The STIM-Orai Pathway: Orai, the Pore-Forming Subunit of the CRAC Channel

3

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Abstract

This chapter focuses on the Orai proteins, Orai1–Orai3, with special emphasis on Orai1, in humans and other mammals, and on the definitive evidence that Orai is the pore subunit of the CRAC channel. It begins by reviewing briefly the defining characteristics of the CRAC channel, then discusses the studies that implicated Orai as part of the store-operated Ca^{2+} entry pathway and as the CRAC channel pore subunit, and finally examines ongoing work that is providing insights into CRAC channel structure and gating.

3.1 The Native CRAC Channel

The CRAC current was originally defined electrophysiologically in T cells and mast cells (Fig. 3.1) (Lewis and Cahalan 1989; Hoth and Penner 1992, 1993; McDonald et al. 1993; Zweifach and Lewis 1993, 1995). Its essential characteristics are that it is activated by a reduction in free Ca^{2+} concentration in endoplasmic reticulum (ER) stores and that under physiological conditions it exhibits a very high selectivity for Ca^{2+} over Na^+ and other ions. Its small unitary current, estimated at ~6 fA in normal physiological solution at −110 mV (Prakriya and Lewis 2006), speaks of an energetic barrier to ion passage through the pore. In part this barrier may represent a purely physical constraint, the narrow pore diameter (~0.39 nm)

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Fig. 3.1 Typical current–voltage (I–V) relation of the CRAC channel. The graph is an idealized rendering of the whole-cell current recorded from T cells or mast cells. In physiological solutions, the inward current at negative transmembrane potentials is carried by Ca^{2+}, and outward current at positive transmembrane potentials is negligible
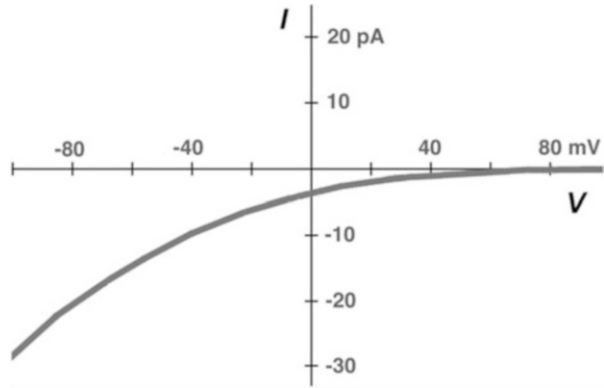
inferred from permeation by a series of organic cations in the absence of Ca^{2+} and Mg^{2+} (Bakowski and Parekh 2002; Prakriya and Lewis 2006). Selective CRAC channel inhibitors have not been available until recently (Prakriya and Lewis 2015), but the classical pharmacological fingerprint of the channel includes blockade by low concentrations of lanthanides (Hoth and Penner 1993; Ross and Cahalan 1995; Aussel et al. 1996) and enhancement of current by low concentrations and block by higher concentrations of 2-aminoethoxydiphenyl borate (2-APB) (Prakriya and Lewis 2001).

CRAC current was observed electrophysiologically in cells other than T cells or mast cells (reviewed in Parekh and Putney 2005). These scattered observations foreshadowed the broad tissue distribution of Orai proteins (Gwack et al. 2007, 2008; Vig et al. 2008; McCarl et al. 2009) and the physiological role of Orai1 in tissues from the skin to secretory epithelia to muscle (Gwack et al. 2008; McCarl et al. 2009; Davis et al. 2015; Concepcion et al. 2016).

3.2   Identification of Orai

Orai was linked to store-operated Ca^{2+} entry by three RNAi screens in *Drosophila* S2 cells (Feske et al. 2006; Vig et al. 2006a; Zhang et al. 2006, reviewed in Hogan et al. 2010). One screen (Feske et al. 2006) scored nuclear localization of the transcription factor NFAT, visualized as a human NFAT-GFP fusion protein, to report on sustained Ca^{2+} influx in response to ER Ca^{2+} store depletion. RNAi treatment identified a handful of *Drosophila* genes whose depletion prevents nuclear import of NFAT-GFP, including a gene annotated at the time as *olf186-F*, now renamed *Drosophila Orai*. This finding meshed with the genetic mapping of severe combined immunodeficiency (SCID) trait in a human family to a region of human chromosome 12 containing *Orai1*, a human homolog of *Drosophila Orai* (Feske et al. 2006). Carriers of the SCID trait were found to be heterozygous for a point mutation that encoded an R91W replacement in the Orai1 protein, and the two

affected SCID patients were homozygous. Reconstitution of T cells from a SCID patient with wildtype Orai1 restored CRAC current.

The two other RNAi screens (Vig et al. 2006a; Zhang et al. 2006), using cytoplasmic Ca^{2+} levels as readout, found a large number of *Drosophila* genes contributing to store-operated Ca^{2+} entry, including *Orai*. These laboratories verified that *Drosophila* Orai or its homolog Orai1 has a role in CRAC current by showing that there was a substantial increase in the store-operated current when Orai was coexpressed with its corresponding ER Ca^{2+} sensor protein, *Drosophila* STIM or human STIM1 (Zhang et al. 2006; Peinelt et al. 2006).

Orai1 deficit is not one of the common causes of inherited immunodeficiency in humans, but a few additional families have been identified with loss of CRAC current due to independent Orai1 mutations (see also Chap. 21, reviewed in Feske et al. 2010; Lacruz and Feske 2015). In addition, rare autosomal dominant mutations in Orai1 lead to increased or constitutive Ca^{2+} influx and cause skeletal myopathy (reviewed in Lacruz and Feske 2015). Studies with cells from $Orai1^{-/-}$ mice show that Orai1 is a main contributor to CRAC current in murine mast cells and mature effector T cells (Vig et al. 2008; Gwack et al. 2008). A residual CRAC-like current in naïve murine T cells has been ascribed to other Orai-family proteins (Vig et al. 2008; Gwack et al. 2008).

3.3 Recombinant Orai Currents

Orai is a plasma membrane protein of mass ~33 kDa with four transmembrane helices (Fig. 3.2). The multimeric Orai1 channel is distributed more or less uniformly in the plasma membrane of resting cells. Following store depletion, Orai1 redistributes to discrete sites on the cell surface, coincident with the STIM1 "puncta" that have been shown to mark sites of Ca^{2+} influx (Luik et al. 2006; Xu et al. 2006; Li et al. 2007; Muik et al. 2008; Navarro-Borelly et al. 2008; Calloway et al. 2009). The physical basis for this redistribution in a STIM-Orai protein-protein interaction is elaborated in Chap. 4. Overexpressed Orai collaborates with overexpressed STIM to produce large CRAC currents, exceeding native CRAC currents in some cases by two orders of magnitude (Zhang et al. 2006; Peinelt et al. 2006; Soboloff et al. 2006; Mercer et al. 2006). Thus, STIM and Orai are the only limiting components of the CRAC channel pathway in the mammalian and *Drosophila* cells tested.

Currents recorded from cells expressing recombinant human or *Drosophila* Orai have precisely the same characteristics as native CRAC currents (Fig. 3.3) (Feske et al. 2006; Prakriya et al. 2006; Zhang et al. 2006; Peinelt et al. 2006; Mercer et al. 2006; Li et al. 2007; Yamashita et al. 2007). The channels are activated by depletion of ER Ca^{2+} stores. They are highly selective for Ca^{2+}. The unitary current is miniscule, and the channel pore is narrow. The pharmacology is that of the CRAC current. Thus, expression of Orai1 gives functional CRAC channels.

