1 c-terminal fragment 233–498 [99].

Functional studies have presented a confusing picture for the roles for the C- and N-terminal sites. In contrast to the complete elimination of STIM1 binding seen in the C-terminal mutations, some early studies found that N-terminal deletions retain significant levels of STIM1–ORAI1 binding, and yet, these deletions abrogated SOCE and ICRAC [37,40,87,108]. A construct with only 73–84 deleted fails to support CRAC activity when co-expressed with STIM [40], yet, some N-terminal deletion mutants formed puncta [87] and supported increases in ORAI1–STIM1 FRET following store depletion [37]. These results led to a modular functional assignment for the roles of the two binding sites, with the C-terminal site thought to mediate STIM1 binding and the N-terminal site thought to strictly regulate only channel gating. However, a more recent study indicates that the N-terminus contributes significantly to the overall stability of STIM1–ORAI1 binding with deletions and mutations at this site strongly diminishing ORAI1 recruitment into puncta and STIM1–ORAI1 binding [109]. This study also found that completely deleting the C-terminal site resulted in nonfunctional channels even when CAD was directly tethered to the ORAI1 C-terminus [109], indicating that the C-terminal site has a role in gating beyond STIM1 binding. These revised results indicate that the C- and N-terminal STIM1 binding sites are both essential for multiple aspects of ORAI1 function including STIM1–ORAI1 association, ORAI1 trapping, and channel gating.

### 33.11 THE SUBUNIT STOICHIOMETRY OF CRAC CHANNELS

There is strong evidence that the ORAI1 subunits interact with each other to form a multi-subunit complex [38,46,60,61,97,99]. However, attempts to evaluate the stoichiometry of this interaction from biochemical and functional assays have proven controversial. Gwack et al. reported that purified ORAI1 co-migrates with STIM1 in glycerol-gradient centrifugation, and that this fraction runs as monomers and dimers on denaturing SDS-PAGE [60]. Maruyama et al. reported that purified ORAI1 is 3× larger than a tetramer [110], and likewise, Park et al. found that purified ORAI1 elutes in a 290 kDa complex [40]. While these studies reaffirmed that ORAI1 exists in a higher order oligomer, they did not provide an easily interpretable ORAI1 stoichiometry.

Two labs applied the subunit-counting approach wherein channel stoichiometry is evaluated by counting the number of photo-bleach steps of GFP fused to ORAI1 monomers [111]. With this approach, these studies concluded that ORAI1 channels stably bound to soluble STIM C-terminus fragment (STIM1-ct) have 4 ORAI1 copies per channel complex [97,112]. However, in the absence of STIM1, the two groups came to differing conclusions. Penna et al. reported that most GFP-ORAI1 complexes bleach in only two steps [97]. Likewise, ORAI3 channels gated directly by the small-molecule, 2-APB, were found to bleach mostly in two steps [113]. These results were interpreted in favor of the model in which ORAI1 exists as a dimer in the resting state, with STIM1 assembling the ORAI1 dimers to form functional, tetrameric channels. In contrast, Ji et al. found that co-expression of STIM1-ct did not affect the number of bleach steps, which occurred in three or four steps in both conditions [112]. In an alternate approach, Madl et al. used a combination of photobleaching and single molecule brightness analysis on the mobile fraction of ORAI1 in resting cells and concluded that ORAI1 predominantly diffuses as a tetramer [114]. They also showed that FRET between ORAI1 dimers was unaltered upon store-depletion suggesting that the stoichiometry of ORAI1 was independent of its association with STIM1 [59].

In yet another approach, Mignen et al. exploited the ability of pore mutants of ORAI1 (e.g., E106Q) to suppress CRAC channel activity through a dominant-negative effect [115]. They found that the ability of (monomeric) ORAI1-E106Q to inhibit Ic_{CRAC} is eliminated when co-expressed with tandem wt ORAI1 constructs containing four protomers [115]. Taken together, these studies concluded that the active channel is a tetramer, although they reached different conclusions on the resting state stoichiometry. All of these studies, however, suffered from the caveat that the underlying results did not rule out the possibility of the stoichiometry being more than four.

