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Structural features of STIM and Orai underlying store-operated calcium entry

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Abstract

Store-operated calcium entry (SOCE) through Orai channels is triggered by receptor-stimulated depletion of Ca^{2+} from the ER. Orai1 is unique in terms of its activation mechanism, biophysical properties, and structure, and its precise regulation is essential for human health. Recent studies have begun to reveal the structural basis of the major steps in the SOCE pathway and how the system is reliably suppressed in resting cells but able to respond reliably to ER Ca^{2+} depletion. In this review we discuss current models describing the activation of ER Ca^{2+} sensor STIM1, its binding to Orai1, propagation of the binding signal from the channel periphery to the central pore, and the resulting conformational changes underlying opening of the highly Ca^{2+} selective Orai1 channel.

Introduction

Ca^{2+} release-activated Ca^{2+} (CRAC) channels are a unique and nearly ubiquitous class of store-operated Ca^{2+} channels that open in response to the loss of Ca^{2+} from the lumen of the ER [1]. They are activated by receptors that release Ca^{2+} from the ER, typically through the generation of inositol 1,4,5-trisphosphate, and are distinguished by an extremely high Ca^{2+} selectivity and low single-channel conductance. Their activity is essential for initiating the adaptive immune response, sustaining contractile activity in muscle, blood clotting by platelets, skin and tooth development and many other functions. Tight regulation of store-operated Ca^{2+} entry (SOCE) is critical, as loss-of-function and gain-of-function mutations in humans create serious health disorders, including severe combined immunodeficiency and autoimmunity, myopathy, ectodermal dysplasia, and Stormorken's Syndrome [2].

The essential components of SOCE are the STIM family of ER Ca^{2+} sensors (STIM1 and STIM2 in vertebrates) and the Orai pore-forming channel proteins (Orai1, 2, and 3 in vertebrates). In resting cells STIM1 and Orai1 diffuse independently in the ER and PM, but

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Conflict of Interest Statement

The author declares no conflict of interest.

ER Ca^{2+} depletion activates STIM1, enabling it to oligomerize and accumulate at ER-plasma membrane junctions where it binds, traps and opens Orai1 channels (Figure 1). In this way, the core machinery of SOCE is assembled on demand through a self-organizing diffusion trap that provides a flexible means of targeting local Ca^{2+} signals to particular locations in the cell.

Recent work has provided new insights into the structural rearrangements of STIM1 that underlie activation, the relation between STIM1 binding and Orai1 channel opening, and how the channel pore may rearrange to allow ion conduction while conferring the extremely high Ca^{2+} selectivity characteristic of the native CRAC channel. This review emphasizes recent progress in understanding these and other structural aspects of STIM and Orai function. Comprehensive reviews provide more complete background information [1,3].

A bimodal switch selects the quiescent and active states of STIM1

STIM1 activity is controlled primarily by regulating access to a cytosolic domain known as the CRAC activation domain (CAD; [4]) or STIM-Orai activation region (SOAR; [5]) to Orai1 (Figure 2A). In resting cells with ~400 μM $[Ca^{2+}]_{ER}$ [6], Ca^{2+} is bound to the two luminal EF hands of the STIM1 dimer [7] and to 4–5 additional surface sites in each EF-SAM domain that are energetically coupled to the EF hand [8] (Figure 2B). Each EF hand envelops a helix of its adjacent SAM domain [7], separating the two EF-SAM domains [8,9]. In this state, the cytosolic domain adopts a compact structure in which the CC1 domain interacts with CAD/SOAR, effectively sequestering it near the ER membrane [10–14]. The ER-proximal CC1α1 helix appears to be most directly involved in binding CAD/SOAR, as suggested by the ability of a truncated STIM1 protein containing CC1α1 to capture soluble CAD/SOAR when ER $[Ca^{2+}]$ is high [12] and FRET between ER-anchored CC1α1 and CC3 fragments [15]. The binding interface is not yet defined, but several critical hydrophobic residues in CC1α1 (L248, L251. L258, and L261) and CC3 (L416, V419, and L423) have been proposed to interact through a coiled-coil based on mutagenesis studies [10,12] (Figure 2B). Mutations or deletions of the CC1α3 domain also cause constitutive STIM1 activation [14,16,17], and the amphipathic nature of the helix appears to be important for its ability to regulate STIM1 activity [18]. However, the underlying mechanism, in particular whether CC1α3 interacts directly with CAD/SOAR, is not clear [12,15].