Fig. 3.2 The Orai1 monomer is the basic building block of the CRAC channel. The 301-residue Orai1 polypeptide has four transmembrane helices (TM1–TM4) and intracellular N and C termini. Features discussed in the text include STIM binding and gating segments in the cytoplasmic regions of Orai; E106 residues that constitute the principal Ca^{2+} binding site in the pore; the TM1–TM2 loop, whose acidic residues account for lanthanide binding and channel blockade; and R91, site of an R > W replacement that underlies an inherited immunodeficiency syndrome

extracellular

TM1-TM2 loop

E106

TM1 TM2 TM3 TM4

R91

Gating

STIM binding

N

C

intracellular
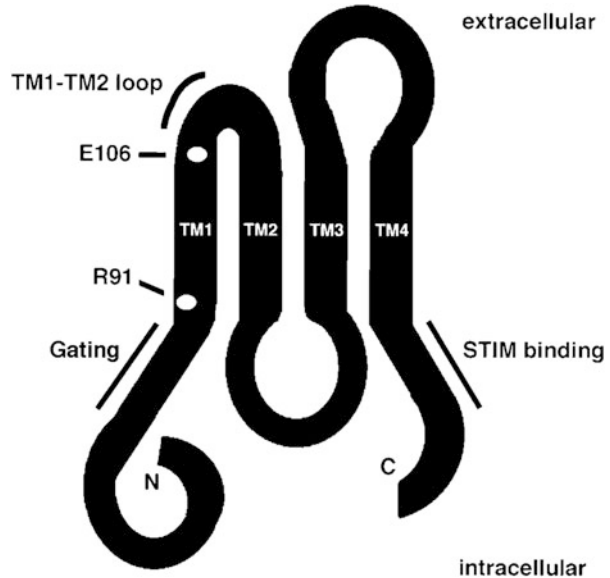
3.4 Orai Is the Pore-Forming Subunit

Several point mutations in Orai1 are sensed by ions permeating the channel, implying that Orai contributes to the CRAC channel pore. The replacements E106A or E106Q, which eliminate the negative charge at E106 in transmembrane helix 1 (TM1), block Ca^{2+} current, even though the mutant Orai proteins are expressed at normal levels at the cell surface (Prakriya et al. 2006; Vig et al. 2006b; Spassova et al. 2008). More tellingly, the point mutation E106D in human Orai1 or the corresponding mutation, E180D, in *Drosophila* Orai alters ion selectivity (Yeromin et al. 2006; Prakriya et al. 2006; Vig et al. 2006b; Spassova et al. 2008) and reduces Ca^{2+} affinity for a site in the pore as measured by Ca^{2+} block of Na^+ currents (Prakriya et al. 2006; Yamashita et al. 2007). These electrophysiological data, together with biochemical and structural evidence discussed below, lead to the conclusion that E106 is part of a Ca^{2+} binding site or sites in the pore. Aside from the effect of mutations at E106, replacement of individual acidic residues in the TM1–TM2 loop alters lanthanide blockade of the channel (Yeromin et al. 2006; McNally et al. 2009), and the replacement E190Q in TM3 has allosteric effects on Ca^{2+} selectivity and pore diameter (Prakriya et al. 2006; Vig et al. 2006b; Yamashita et al. 2007; McNally et al. 2009; Zhou et al. 2010b). The latter findings are additional strong evidence that Orai1 is part of the channel, although the TM1–TM2 loop is not an essential Ca^{2+} binding site and E190 is not in the permeation pathway.

There is further evidence that only Orai is needed to assemble functional CRAC channels in the plasma membrane. In the STIM and Orai coexpression studies,
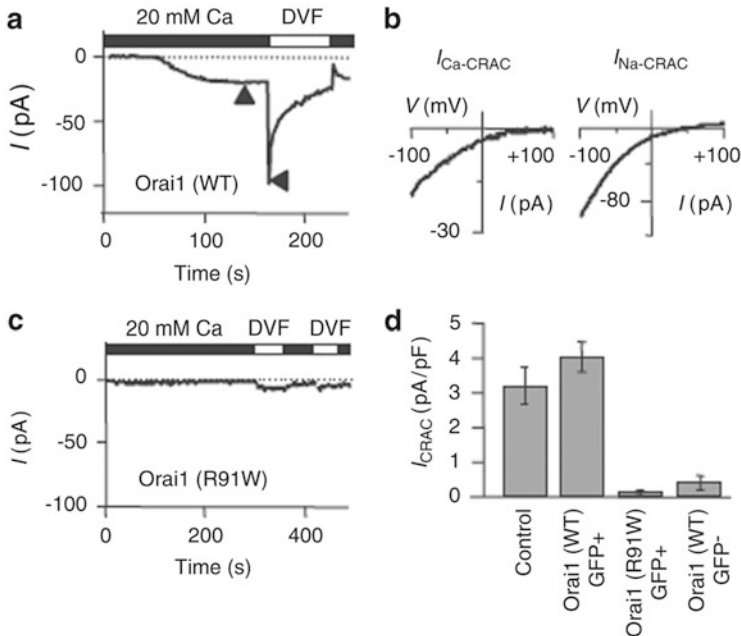
Fig. 3.3 Expression of Orai1 restores CRAC channel function in Orai1(R91W) SCID T cells. (**a**) Development of CRAC current upon store depletion in a SCID T cell expressing recombinant wildtype Orai1. Divalent-free (*DVF*) extracellular solution is used to examine the current carried by Na^+ in the absence of Ca^{2+} and Mg^{2+}. Na^+ does not carry appreciable current when divalent ions are present. (**b**) Typical inward rectifying *I–V* curves for CRAC current carried by Ca^{2+} or by Na^+ at the times indicated by *arrows* in **a**. (**c**) CRAC current is not observed upon store depletion in SCID T cells expressing recombinant Orai1(R91W). (**d**) Peak CRAC current densities in normal T cells, in SCID T cells expressing wildtype Orai1 or Orai1(R91W), and in cells from the cultures transduced with wildtype Orai1 that received little or no expression vector as indicated by the absence of the marker GFP (reproduced from Feske et al. 2006; Fig. 6)

labeled STIM stays in the ER (Mercer et al. 2006; Xu et al. 2006), so only Orai is overexpressed in the plasma membrane. Recombinant Orai1 in isolated yeast membrane vesicles is gated by a purified soluble STIM1 fragment (Zhou et al. 2010a). Because *S. cerevisiae* does not have a STIM-Orai Ca^{2+} signaling mechanism, nor indeed an ER-based Ca^{2+} signaling mechanism, the yeast expression host is unlikely to have contributed an essential channel subunit. Finally, purified recombinant Orai1 reconstituted into liposomes conducts Ca^{2+} when gated by soluble STIM1 (Gudlur et al. 2014). Recombinant Orai1 solubilized and purified from insect, HEK293, and yeast cells is a homomultimer (Park et al. 2009; Maruyama et al. 2009; Hogan 2012), indicating that the basic channel complex consists only of Orai. All of these studies involve overexpression of Orai, however, and they do not establish that overexpressed Orai is gated as efficiently as Orai in the native CRAC channel of T cells and mast cells. In mammalian cells, other

associated proteins may modulate the efficiency of CRAC current activation (Srikanth et al. 2010; Krapivinsky et al. 2011; Palty et al. 2012; Jing et al. 2015; Sharma et al. 2013; Quintana et al. 2015; reviewed in Soboloff et al. 2012).

3.5   Channel Architecture

The Orai1 channel pore architecture was first deduced from Cd^{2+} blockade of current and from disulfide crosslinking experiments on Orai channels harboring single engineered cysteine residues. The Cd^{2+} blockade data showed that TM1 helices line the pore, with residues R91C, L95C, G98C, and V102C facing into the conductance pathway (McNally et al. 2009). Disulfide bridge formation between engineered cysteine residues came to the same conclusion, with sharp peaks of crosslinking at A88C, L95C, and V102C (Zhou et al. 2010b). Engineered E106C residues were readily crosslinked, demonstrating the physical proximity of E106 residues of separate monomers in the channel (McNally et al. 2009; Zhou et al. 2010b). Since the Cd^{2+} blockade experiments report on occlusion of the conductance pathway in the open channel, and covalent crosslinking on the preferred position of side chains in the resting channel, the close similarity of the results suggested that Orai gating movements are subtle. The deduced pore architecture has been confirmed by a 3.35 Å structure of an inactive *Drosophila* Orai channel (Hou et al. 2012) (Fig. 3.4). Importantly, the E106 residues form a negatively charged ring encircling the extracellular opening of the pore, and crystals soaked in the permeant ion Ba^{2+} or the pore blocker Gd^{3+} exhibit electron density for those ions at the E106 ring (Hou et al. 2012). (A second lanthanide-binding site that has been observed in electrophysiological experiments (Yeromin et al. 2006; McNally et al. 2009) is not observed in the crystal structure.) The biochemical and structural verification that E106 residues constitute a Ca^{2+} binding site or sites completes the argument that originated from electrophysiological studies.