The most definitive evidence for the subunit stoichiometry of the CRAC channel has come from the recent crystal structure of the *Drosophila* ORAI1 channel [105]. This structure of the closed channel (crystallized in the absence of STIM1) revealed a multimeric complex composed of six ORAI1 subunits arranged in a threefold axis of symmetry (Figure 33.2a). The C-termini of neighboring subunits were found to be self-associated in an interesting anti-parallel coiled-coil configuration in this structure. Moreover, the cytoplasmic N-terminus was found to extend the pore considerably longer into previously suspected, resulting in an unusually long ion putative ion conduction pathway of ~55 Å (Figure 33.2b) [105]. The hexameric stoichiometry is consistent with the published evidence from size-exclusion and light scattering studies showing a complex of 290 kDa [40]. Thus, the structure of the purified ORAI complex has produced a model that differs from all previous studies of stoichiometry. It remains unclear, however, whether the purified
hexameric complex exhibits the canonical properties of CRAC channels including high Ca\textsuperscript{2+} selectivity and low permeability to Cs\textsuperscript{+}. Indeed, a recent study using concatenated ORAI1 monomers has contended that an overexpressed hexameric concatemer does not produce currents with the signature high Ca\textsuperscript{2+} of CRAC channels [116]. Thus, additional studies are clearly needed to evaluate findings predicted from the structure. Electrophysiological and molecular approaches promise to provide healthy debate on this issue.

### 33.12 HOW MANY STIM MOLECULES DOES IT TAKE TO ACTIVATE CRAC CHANNELS?

An important mechanistic attribute of CRAC channels with far-reaching consequences for CRAC channel operation is the functional stoichiometry of channel activation: How many STIM1 molecules does it take to drive the opening of CRAC channels? To tackle this issue, Li et al. used tandem constructs to determine the effect of STIM1:ORAI1 ratio on CRAC channel activity [117]. In their approach, tandem constructs with varying number of ORAI1 protomers were fused to a STIM1 region containing the minimal activation domain (amino acids 336–485, called S). Their results indicated that for tandem constructs in which the S:ORAI1 ratio was 1:1 or 1:2, addition of a tandem S–S construct increased \( I_{\text{CRAC}} \) magnitude, but for complexes where the ratio is 2:1, the exogenous expression of S–S had no impact on current magnitude [117]. Moreover, (when expressed alone) \( I_{\text{CRAC}} \) was largest for constructs with a 2:1 S-ORAI1 ratio, and decreased as the STIM1–ORAI1 ratio decreased [117]. Their data suggested that a 2:1 STIM1:ORAI1 ratio gives optimal CRAC activity. If each complex contains four copies of ORAI1, then the active complex would have eight copies of STIM1. Crucially, their data showed that if the STIM1:ORAI1 ratio is less than optimal, \( I_{\text{CRAC}} \) is diminished but not eliminated.

In an alternate approach, Hoover and Lewis varied the relative expression of full-length STIM1 and ORAI1 proteins fused to mCherry and GFP to study the functional requirements of ORAI1 activation as well as trapping at the ER-PM junctions [118]. Their results confirmed that activation is graded with increasing STIM1 concentration, but in contrast to the findings using concatenated constructs, this study found that the dependence on STIM1 concentration is highly nonlinear [118]. Maximal CRAC current activation requires the binding of two STIM1 molecules per ORAI1 subunit, and declines sharply with diminishing STIM1 such that the minimal stoichiometry for trapping ORAI proteins into puncta fails to evoke significant activation [118]. These results were interpreted in terms of a model in which once an optimal STIM threshold is reached, individual channels open abruptly to their fully active state in an all-or-none manner due to high cooperativity of channel opening.

At face value, this model is consistent with findings indicating that the slow increase in \( I_{\text{CRAC}} \) following store depletion occurs from the stepwise recruitment of closed channels to a very high \( P_o \) state [21]. However, whether this type of modal gating occurs due to STIM1 binding or represents an intrinsic property of ORAI proteins remains to be resolved.