Following store depletion, Ca^{2+} unbinds from the EF hands and the additional luminal binding sites with an overall $K_{1/2}$ of ~200 μM, allowing the two EF-SAM domains to dimerize [8,9,19] and bring together the two TM domains to form a coiled-coil [20] (Figure 2B). This rearrangement releases the two CC1α1 domains from CAD/SOAR, which then associate to extend the coiled-coil beyond the TM domains [20]. In this way, the CC1α1 domain serves a dual function: it pairs with CAD/SOAR to stabilize the quiescent state and dimerizes after store depletion to release and move it towards Orai1 in the active state. A critical requirement noted by Hirve et al. [20] is to balance the two conformations so that the quiescent and active forms are stable yet interchangeable. This balance may be established by an imperfect heptad repeat and several sentinel residues (N234, S237, D247) in the proximal part of CC1α1, which are thought to destabilize the coiled-coil enough to make it

unfavorable under resting conditions when the TM domains are held apart, but allow it to form after the EF-SAM domains dimerize and the TM domains associate following Ca^{2+} release [20].

Whether the CC1α2 and CC1α3 domains also pair via coiled-coil interactions is not known, but they are likely to coordinate with CC1α1 during STIM1 activation. The R304W mutation activates STIM1 and causes Stormorken syndrome [2]. Located just before the linker region between CC1α2 and CC1α3, the R304W mutation appears to extend the helical domain through the linker, straightening this region and driving CC1α1 to unbind from CAD/SOAR [21] (Figure 2B). If a similar structural change occurs during activation of WT STIM1 by store depletion, extension of CC1α2 and CC1α3 would complement the pairing of CC1α1's to promote CAD/SOAR release and could move it towards the PM by as much as ~15 nm, a truly dramatic conformational switch.

CRAC channel structure and STIM1-Orai1 binding

The first crystal structure of the *Drosophila* Orai (dOrai) channel was a critical breakthrough in the field, demonstrating a hexameric arrangement of 4-TM subunits [22] that countered the prevailing non-structural evidence for a tetramer (reviewed in [1,23]) (Figure 3A, B). The hexameric stoichiometry was tested functionally through electrophysiological studies of hexameric Orai1 concatemers [24,25]. Importantly, the pore properties of hexameric concatemers, including Ca^{2+} blocking affinity, unitary conductance, and the Cs^{+}/Na^{+} permeability ratio all matched those of native CRAC channels [25]. Because these properties are determined by the local geometry of the pore helices (which would be quite different for tetrameric and hexameric configurations), the electrophysiological data strongly imply that the native CRAC channel functions as a hexamer of Orai1 subunits.

As predicted from earlier cysteine-scanning studies [26,27], the Orai channel structure shows that the six TM1s line the entire ion permeation pathway, comprising several domains in series: the selectivity filter, a 15 Å-long hydrophobic region and a 15 Å-long basic region (Figure 3C). The pore is surrounded by a shell of interlocking TM2 and TM3 helices and TM4s at the perimeter of the channel (Figure 3B). P245 creates a kink in TM4 which enables adjacent cytosolic M4 extensions (M4ext) to bend in opposite directions and interact through antiparallel coiled-coils, resulting in three crossed helical pairs (Figure 3B and3D, *left*). The M4 extensions are widely accepted to be the major binding site for STIM1, as they bind STIM1 in vitro, and deletion and mutagenesis show they are required for STIM1 binding, puncta formation, and Orai1 activation in vivo [1]. Human STIM1 does not bind the Orai1 2–3 loop in vitro, although binding has been detected in *C. elegans* which appears to utilize a somewhat different activation mechanism [28]. Binding to the N-terminus and its functional significance has been debated. The cytoplasmic extension of TM1 (aa 73–91) is required for STIM1-mediated CRAC channel activity and isolated fragments bind weakly to STIM1 and CAD/SOAR [4,29–31], but recent reports reveal it is also required to support constitutive activity of mutant Orai1 channels in the absence of STIM1 [32,33]. While these results do not rule out binding to STIM1, a simple interpretation is that channel opening requires the N terminus to interact with other parts of Orai1, possibly the 2–3 loop [34].