The *Drosophila* channel structure also showed that Orai is a hexameric channel. This finding prompted considerable debate, as reviewed in detail by Amcheslavsky et al. (2015), since studies with Orai1 concatemers, as well as technically difficult single-molecule fluorescence experiments, had favored the idea that the channel was a tetramer. The conclusion from the crystal structure that Orai is a hexamer was reinforced by determination of the molecular mass of the purified channel complex using light scattering/UV absorbance/refractive index measurements and by protein cross-linking of *Drosophila* Orai expressed in mammalian HEK293 cell membranes that produced a clearly resolvable ladder of multimers up to the hexamer (Hou et al. 2012). Two careful new studies examining Orai1 concatemers explain the earlier concatemer results and support a hexameric structure (Yen et al. 2016; Cai et al. 2016).
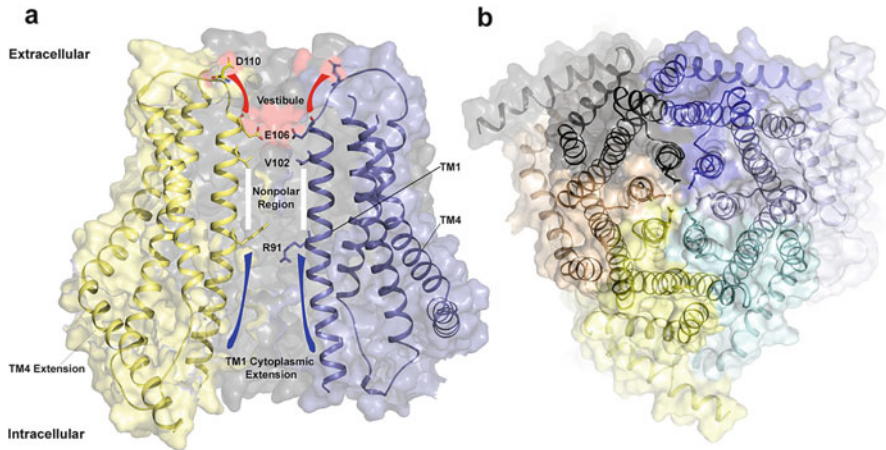
Fig. 3.4 Structural overview of the Orai channel. (**a**) A cutaway view of a human Orai1 model, generated using the *Drosophila* Orai structure PDB:4HKR, highlighting key features of the channel. Some of the subunits and surfaces have been culled for clarity. Surfaces corresponding to E106 and D110 are *shaded red*. The TM1–TM2 vestibule at the extracellular mouth of the channel is marked approximately by *red lines*, the narrow nonpolar region by *white lines*, and the TM1 cytoplasmic extension region by *blue lines*. Residues D110, E106, V102, and R91 are shown in *stick* representation. Note that parts of this apparently concrete model derive from in silico predictions, since the vestibule TM1–TM2 loops and the cytoplasmic TM2–TM3 loops were not resolved in the *Drosophila* Orai crystal structure, and the structure of the *Drosophila* TM3–TM4 loops is not informative for the human protein. (**b**) A snapshot of the channel as viewed from the extracellular side. Each subunit of the hexamer is represented in a *different color* to accent the hexameric organization of the channel. E106 residues are shown in *stick* representation

3.6 The Conductance Pathway

The conductance pathway from outside to inside comprises an outer vestibule, the Ca^{2+} binding site or sites in the vicinity of the E106 ring, a pore segment lined by nonpolar TM1 side chains, and a pore segment flanked by a more polar region of the TM1 helices and their cytoplasmic extensions (Fig. 3.4). Two of these regions have plausible connections to two defining properties of the CRAC channel—the E106 Ca^{2+} binding site(s) to the Ca^{2+} selectivity of the channel and the nonpolar pore segment to the very small single-channel current. However, important mechanistic details of Ca^{2+} ion conductance remain to be unraveled, as noted below.

The vestibule has not yet been defined structurally. The TM1–TM2 loops are not resolved in the *Drosophila* Orai structure—presumably because they are flexible and can adopt multiple conformations (McNally et al. 2009)—and the neighboring TM3–TM4 loops of *Drosophila* Orai, which are resolved, differ substantially in length and sequence from those of Orai1. What is known is that the vestibule immediately external to E106 is relatively wide, given that introduced cysteine residues 107C–110C are accessible to the reagent MTS-TEAE, which has an

8 Å-diameter headgroup (McNally et al. 2009). It has been established by comparison of wildtype and D110A channels that the negatively charged D110 side chains can facilitate Ca^{2+} delivery into the pore (Frischauf et al. 2015). In contrast, D > A replacements indicate that D112 and D114 individually do not contribute significantly to Ca^{2+} current (Frischauf et al. 2015). The latter finding is not surprising, since continuity of the protein backbone to TM2 implies that these residues are more distant from the pore opening than D110, in a region where their negative charge may be countered by the basic residues in the TM3–TM4 loop of human Orai1. The channel vestibule also appears to influence the configuration of the ion selectivity filter, directly or indirectly, since the more extensive vestibule substitutions D110A/D112A and D110A/D112A/D114A decrease Ca^{2+} selectivity and increase pore diameter (Yeromin et al. 2006; Vig et al. 2006b; Yamashita et al. 2007).

The Ca^{2+} binding site at the mouth of channel is a main determinant of Ca^{2+} selectivity. The blockade of Na^+ currents through the wildtype CRAC channel by Ca^{2+} at low micromolar concentrations had been interpreted as a block by Ca^{2+} in transit through the pore (Lepple-Wienhues and Cahalan 1996; Bakowski and Parekh 2002; Prakriya and Lewis 2006) and can now be referred specifically to binding in the vicinity of the E106 ring. This has a functional parallel in the Ca^{2+} binding site that underlies discrimination between Ca^{2+} and Na^+ in the L-type Ca^{2+} channel (Yang et al. 1993; Ellinor et al. 1995). There is another layer of complexity under this seemingly straightforward conclusion. Certain experimental observations, exemplified, for example, by the anomalous mole fraction behavior of Ca^{2+} and Ba^{2+} currents (Hoth 1995), are inconsistent with a model in which the CRAC channel binds only one Ca^{2+} at a time. Where could the additional Ca^{2+} ion (s) bind? One possibility is the E106 ring itself. Note that Ca^{2+} binding "site" of the L-type Ca^{2+} channel—sometimes more properly termed a Ca^{2+} binding "locus"—is understood to be capable of binding two Ca^{2+} ions simultaneously during Ca^{2+} influx (Yang et al. 1993; Ellinor et al. 1995). The E106 ring of the Orai channel, with two more acidic side chains than the acidic ring in the L-type channel, should arguably also be able to bind more than one Ca^{2+}. Another candidate is the Gd^{3+}/La^{3+} site (or, again, locus) in the channel vestibule, which has been rigorously documented in electrophysiological experiments (Yeromin et al. 2006; McNally et al. 2009). Gd^{3+} binding at this physiologically defined locus was not evident in the crystal structure, presumably either because the TM1–TM2 loop is disordered and Gd^{3+} binding has no one preferred configuration or because Gd^{3+} does not bind tightly to these sites in the closed channel. Defining how individual Ca^{2+} ions interact with available ligands as they traverse the channel will be challenging. The relevant configurations are necessarily fleeting and—given the low channel conductance—infrequent. Nonetheless, understanding the possible Ca^{2+} trajectories through the pore is inextricably linked to understanding the very high Ca^{2+} selectivity of the Orai channel.

The highly conserved nonpolar segment of TM1 spans three turns of the helix just internal to the E106 site. It is anchored by the region from residues 99–104, whose relative structural rigidity is evidenced by low rotational mobility in the intermonomer disulfide crosslinking assay and by low temperature factors in the

corresponding region of the *Drosophila* Orai crystal structure (Zhou et al. 2010b; Hou et al. 2012). The nonpolar segment of the pore presents a barrier to ion flux that can be traced in silico in the free energy profile for Na^+ traversing the closed wildtype channel (Dong et al. 2013) and experimentally in the barrier that prevents constitutive Ca^{2+} flux in a channel truncated to remove other proposed barriers at R91 and in the TM1 cytoplasmic extensions (Gudlur et al. 2014). The several lines of evidence that this barrier is displaced during STIM-dependent channel gating are discussed below.