Interestingly, there is strong evidence that the STIM1:ORAI1 ratio affects not only STIM1-dependent activation, but also calcium-dependent fast inactivation (CDI) [119,120] and as described further later, the ion selectivity of CRAC channels [121]. An acidic region in the C-terminus of STIM1 (amino acids 470–491) is critical for CDI although the precise mechanism by which this domain confers fast inactivation remains unclear [120,122,123]. STIM1/ORAI1 ratio also

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**Figure 33.2** Crystal structure of *Drosophila* ORAI in the closed state. (a) Cross-sectional view of ORAI1 from the extracellular side showing hexameric ORAI complex arranged around a central axis. The transmembrane helices of a single subunit are highlighted in color. (b) Architecture of the ORAI1 pore showing two TM1 helices. The predicted pore-lining residues in TM1 are highlighted in yellow along with their side chains. The corresponding residues for human ORAI1 are shown in parentheses. Residues in the ORAI1 N-terminus including K159, R155, and Q152 (K87, R83, and Y80 in human ORAI1) that may be a part of the ion conduction pathway are highlighted in orange.
affects permeation to Ba²⁺ and Sr²⁺, as well as inhibition by the compound, 2-APB [119]. These results suggest that in addition to serving as a ligand to promote channel activity, STIM1 likely serves as a mobile subunit, influencing many key functional attributes of the channel.

33.13 MECHANISMS OF Ca²⁺ SELECTIVITY AND PERMEATION

CRAC channels are widely noted for their exquisite Ca²⁺ selectivity (Pₐₕ/Pₐₜ > 1000), which places them in a unique category of highly Ca²⁺ selective channels together with voltage-gated Ca²⁺ (Caᵥ) channels [19]. As noted earlier, high Ca²⁺ selectivity is only manifested in Ca²⁺-containing solutions: In divalent-free solutions, CRAC channels readily conduct a variety of small monovalent ions (Na⁺, Li⁺, and K⁺) [20,22,23] illustrating that Ca²⁺ selectivity is not due to steric exclusion of monovalent ions. Supplementing divalent free solutions with micromolar concentrations of Ca²⁺ (Kᵥ ∼ 20 µM at −100 mV) [19–24] blocks monovalent currents, revealing that Ca²⁺ ions occlude monovalent flux through ion–ion and ion–pore interactions. As expected for a binding site within the pore, Ca²⁺ block is voltage-dependent [21,25]. These characteristics are qualitatively reminiscent of the properties of L-type Caᵥ channels, in which Ca²⁺ ions similarly bind tightly to a high-affinity binding site within the pore to occlude Na⁺ flux [26,27]. Unlike Caᵥ channels, however, the affinity of Ca²⁺ blockade is significantly lower in CRAC channels (0.7 µM in L-type Caᵥ channels vs. 20–25 µM in CRAC channels) [22,23,25,26,124,125]. This key functional difference suggests that from a biophysical standpoint, the underlying mechanisms that confer Ca²⁺ selectivity are likely to be different between the two classes of highly Ca²⁺ selective channels. Indeed, a recent study has argued that in addition to Ca²⁺ binding at the selectivity filter, CRAC channels achieve high Ca²⁺ selectivity by restricting the flux of ions (both preferred and nonpreferred) by high energy barriers [125]. Enhancing Na⁺ and Ca²⁺ flux rates by lowering the entry and exit barriers paradoxically reduces Ca²⁺ selectivity, as seen in ORA11 channels gated directly by the small molecule, 2-APB [125]. Thus, both high affinity Ca²⁺ binding as well as kinetic factors seem to contribute to high Ca²⁺ selectivity in CRAC channels.

It is widely accepted that ion permeation is governed by the chemistry and arrangement of pore-lining residues, prompting considerable interest in determining the identity of the pore-lining residues in CRAC channels. Toward this goal, one study applied the substituted cysteine accessibility method (SCAM) [58]. In this approach, residues in the pore-lining region are mutated individually to Cys and the sensitivity of the mutated channels to blockade by aqueous thiol-labeling reagents, such as MTS reagents, is assessed [126]. This study indicated that residues in TM1 flank the pore, and ruled out TM3, and specifically, E190, as pore-lining residues [58]. Similar conclusions were reached in an independent report that examined the pattern of disulfide cross-linking of Cys residues introduced into ORA11 [59]. The SCAM study also indicated that the TM1–TM2 loop segments interact tightly with both large (>8 Å) and small (<3 Å), and with positively charged and negatively charged probes, suggesting that these loops form an outer vestibule with sufficient flexibility to accommodate ions of different size and charge [58]. Strong Cd²⁺ reactivity of several residues in TM1 indicated that the centrally located TM1 helices are close to one another and therefore line a narrow pore, a feature that is likely to account for the low permeability of the CRAC channel to large cations (>3.8 Å) and its low unitary conductance. Moreover, differences in the accessibility of probes of different sizes showed that the pore narrows sharply at the base of the vestibule, near the Ca²⁺ binding site formed by E106. These results provided the first step toward building a structural model of the open pore, and were largely confirmed by the recent crystal structure of the Drosophila ORA1 protein [105].