The role of the TM1 cytoplasmic extensions remains uncertain. Intermonomer crosslinking at residue A88C of the inactive channel (Zhou et al. 2010b), the partial Cd^{2+} blockade at residue R91C of the open channel (McNally et al. 2009), and the effect of diamide crosslinking on current through the R91C channel (Zhang et al. 2011) had established that separate TM1 helices can come into close apposition near the cytoplasmic boundary of the membrane. The *Drosophila* Orai crystal structure visualizes helices projecting roughly 2 nm beyond the membrane boundary, to a position corresponding to Q72 in human Orai1 (Hou et al. 2012). Any interpretation relating the TM1 extensions to channel conductance properties is tentative, because it is uncertain whether the configuration of the helices observed for channels in detergent-lipid micelles represents that in a native lipid environment and whether the position of the helices in the crystallized closed channel reflects their configuration in the open channel. In this connection, for instance, it is not obvious from the *Drosophila* Orai structural model how residues 74–83 would bind cholesterol and decrease channel activity (Derler et al. 2016). The TM1 extensions are stabilized in the crystallized form by an iron-containing anionic complex, and it is a further open question whether physiological anions give the same stabilization in cells. All these questions will be answered, of course, and the answers will lead to a clearer understanding of how the TM1 extensions contribute to channel function.

3.7   Channel Gating

Channel gating depends on a direct interaction of Orai1 channels with STIM (reviewed extensively in Gudlur et al. 2013). The initial interaction in cells that recruits Orai to junctions requires the C-terminal cytoplasmic regions of Orai (Li et al. 2007). The region of interaction in Orai1 has been mapped roughly to residues 267–283 by truncations and mutations (Muik et al. 2008; Navarro-Borelly et al. 2008; Yuan et al. 2009; Park et al. 2009; Frischauf et al. 2009; Lee et al. 2009) and by direct binding measurements with Orai1 peptide fragments (Muik et al. 2008; Yuan et al. 2009; Park et al. 2009; Zhou et al. 2010a). The solution NMR structure of a fragment of STIM1 complexed with Orai1(272–292) illustrates one way in which a STIM1 dimer could bind to a pair of adjacent Orai1 C-terminal helices (Stathopulos et al. 2013). Other experiments have pointed to an alternative binding model in which Orai C-terminal helices interact individually with STIM1 (Hou et al. 2012; Zhou et al. 2015, Tirado-Lee et al. 2015, Palty et al. 2015). It is, of course, conceivable that both modes of binding occur during the physiological

interaction of STIM1 with the channel. The issue will be clarified by further experiments.

The N-terminal region of Orai1 spanning residues 73–91 is essential for STIM-dependent activation of the channel (Li et al. 2007; McNally et al. 2013; Derler et al. 2013; Zheng et al. 2013; Palty and Isacoff 2016). Some specific residues that contribute to gating have been mapped in mutational studies (Lis et al. 2010; Derler et al. 2013; Gudlur et al. 2014; Zhou et al. 2016). Most strikingly, the introduction of the three mutations ^{81}LSRAK^{85} > ^{81}AARAE^{85} or the single mutation L81A disrupts gating by STIM1, despite STIM-Orai binding through an intact Orai1 C-terminus (Gudlur et al. 2014; Zhou et al. 2016). The isolated peptides Orai1 (68–87) and Orai1(65–91) bind to STIM1 (Park et al. 2009; Zhou et al. 2010a), and deletions or mutations in this region decrease the interaction of Orai1 with full-length STIM1 or with a soluble STIM1 fragment in cells (McNally et al. 2013; Derler et al. 2013), suggesting that STIM binds directly to the N-terminal region to gate Orai. Other contrasting evidence favors the possibility that STIM binding to the Orai C-terminus is all that is required for gating (Zhou et al. 2015, 2016). In the latter case, the impairment of gating by mutations in the N-terminal segment might indicate that contacts between this segment and other parts of the Orai channel itself are necessary to stabilize the open conformation of the channel. A clearer view of the positioning of this region in the STIM-gated channel will be crucial in understanding physiological channel gating.

While the detailed STIM-Orai interactions and the detailed conformational rearrangements linking STIM binding to channel gating remain to be defined, there has been progress in delineating how gating affects the pore itself. Specifically, productive interaction with STIM1 leads to a rearrangement of the TM1 helices at E106 and at V102 in the nonpolar segment of the pore. The first direct evidence locating a gate in the ion-conducting path of Orai1 was the state-dependent accessibility of G98C to the covalent modifier MTSEA (McNally et al. 2012). More recently, a gating movement of the wildtype channel has been observed directly by monitoring luminescence of Tb^{3+} bound in the Orai1 Ca^{2+} binding site (Gudlur et al. 2014) (Fig. 3.5). The luminescence of bound Tb^{3+} increases when STIM1 binds under conditions that trigger ion flux, indicating that there is a structural rearrangement in the vicinity of E106. This in vitro conformational change is sensitive to the ^{81}LSRAK^{85} > ^{81}AARAE^{85} mutation and is blocked—as is STIM1-dependent current in cells—by the Orai N-terminal peptide Orai1(66–91). Additionally, an increase in intermonomer disulfide crosslinking triggered by soluble STIM1 in the V102C channel strengthens the argument that the hydrophobic region near V102 moves during gating (Gudlur et al. 2014). Completing the argument that the rearrangements detected are essential to gating, an intact L95–V102 segment largely blocks ion flux in a closed channel, even in the absence of R91 and the TM1 extensions (Gudlur et al. 2014), indicating that movement of the nonpolar segment is necessary for ion conductance.

It is informative to compare the physiologically gated wildtype channel with the constitutively conducting channels produced by certain V102X replacements. Replacement of V102 by the small and more polar residues C, S, T, G, or A results
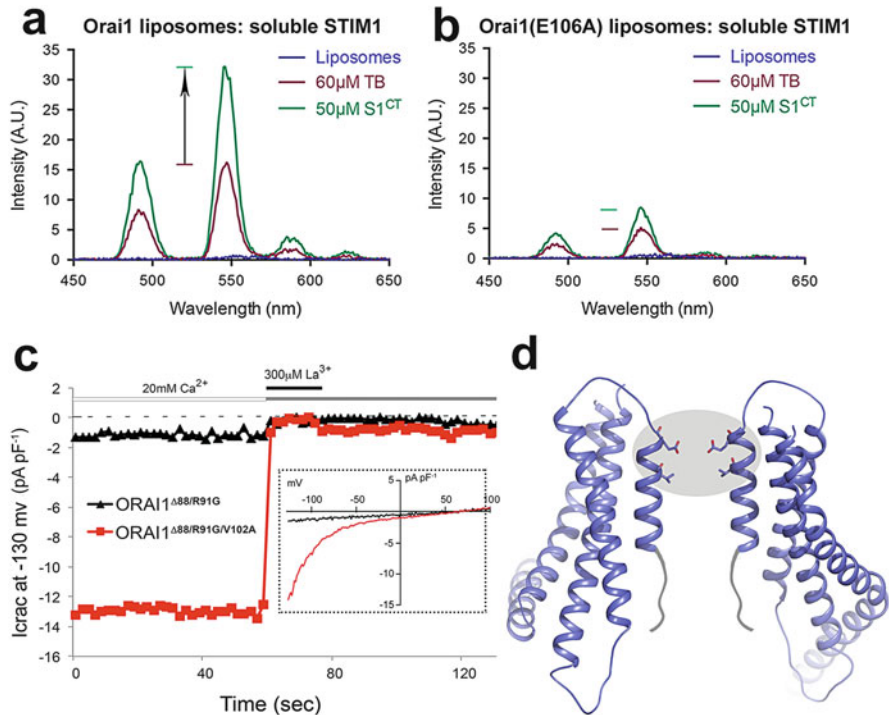
Fig. 3.5 A gating movement in the Orai channel. (**a**) Tb^{3+} binding to purified Orai1 channels reconstituted into liposomes is detected as a luminescence signal following Tb^{3+} addition (*red curve*). Soluble STIM1 ($S1^{CT}$) causes a further increase in luminescence of Tb^{3+} bound to Orai1 (*green curve*), indicating a structural rearrangement at the Tb^{3+} binding site. (**b**) The purified E106A channel in liposomes exhibits very little Tb^{3+} binding (*red curve*) and a minimal response following addition of soluble STIM1 (*green curve*), identifying the ring of E106 side chains as the main site of Tb^{3+} binding and STIM1-dependent rearrangement. Other evidence that STIM elicits movement of the TM1 segment G98–E106 is cited in the text. (**c**) Analysis of constitutive whole-cell currents in cells expressing truncated Orai channels with Orai residues 1–88 replaced by a short unstructured peptide and residue R91 replaced by glycine. Truncated V102A Orai1 channels (*red*) exhibit constitutive currents comparable in amplitude to those of full-length V102A channels expressed at the same level. In contrast, cells expressing corresponding truncated wildtype channels (*black*) have little or no constitutive current. Thus the nonpolar pore region of the Orai channel in itself forms a major barrier to ion permeation. *Inset*: I–V curves of constitutive currents in cells expressing the truncated wildtype and V102A Orai1 channels. (**d**) A schematic view of the pore of the truncated wildtype channel in panel **c**, showing the locus probed by Tb^{3+} in the experiment of panel **a** (*gray ellipse*) and the short unstructured regions replacing the TM1 helix extensions for the experiment of panel **c** (*gray*). Only two of the six channel subunits are depicted (**a**–**c** from Gudlur et al. 2014; Figs. 2b, 2d, and 6b–c)

in a constitutively conducting and less selective channel (McNally et al. 2012; Derler et al. 2013). In silico calculations for the *Drosophila* V174A channel—corresponding to a human V102A channel—suggest that it retains the closed pore conformation of the wildtype *Drosophila* channel but presents a markedly lower

energetic barrier to ion conductance (Dong et al. 2013). Consistent with the idea that the channel is not already in the STIM-gated conformation, the relatively nonselective V102X channels undergo a STIM-dependent conformational change to more selective channels (McNally et al. 2012; Derler et al. 2013). The Tb^{3+} luminescence assay also detects a STIM-dependent conformational change in the V102A channel (Gudlur et al. 2014). This evidence indicating that V102X replacements produce a leaky channel without a gating conformational change further supports the conclusion that the nonpolar segment is a barrier to passage of ions in the closed state of the channel.

The SCID mutant R91W channel also offers some insight. The R91W protein inserts normally into the plasma membrane (Feske et al. 2006) and assembles into a multimeric Orai complex (Muik et al. 2008; Navarro-Borelly et al. 2008). STIM engages R91W Orai upon store depletion, as gauged in store-depleted cells by recruitment of Orai to puncta, by FRET between appropriately labeled STIM and Orai proteins, and by the detectable change in FRET between C-terminally labeled R91W Orai monomers and in vitro by the comigration of soluble STIM1 with cell membranes containing R91W channels on a sucrose density gradient (Muik et al. 2008; Navarro-Borelly et al. 2008; Derler et al. 2009, 2013; Gudlur et al. 2014). A circumstantial case could be made that the SCID mutation blocks ion flux through an otherwise open channel in store-depleted cells. The R91W tryptophan ring is seen to occlude the channel in the *Drosophila* Orai crystal structure (Hou et al. 2012), and experimentally the R91W replacement overrides the constitutive conductance of the V102C mutant (McNally et al. 2012). However, both observations refer to the closed conformation of the channel. Importantly, R91W channels do not exhibit STIM1-dependent gating as measured by Tb^{3+} luminescence or by V102C crosslinking (Gudlur et al. 2014). Therefore, the simplest explanation of the failure to conduct ions is that packing of the six tryptophan residues stabilizes the resting configuration of the Orai N-terminus and disallows a productive gating interaction of the N-terminal segment either with STIM1 or with other parts of the Orai channel.

Gating might additionally involve widening of the pore by an outward movement of residues 76–95 (Zhang et al. 2011; Hou et al. 2012; Derler et al. 2013; Rothberg et al. 2013). It has been proposed, specifically, that the several basic pore-facing residues in this region—R83, K87, and R91—constitute a barrier to ion flux that is repositioned upon gating. However, empirical support for this hypothesis remains equivocal. R91 has been the most prominent candidate to form a barrier, but the R91G, R91D, and R91E Orai1 channels are all closed at rest and open normally upon store depletion, seemingly ruling out an essential role for R91 in gating (Derler et al. 2009; Zhang et al. 2011). The absence of constitutive current through the Orai1(R83A/K87A) channel (Derler et al. 2013) further suggests that R83 and K87 are not required elements of the channel gate. The latter mutant raises some unanswered questions, though, since even the Orai1(R83A/K87A/V102A) channel does not conduct in resting cells, but it is opened by STIM1 to a Ca^{2+}-selective channel (Derler et al. 2013). Other substitutions at positions 83 and 87 might provide further insight. In any case, it is unlikely that the segment

spanning residues 76–95 acts independently as a gate, given that the nonpolar segment from L95–V102 by itself, in a truncated channel lacking the basic residues and the TM1 helix extension, allows little or no constitutive ion flux (Gudlur et al. 2014) (Fig. 3.5).

Mechanistic studies of CRAC channel gating and kinetics have been hampered in the past by the inability to resolve either single-channel currents or gating currents. Recently developed alternative methods, such as detecting the gating conformational change with Tb^{3+} or other fluorescent probes (Gudlur et al. 2014) or recording optically from single Orai1 channels tagged with genetically encoded calcium indicators (Dynes et al. 2016), may offer a way around these limitations.

3.8   Orai2 and Orai3 Channels

Orai2 and Orai3 are reviewed in detail elsewhere (Hoth and Niemeyer 2013), and hence only a few essential facts with regard to pore formation and CRAC activity are outlined here. Both proteins exhibit high sequence similarity to Orai1, particularly in their transmembrane segments. Consistent with this similarity, overexpression of Orai2 or Orai3 together with STIM1 results in store-dependent Ca^{2+} influx and Ca^{2+}-selective currents (Gwack et al. 2007; Lis et al. 2007; DeHaven et al. 2007). Orai3 channels account for a major fraction of the CRAC current in the MCF-7 breast adenocarcinoma cell line (Motiani et al. 2010), and Orai1–Orai3 heteromultimers underlie an arachidonate/leukotriene C_4-regulated Ca^{2+}-selective current, or ARC current, elicited by receptor-phospholipase A_2 signaling (Mignen et al. 2008a, 2009; Gonzalez-Cobos et al. 2013; Zhang et al. 2014). Thus, the other Orai-family proteins are pore-forming channel subunits, and Orai3 has a documented physiological role as a CRAC channel subunit, but the spectrum of physiological Orai channels extends beyond the classical CRAC channel.

Orai3 channels, and to a lesser extent Orai1 channels, can be gated by 2-APB without the intervention of STIM (Peinelt et al. 2008; Zhang et al. 2008; DeHaven et al. 2008; Schindl et al. 2008). STIM and 2-APB trigger the same basic gating and permeation mechanism: The Orai3 correlate of the E106A replacement, E81A, depresses both store-operated current and 2-APB-dependent current (Zhang et al. 2008); and the Orai3 correlate of the R91W mutation, R66W, abolishes the inward current triggered by either stimulus (Schindl et al. 2008). However, in clear contrast to the STIM-gated Orai3 channel, which is selective for Ca^{2+}, the 2-APB-treated Orai3 channel undergoes pore dilation and loses selectivity for Ca^{2+} (Peinelt et al. 2008; Zhang et al. 2008; DeHaven et al. 2008; Schindl et al. 2008, Amcheslavsky et al. 2014). This difference may limit the usefulness of 2-APB as a tool for understanding physiological STIM1-dependent gating.

The close sequence similarity of Orai-family proteins has aided in constructing chimeric Orai proteins to dissect specific channel properties, an approach that has been especially fruitful in dissecting STIM-Orai coupling (Frischauf et al. 2009), as

discussed in Chap. 4. The same approach has been applied to other regions of Orai proteins (Zhang et al. 2008; Frischauf et al. 2011).