The crystal structure of Drosophila ORA1 confirmed that TM1 flanks the ion conduction pathway, and, with the exception of F99, the pore-lining residues observed in the structure matched the residues found from cysteine accessibility analysis, including E106, V102, L95, and R91 (Figure 33.2b) [105]. The difference at F99 (G98 in the cysteine scan study) could indicate a possible structural alteration caused by introducing the Cys mutation, but may also reflect a difference between the structures of closed and open channels. The closed x-ray structure also revealed a Ca²⁺ ion density a few angstroms above the predicted selectivity filter formed by E106 rather than at the selectivity filter itself [105]. Why the Ca²⁺ ion density is not localized to the predicted center of the selectivity filter remains unclear, but this may be related to the presumed closed, nonconductive state of the crystallized channels. The cysteine accessibility studies and the x-ray crystal structure have provided us with a firm framework for gaining a better understanding of the mechanisms of ion conduction in CRAC channels, but clearly much more needs to be understood before the structure can be incorporated into plausible models for selectivity and permeation. Ultimately, the channel structures of both the closed and open states are required to illuminate the dynamics of the steps of CRAC channel ion transport cycle.

33.14 STIM1 REGULATES CRAC CHANNEL ION SELECTIVITY

A surprising functional aspect of CRAC channels revealed by analysis of the ion selectivity of a mutant ORA11 channel is that STIM1 not only controls CRAC channel gating, but also bestows many fundamental features that have historically defined the fingerprint of the CRAC channel pore [121]. A variety of substitutions at the pore-lining residue, V102, including substitutions to Cys, Ala, Ser, and Thr, produce constitutively open channels that are open even in the absence of bound STIM1 [121]. The ion selectivities of the STIM1-bound and -free channels, however, are strikingly different. STIM1-free V102C mutant channels exhibit poor Ca²⁺ selectivity and allow permeation of Cs⁺ and several other large cations that are normally impermeable through CRAC channels [121]. However, interaction of the mutant channels with STIM1 restores high Ca²⁺ selectivity to the poorly selective STIM1-free channels. In effect, the aberrant ion selectivity of the STIM1-free mutant channels is corrected following STIM1 binding [121]. These changes are accompanied by alterations in the pore geometry, specifically, significant narrowing of the pore to state that more closely resembles the dimensions seen for WT ORA11 channels. The tuning of ORA11 ion selectivity by STIM1 is not unique to
the V102C mutant channels, but is also seen in wild-type ORAI1 channels as the amount of STIM1 bound to ORAI1 is increased [121], suggesting that the V102X mutations merely unmask a native intermediate channel activation state due to a leaky gate.

The regulation of Ca\(^{2+}\) selectivity of ORAI1 channels by STIM1 is surprising, for this feature implies that STIM1-free ORAI1 channels are intrinsically poorly Ca\(^{2+}\) selective. Instead, the distinguishing characteristics of CRAC channels including high Ca\(^{2+}\) selectivity, low Cs\(^{+}\) permeability, and a narrow pore are bestowed to the otherwise poorly Ca\(^{2+}\) selective ORAI1 channels by STIM1. How does STIM1 modulate ORAI1 ion selectivity? Given that the ORAI1 N-terminus bears a STIM1 binding site in proximity to the pore-forming TM1 segment (Figure 33.1b), it is not difficult to envision that STIM1 binding to the N-terminus could exert powerful effects on the energetic stability of the selectivity filter.

In addition to biophysical implications, the finding that STIM1 regulates ORAI1 selectivity has many important implications for the nature of Ca\(^{2+}\) signals arising from opening of ORAI1 channels under different conditions. The ability of CRAC channels to conduct Na\(^{+}\) under certain conditions may expand their potential functions to include novel modes by which they encode and process cellular information. Because emerging evidence suggests that CRAC channels, aside from activation by STIM1, can also be activated in a STIM1-independent fashion by other ligands, including the small molecule, 2-APB [104,127–130], and the Golgi Ca\(^{2+}\)-ATPase, SPCA2 [77], these findings raise the possibility that ORAI1 channels may function either as highly Ca\(^{2+}\) selective channels or nonselective channels depending on the nature of the upstream activation signal. In addition, the tight coupling of permeation and gating found for CRAC channels provides an alternative perspective on ion channel gating that contradicts conventional ion channel postulates on the separation of gating and selectivity. The picture that emerges is of a hydrophobic gate (V102) located in proximity to the selectivity filter (E106); the proximity of the two structures likely results in a variety of conformational alterations in the selectivity filter during gating. These findings reinforce the emerging viewpoint that there is much more happening in the vicinity of the selectivity filter in ion channels than initially imagined [131,132].