3.9 Conclusions

Classical electrophysiological studies defined the CRAC current as a store-operated Ca^{2+} current responsible for physiological activation of T cells and mast cells. The CRAC channel is now known to be widely expressed. RNAi screens identified Orai as a protein essential for CRAC channel function, and a combination of studies using human SCID T cells, protein biochemistry, and electrophysiology established that Orai is the pore subunit of the channel. Ongoing work has illuminated the pore architecture of the channel and the basis for its signature electrophysiological features and has provided insights into channel gating. Despite these considerable advances, a thorough understanding of this specialized Ca^{2+} channel will require further high-resolution structural studies, biophysical probing of conducting Orai channels, and in silico simulations of channel gating and Ca^{2+} permeation.

References

Amcheslavsky A, Safrina O, Cahalan MD (2014) State-dependent block of Orai3 TM1 and TM3 cysteine mutants: insights into 2-APB activation. J Gen Physiol 143:621–631

Amcheslavsky A, Wood ML, Yeromin AV, Parker I, Freites JA, Tobias DJ, Cahalan MD (2015) Molecular biophysics of Orai store-operated Ca2+ channels. Biophys J 108:237–246

Aussel C, Marhaba R, Pelassy C, Breittmayer JP (1996) Submicromolar La3+ concentrations block the calcium release-activated channel, and impair CD69 and CD25 expression in CD3- or thapsigargin-activated jurkat cells. Biochem J 313:909–913

Bakowski D, Parekh AB (2002) Monovalent cation permeability and Ca2+ block of the store-operated Ca2+ current ICRAC in rat basophilic leukemia cells. Pflugers Arch 443:892–902

Cai X, Zhou Y, Nwokonko RM, Loktionova NA, Wang X, Xin P, Trebak M, Wang Y, Gill DL (2016) The Orai1 store-operated calcium channel functions as a hexamer. J Biol Chem 291:25764–25775

Calloway N, Vig M, Kinet JP, Holowka D, Baird B (2009) Molecular clustering of STIM1 with Orai1/CRACM1 at the plasma membrane depends dynamically on depletion of Ca2+ stores and on electrostatic interactions. Mol Biol Cell 20:389–399

Concepcion AR, Vaeth M, Wagner LE 2nd, Eckstein M, Hecht L, Yang J, Crottes D, Seidl M, Shin HP, Weidinger C, Cameron S, Turvey SE, Issekutz T, Meyts I, Lacruz RS, Cuk M, Yule DI, Feske S (2016) Store-operated Ca2+ entry regulates Ca2+-activated chloride channels and eccrine sweat gland function. J Clin Invest 126:4303–4318

Davis FM, Janoshazi A, Janardhan KS, Steinckwich N, D'Agostin DM, Petranka JG, Desai PN, Roberts-Thomson SJ, Bird GS, Tucker DK, Fenton SE, Feske S, Monteith GR, Putney JW Jr (2015) Essential role of Orai1 store-operated calcium channels in lactation. Proc Natl Acad Sci U S A 112:5827–5832

DeHaven WI, Smyth JT, Boyles RR, Putney JW Jr (2007) Calcium inhibition and calcium potentiation of Orai1, Orai2, and Orai3 calcium release-activated calcium channels. J Biol Chem 282:17548–17556

DeHaven WI, Smyth JT, Boyles RR, Bird GS, Putney JW Jr (2008) Complex actions of 2-aminoethyldiphenyl borate on store-operated calcium entry. J Biol Chem 283:19265–19273

Derler I, Fahrner M, Carugo O, Muik M, Bergsmann J, Schindl R, Frischauf I, Eshaghi S, Romanin C (2009) Increased hydrophobicity at the N terminus/membrane interface impairs gating of the severe combined immunodeficiency-related ORAI1 mutant. J Biol Chem 284:15903–15915

Derler I, Plenk P, Fahrner M, Muik M, Jardin I, Schindl R, Gruber HJ, Groschner K, Romanin C (2013) The extended transmembrane Orai1 N-terminal (ETON) region combines binding interface and gate for Orai1 activation by STIM1. J Biol Chem 288:29025–29034

Derler I, Jardin I, Stathopulos PB, Muik M, Fahrner M, Zayats V, Pandey SK, Poteser M, Lackner B, Absolonova M, Schindl R, Groschner K, Ettrich R, Ikura M, Romanin C (2016) Cholesterol modulates Orai1 channel function. Sci Signal 9:ra10

Dong H, Fiorin G, Carnevale V, Treptow W, Klein ML (2013) Pore waters regulate ion permeation in a calcium release-activated calcium channel. Proc Natl Acad Sci U S A 110:17332–17337

Dynes JL, Amcheslavsky A, Cahalan MD (2016) Genetically targeted single-channel optical recording reveals multiple Orai1 gating states and oscillations in calcium influx. Proc Natl Acad Sci U S A 113:440–445

Ellinor PT, Yang J, Sather WA, Zhang JF, Tsien RW (1995) Ca2+ channel selectivity at a single locus for high-affinity Ca2+ interactions. Neuron 15:1121–1132

Feske S, Gwack Y, Prakriya M, Srikanth S, Puppel SH, Tanasa B, Hogan PG, Lewis RS, Daly M, Rao A (2006) A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. Nature 441:179–185

Feske S, Picard C, Fischer A (2010) Immunodeficiency due to mutations in ORAI1 and STIM1. Clin Immunol 135:169–182

Frischauf I, Muik M, Derler I, Bergsmann J, Fahrner M, Schindl R, Groschner K, Romanin C (2009) Molecular determinants of the coupling between STIM1 and Orai channels: differential activation of Orai1-3 channels by a STIM1 coiled-coil mutant. J Biol Chem 284:21696–21706

Frischauf I, Schindl R, Bergsmann J, Derler I, Fahrner M, Muik M, Fritsch R, Lackner B, Groschner K, Romanin C (2011) Cooperativeness of Orai cytosolic domains tunes subtype-specific gating. J Biol Chem 286:8577–8584

Frischauf I, Zayats V, Deix M, Hochreiter A, Jardin I, Muik M, Lackner B, Svobodova B, Pammer T, Litvinukova M, Sridhar AA, Derler I, Bogeski I, Romanin C, Ettrich RH, Schindl R (2015) A calcium-accumulating region, CAR, in the channel Orai1 enhances Ca2+ permeation and SOCE-induced gene transcription. Sci Signal 8:ra131

Gonzalez-Cobos JC, Zhang X, Zhang W, Ruhle B, Motiani RK, Schindl R, Muik M, Spinelli AM, Bisaillon JM, Shinde AV, Fahrner M, Singer HA, Matrougui K, Barroso M, Romanin C, Trebak M (2013) Store-independent Orai1/3 channels activated by intracrine leukotriene C4: role in neointimal hyperplasia. Circ Res 112:1013–1025

Gudlur A, Zhou Y, Hogan PG (2013) STIM-ORAI interactions that control the CRAC channel. Curr Top Membr 71:33–58

Gudlur A, Quintana A, Zhou Y, Hirve N, Mahapatra S, Hogan PG (2014) STIM1 triggers a gating rearrangement at the extracellular mouth of the ORAI1 channel. Nat Commun 5:5164

Gwack Y, Srikanth S, Feske S, Cruz-Guilloty F, Oh-hora M, Neems DS, Hogan PG, Rao A (2007) Biochemical and functional characterization of Orai proteins. J Biol Chem 282:16232–16243

Gwack Y, Srikanth S, Oh-Hora M, Hogan PG, Lamperti ED, Yamashita M, Gelinas C, Neems DS, Sasaki Y, Feske S, Prakriya M, Rajewsky K, Rao A (2008) Hair loss and defective T- and B-cell function in mice lacking ORAI1. Mol Cell Biol 28:5209–5222

Hogan PG (2012) STIM1-ORAI1 store-operated calcium current. In: Egelman E (ed) Comprehensive biophysics, vol 6. Academic, Oxford, pp 223–233

Hogan PG, Lewis RS, Rao A (2010) Molecular basis of calcium signaling in lymphocytes: STIM and ORAI. Annu Rev Immunol 28:491–533

Hoth M (1995) Calcium and barium permeation through calcium release-activated calcium (CRAC) channels. Pflugers Arch 430:315–322

Hoth M, Niemeyer BA (2013) The neglected CRAC proteins: Orai2, Orai3, and STIM2. Curr Top Membr 71:237–271

Hoth M, Penner R (1992) Depletion of intracellular calcium stores activates a calcium current in mast cells. Nature 355:353–356

Hoth M, Penner R (1993) Calcium release-activated calcium current in rat mast cells. J Physiol 465:359–386

Hou X, Pedi L, Diver MM, Long SB (2012) Crystal structure of the calcium release-activated calcium channel Orai. Science 338:1308–1313

Jing J, He L, Sun A, Quintana A, Ding Y, Ma G, Tan P, Liang X, Zheng X, Chen L, Shi X, Zhang SL, Zhong L, Huang Y, Dong MQ, Walker CL, Hogan PG, Wang Y, Zhou Y (2015) Proteomic mapping of ER-PM junctions identifies STIMATE as a regulator of Ca^{2+} influx. Nat Cell Biol 17:1339–1347

Krapivinsky G, Krapivinsky L, Stotz SC, Manasian Y, Clapham DE (2011) POST, partner of stromal interaction molecule 1 (STIM1), targets STIM1 to multiple transporters. Proc Natl Acad Sci U S A 108:19234–19239

Lacruz RS, Feske S (2015) Diseases caused by mutations in ORAI1 and STIM1. Ann N Y Acad Sci 1356:45–79

Lee KP, Yuan JP, Zeng W, So I, Worley PF, Muallem S (2009) Molecular determinants of fast Ca2+-dependent inactivation and gating of the orai channels. Proc Natl Acad Sci U S A 106:14687–14692

Lepple-Wienhues A, Cahalan MD (1996) Conductance and permeation of monovalent cations through depletion-activated Ca2+ channels (ICRAC) in Jurkat T cells. Biophys J 71:787–794

Lewis RS, Cahalan MD (1989) Mitogen-induced oscillations of cytosolic Ca2+ and transmembrane Ca2+ current in human leukemic T cells. Cell Regul 1:99–112

Li Z, Lu J, Xu P, Xie X, Chen L, Xu T (2007) Mapping the interacting domains of STIM1 and Orai1 in Ca2+ release-activated Ca2+ channel activation. J Biol Chem 282:29448–29456

Lis A, Peinelt C, Beck A, Parvez S, Monteilh-Zoller M, Fleig A, Penner R (2007) CRACM1, CRACM2, and CRACM3 are store-operated Ca2+ channels with distinct functional properties. Curr Biol 17:794–800

Lis A, Zierler S, Peinelt C, Fleig A, Penner R (2010) A single lysine in the N-terminal region of store-operated channels is critical for STIM1-mediated gating. J Gen Physiol 136:673–686

Luik RM, Wu MM, Buchanan J, Lewis RS (2006) The elementary unit of store-operated Ca2+ entry: local activation of CRAC channels by STIM1 at ER-plasma membrane junctions. J Cell Biol 174:815–825

Maruyama Y, Ogura T, Mio K, Kato K, Kaneko T, Kiyonaka S, Mori Y, Sato C (2009) Tetrameric Orai1 is a teardrop-shaped molecule with a long, tapered cytoplasmic domain. J Biol Chem 284:13676–13685

McCarl CA, Picard C, Khalil S, Kawasaki T, Röther J, Papolos A, Kutok J, Hivroz C, Ledeist F, Plogmann K, Ehl S, Notheis G, Albert MH, Belohradsky BH, Kirschner J, Rao A, Fischer A, Feske S (2009) ORAI1 deficiency and lack of store-operated Ca2+ entry cause immunodeficiency, myopathy, and ectodermal dysplasia. J Allergy Clin Immunol 124:1311–1318

McDonald TV, Premack BA, Gardner P (1993) Flash photolysis of caged inositol 1,4,5-trisphosphate activates plasma membrane calcium current in human T cells. J Biol Chem 268:3889–3896

McNally BA, Yamashita M, Engh A, Prakriya M (2009) Structural determinants of ion permeation in CRAC channels. Proc Natl Acad Sci U S A 106:22516–22521

McNally BA, Somasundaram A, Yamashita M, Prakriya M (2012) Gated regulation of CRAC channel ion selectivity by STIM1. Nature 482:241–245

McNally BA, Somasundaram A, Jairaman A, Yamashita M, Prakriya M (2013) The C- and N-terminal STIM1 binding sites on Orai1 are required for both trapping and gating CRAC channels. J Physiol 591:2833–2850

Mercer JC, Dehaven WI, Smyth JT, Wedel B, Boyles RR, Bird GS, Putney JW Jr (2006) Large store-operated calcium selective currents due to co-expression of Orai1 or Orai2 with the intracellular calcium sensor, Stim1. J Biol Chem 281:24979–24990

Mignen O, Thompson JL, Shuttleworth TJ (2008a) Both Orai1 and Orai3 are essential components of the arachidonate-regulated Ca2+-selective (ARC) channels. J Physiol 586:185–195

Mignen O, Thompson JL, Shuttleworth TJ (2009) The molecular architecture of the arachidonate-regulated Ca2+-selective ARC channel is a pentameric assembly of Orai1 and Orai3 subunits. J Physiol 587:4181–4197

Motiani RK, Abdullaev IF, Trebak M (2010) A novel native store-operated calcium channel encoded by Orai3: selective requirement of Orai3 versus Orai1 in estrogen receptor-positive versus estrogen receptor-negative breast cancer cells. J Biol Chem 285:19173–19183

Muik M, Frischauf I, Derler I, Fahrner M, Bergsmann J, Eder P, Schindl R, Hesch C, Polzinger B, Fritsch R, Kahr H, Madl J, Gruber H, Groschner K, Romanin C (2008) Dynamic coupling of the putative coiled-coil domain of ORAI1 with STIM1 mediates ORAI1 channel activation. J Biol Chem 283:8014–8022

Navarro-Borelly L, Somasundaram A, Yamashita M, Ren D, Miller RJ, Prakriya M (2008) STIM1-Orai1 interactions and Orai1 conformational changes revealed by live-cell FRET microscopy. J Physiol 586:5383–5401

Palty R, Isacoff EY (2016) Cooperative binding of stromal interaction molecule 1 (STIM1) to the N and C termini of calcium release-activated calcium modulator 1 (Orai1). J Biol Chem 291:334–341

Palty R, Raveh A, Kamisky I, Meller R, Reuveny E (2012) SARAF inactivates the store operated calcium entry machinery to prevent excess calcium refilling. Cell 149:425–438

Palty R, Stanley C, Isacoff EY (2015) Critical role for Orai1 C-terminal domain and TM4 in CRAC channel gating. Cell Res 25:963–980

Parekh AB, Putney JW Jr (2005) Store-operated calcium channels. Physiol Rev 85:757–810

Park CY, Hoover PJ, Mullins FM, Bachhawat P, Covington ED, Raunser S, Walz T, Garcia KC, Dolmetsch RE, Lewis RS (2009) STIM1 clusters and activates CRAC channels via direct binding of a cytosolic domain to Orai1. Cell 136:876–890

Peinelt C, Vig M, Koomoa DL, Beck A, Nadler MJ, Koblan-Huberson M, Lis A, Fleig A, Penner R, Kinet JP (2006) Amplification of CRAC current by STIM1 and CRACM1 (Orai1). Nat Cell Biol 8:771–773

Peinelt C, Lis A, Beck A, Fleig A, Penner R (2008) 2-Aminoethoxydiphenyl borate directly facilitates and indirectly inhibits STIM1-dependent gating of CRAC channels. J Physiol 586:3061–3073

Prakriya M, Lewis RS (2001) Potentiation and inhibition of Ca2+ release-activated Ca2+ channels by 2-aminoethyldiphenyl borate (2-APB) occurs independently of IP(3) receptors. J Physiol 536:3–19

Prakriya M, Lewis RS (2006) Regulation of CRAC channel activity by recruitment of silent channels to a high open-probability gating mode. J Gen Physiol 128:373–386