### 33.15 REGULATION OF CRAC CHANNELS BY CALCIUM: CALCIUM-DEPENDENT INACTIVATION (CDI)

Ca\(^{2+}\)-dependent inactivation is a prominent hallmark of CRAC channels involving feedback inhibition of channel activity by the high local (Ca\(^{2+}\)) around individual CRAC channels, resulting in current decay over 100–300 ms steps during hyperpolarizing steps [19,133,134]. Multiple protein–protein interactions and motifs appear to be involved in this process, including an acidic region of the C-terminal region of STIM1 and calmodulin (CaM) binding to the N-terminus of ORAI1 [120,122,123]. An early indication of a role for STIM1 came from a study showing that increasing the STIM1:ORAI1 transfection ratio increases the extent, rate, and calcium dependence of fast inactivation [119]. This also suggested that multiple STIM1 must likely bind the CRAC channel to evoke fast inactivation. It light of this finding, much of the available data on fast inactivation is difficult to interpret because it is not clear in these studies whether the STIM1:ORAI1 ratio was controlled between the different conditions. For example, mutations of several ORAI1 regions (C-terminus, N-terminus, and the II–III loop are reported to affect inactivation, but it is difficult to know if these effects were really to the mutations or simply due to the mutants expressing at a different level than wt ORAI1.

CDI is not affected by mutations of STIM1 in the N-terminus, including the D76A mutation, which renders STIM1 constitutively active [122]. However, mutations or deletions of the region -474–490 in the C-terminal domain of STIM1 significantly affect fast inactivation. In particular, neutralizing a set of negative charges in this region can enhance or inhibit fast inactivation [120,122,123]. However, while calcium binding to this region was found to affect CDI, it does not appear to be related to Ca\(^{2+}\) binding in a straightforward manner since some mutants exhibit reduced calcium binding affinity but increased CDI [120]. Thus, the precise allosteric mechanism by which this putative Ca\(^{2+}\) binding module within STIM1 regulates CDI remains to be clarified.

Interestingly, mutations in ORAI1 selectivity filter that diminish ion selectivity also strongly attenuate fast inactivation [25]. Diminished inactivation is not due to differences in channel expression or because of lower Ca\(^{2+}\) permeability of mutant channels [25]. The molecular basis of this effect remains unknown. One possibility is that the inactivation gating mechanism is closely coupled to ion permeation, such that mutations that alter permeation also have effects on inactivation gating [25]. A second possibility is that the mutations allosterically affect the gating mechanism, which is located elsewhere. Although the location of the inactivation gate is poorly understood, Srikanth et al. showed that mutations in the loop 2–3 region greatly decrease fast inactivation and enhance SOCE and I\(_{\text{CRAC}}\) amplitudes [135]. Overexpressing a 37 amino acid peptide encompassing the intracellular 2–3 loop or including this peptide directly in the patch pipette resulted in dramatically reduced CRAC currents. These results were interpreted in terms of a model in which the intracellular loop acts as a blocking peptide to produce open channel blockade at the intracellular mouth of the channel [135]. More tests are needed to elucidate if this peptide truly comprises the inactivation gate and determine how mutations in the selectivity filter might alter its function.

### 33.16 CONCLUSIONS

The identification of the STIM and ORAI protein families has produced dramatic advances in many aspects of CRAC channel function. In particular, we now have a firm mechanistic framework for understanding how CRAC channels are activated by the depletion of intracellular Ca\(^{2+}\) stores, and we know the key structural elements involved in ion permeation and selectivity. However, there remain many broad unresolved issues. A major unknown is the gating mechanism of the channel: How does STIM1 binding to ORAI1 open the pore? As described earlier, the structure of the closed channel is now available, many critical steps of STIM1 activation have been described in considerable...
Ion channel families


Store-operated CRAC channels


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