Prakriya M, Lewis RS (2015) Store-operated calcium channels. Physiol Rev 95:1383–1436

Prakriya M, Feske S, Gwack Y, Srikanth S, Rao A, Hogan PG (2006) Orai1 is an essential pore subunit of the CRAC channel. Nature 443:230–233

Quintana A, Rajanikanth V, Farber-Katz S, Gudlur A, Zhang C, Jing J, Zhou Y, Rao A, Hogan PG (2015) TMEM110 regulates the maintenance and remodeling of mammalian ER-plasma membrane junctions competent for STIM-ORAI signaling. Proc Natl Acad Sci U S A 112: E7083–E7092

Ross PE, Cahalan MD (1995) Ca2+ influx pathways mediated by swelling or stores depletion in mouse thymocytes. J Gen Physiol 106:415–444

Rothberg BS, Wang Y, Gill DL (2013) Orai channel pore properties and gating by STIM: implications from the Orai crystal structure. Sci Signal 6:pe9

Schindl R, Bergsmann J, Frischauf I, Derler I, Fahrner M, Muik M, Fritsch R, Groschner K, Romanin C (2008) 2-Aminoethoxydiphenyl borate alters selectivity of Orai3 channels by increasing their pore size. J Biol Chem 283:20261–20267

Sharma S, Quintana A, Findlay GM, Mettlen M, Baust B, Jain M, Nilsson R, Rao A, Hogan PG (2013) An siRNA screen for NFAT activation identifies septins as coordinators of store-operated Ca2+ entry. Nature 499:238–242

Soboloff J, Spassova MA, Tang XD, Hewavitharana T, Xu W, Gill DL (2006) Orai1 and STIM reconstitute store-operated calcium channel function. J Biol Chem 281:20661–20665

Soboloff J, Rothberg BS, Madesh M, Gill DL (2012) STIM proteins: dynamic calcium signal transducers. Nat Rev Mol Cell Biol 213:549–565

Spassova MA, Hewavitharana T, Fandino RA, Kaya A, Tanaka J, Gill DL (2008) Voltage gating at the selectivity filter of the Ca2+ release-activated Ca2+ channel induced by mutation of the Orai1 protein. J Biol Chem 283:14938–14945

Srikanth S, Jung HJ, Kim KD, Souda P, Whitelegge J, Gwack Y (2010) A novel EF-hand protein, CRACR2A, is a cytosolic Ca2+ sensor that stabilizes CRAC channels in T cells. Nat Cell Biol 12:436–446

Stathopulos PB, Schindl R, Fahrner M, Zheng L, Gasmi-Seabrook GM, Muik M, Romanin C, Ikura M (2013) STIM1/Orai1 coiled-coil interplay in the regulation of store-operated calcium entry. Nat Commun 4:2963

Tirado-Lee L, Yamashita M, Prakriya M (2015) Conformational changes in the Orai1 C-terminus evoked by STIM1 binding. PLoS One 10:e0128622

Vig M, Peinelt C, Beck A, Koomoa DL, Rabah D, Koblan-Huberson M, Kraft S, Turner H, Fleig A, Penner R, Kinet JP (2006a) CRACM1 is a plasma membrane protein essential for store-operated Ca2+ entry. Science 312:1220–1223

Vig M, Beck A, Billingsley JM, Lis A, Parvez S, Peinelt C, Koomoa DL, Soboloff J, Gill DL, Fleig A, Kinet JP, Penner R (2006b) CRACM1 multimers form the ion-selective pore of the CRAC channel. Curr Biol 16:2073–2079

Vig M, DeHaven WI, Bird GS, Billingsley JM, Wang H, Rao PE, Hutchings AB, Jouvin MH, Putney JW Jr, Kinet JP (2008) Defective mast cell effector functions in mice lacking the CRACM1 pore subunit of store-operated calcium release-activated calcium channels. Nat Immunol 9:89–96

Xu P, Lu J, Li Z, Yu X, Chen L, Xu T (2006) Aggregation of STIM1 underneath the plasma membrane induces clustering of Orai1. Biochem Biophys Res Commun 350:969–976

Yamashita M, Navarro-Borelly L, McNally BA, Prakriya M (2007) Orai1 mutations alter ion permeation and Ca2+-dependent fast inactivation of CRAC channels: evidence for coupling of permeation and gating. J Gen Physiol 130:525–540

Yang J, Ellinor PT, Sather WA, Zhang JF, Tsien RW (1993) Molecular determinants of Ca2+ selectivity and ion permeation in L-type Ca2+ channels. Nature 366:158–161

Yen M, Lokteva LA, Lewis RS (2016) Functional analysis of Orai1 concatemers supports a hexameric stoichiometry for the CRAC channel. Biophys J 111:1897–1907

Yeromin AV, Zhang SL, Jiang W, Yu Y, Safrina O, Cahalan MD (2006) Molecular identification of the CRAC channel by altered ion selectivity in a mutant of Orai. Nature 443:226–229

Yuan JP, Zeng W, Dorwart MR, Choi YJ, Worley PF, Muallem S (2009) SOAR and the polybasic STIM1 domains gate and regulate Orai channels. Nat Cell Biol 11:337–343

Zhang SL, Yeromin AV, Zhang XH, Yu Y, Safrina O, Penna A, Roos J, Stauderman KA, Cahalan MD (2006) Genome-wide RNAi screen of Ca2+ influx identifies genes that regulate Ca2+ release-activated Ca2+ channel activity. Proc Natl Acad Sci U S A 103:9357–9362

Zhang SL, Kozak JA, Jiang W, Yeromin AV, Chen J, Yu Y, Penna A, Shen W, Chi V, Cahalan MD (2008) Store-dependent and -independent modes regulating Ca2+ release-activated Ca2+ channel activity of human Orai1 and Orai3. J Biol Chem 283:17662–17671

Zhang SL, Yeromin AV, Hu J, Amcheslavsky A, Zheng H, Cahalan MD (2011) Mutations in Orai1 transmembrane segment 1 cause STIM1-independent activation of Orai1 channels at glycine 98 and channel closure at arginine 91. Proc Natl Acad Sci U S A 108:17838–17843

Zhang X, Zhang W, Gonzalez-Cobos JC, Jardin I, Romanin C, Matrougui K, Trebak M (2014) Complex role of STIM1 in the activation of store-independent Orai1/3 channels. J Gen Physiol 143:345–359

Zheng H, Zhou MH, Hu C, Kuo E, Peng X, Hu J, Kuo L, Zhang SL (2013) Differential roles of the C and N termini of Orai1 protein in interacting with stromal interaction molecule 1 (STIM1) for Ca2+ release-activated Ca2+ (CRAC) channel activation. J Biol Chem 288:11263–11272

Zhou Y, Meraner P, Kwon HT, Machnes D, Oh-hora M, Zimmer J, Huang Y, Stura A, Rao A, Hogan PG (2010a) STIM1 gates the store-operated calcium channel ORAI1 in vitro. Nat Struct Mol Biol 17:112–116

Zhou Y, Ramachandran S, Oh-Hora M, Rao A, Hogan PG (2010b) Pore architecture of the ORAI1 store-operated calcium channel. Proc Natl Acad Sci U S A 107:4896–4901

Zhou Y, Wang X, Wang X, Loktionova NA, Cai X, Nwokonko RM, Vrana E, Wang Y, Rothberg BS, Gill DL (2015) STIM1 dimers undergo unimolecular coupling to activate Orai1 channels. Nat Commun 6:8395

Zhou Y, Cai X, Loktionova NA, Wang X, Nwokonko RM, Wang X, Wang Y, Rothberg BS, Trebak M, Gill DL (2016) The STIM1-binding site nexus remotely controls Orai1 channel gating. Nat Commun 7:13725

Zweifach A, Lewis RS (1993) Mitogen-regulated Ca2+ current of T lymphocytes is activated by depletion of intracellular Ca2+ stores. Proc Natl Acad Sci U S A 90:6295–6299

Zweifach A, Lewis RS (1995) Rapid inactivation of depletion-activated calcium current (ICRAC) due to local calcium feedback. J Gen Physiol 105:209–226