Orai1 opening is a remarkably steep function of STIM1 binding. Inhibition of STIM1 binding to just a single M4ext carrying an L273D mutation reduces the open probability to <10% of the WT channel [35]. In addition, incomplete STIM1 binding alters the pore properties [36]; the single L273D mutation triples the unitary conductance while reducing Ca^{2+} block affinity and selectivity for Na^{+} over Cs^{+} [35]. These dramatic effects imply that STIM1 binding to all six Orai1 subunits is required not only to effectively open the channel gate but also to properly configure the pore of the native CRAC channel to achieve its normal conduction and selectivity.

Two general models have been proposed to describe STIM1 binding to the Orai1 C terminus. In the dimeric model, each STIM1 dimer engages a pair of adjacent M4 extensions; this proposal was originally based on the NMR solution structure of a complex of STIM1 and Orai1 fragments, in which the CC2 domains of the CAD/SOAR dimer fold to create a pair of binding pockets for two crossed, antiparallel M4 extensions [37]. That STIM1 interacts with pairs of Orai1 subunits is supported to some extent by the ability of a mutant L273D Orai1 subunit deficient in STIM1 binding to enhance STIM1-Orai1 FRET when located next to a WT subunit [35]. However, there is as yet no direct evidence for simultaneous binding of STIM1 or CAD/SOAR dimers to adjacent Orai1 subunits, and a recent open-channel dOrai structure [38] (discussed below) is not easily reconciled with the antiparallel M4 extensions of the dimeric STIM-Orai complex described by NMR [37].

Other studies support a monomeric binding model in which each STIM1 dimer engages only one Orai1 C terminus, through one of its two subunits. This model is based on the ability of CAD/SOAR dimers with one non-binding F394H subunit to bind and activate Orai1 to the same extent as WT CAD/SOAR [39]. Interestingly, the free CAD/SOAR subunit would help explain how CAD/SOAR expression crosslinks channels into clusters and slows Orai1 diffusion in the PM [4,40]. Crosslinking could also account for the preferred ~15-nm spacing of Orai1 particles at ER-PM junctions [41], approximating the dimensions of two channels separated by a CAD/SOAR dimer [40]. It is worth noting that the monomeric and dimeric models need not be mutually exclusive, if they represent different stages of the activation process (as in a sequential binding mechanism [42]), or if the NMR structure [37] engages two channels rather than adjacent subunits on the same channel. A full resolution to the question of how STIM1 binds to Orai1 awaits a structural description of the full-length STIM1-Orai1 complex, a particularly challenging goal given that the proteins are flexible, reside in separate membranes, and interact with low affinity.

From STIM1 binding to Orai1 opening

Mutagenesis and the closed dOrai structure have offered important new insights into how STIM1 binding at the channel periphery is allosterically transmitted through the TM2-TM3 shell to the TM1 pore and gate. Orai1 mutations at over 20 locations, predominantly in TM2-TM4, constitutively activate the channel [32,33,36,43–47]. These findings suggest that the resting closed state is stabilized by multiple interactions between the TM domains and that the energy barrier to opening is quite low [32]. While most of constitutively active mutants are non-selective, several mutations produce highly Ca^{2+}-selective channels without STIM1 by interfering with interhelical contacts. Replacing $_{261}$LVSHK$_{265}$ with ANSGA in

the M4ext is likely to disrupt hydrophobic contacts between M4 and M3 [33] (Figure 3B), while P245L in TM4 [44] may break the same contacts by straightening a kink in the TM4 helix and disrupting the coiled-coil arrangement of M4ext helices [38] (Figure 3D). H134 mutations in TM2 that activate Orai1 [32,47] disrupt a contact between TM2 and TM1 within a region of alternating stripes of interacting polar and hydrophobic residues that appears critical for gating and selectivity of Orai1 [32] (Figure 3F). Above this location and lateral to the hydrophobic pore region, a tightly packed hydrophobic stack of interacting TM1, TM2 and TM3 residues is thought to transmit force from the TM2/TM3 ring to the pore gate, based on the inhibitory effects of alanine substitutions [32]. In addition, as noted above the cytoplasmic extension of TM1 is likely to interact with other parts of Orai1 during channel activation. The ways in which the forces from STIM1 binding are funnelled from the periphery to the pore are clearly multifaceted and an exciting focus for further research.

Two regions have been implicated in Orai gating: the hydrophobic region near the extracellular end of the pore [29,36,43], and the more interior polybasic region [22] (Figure 3C). Several models have recently been proposed for the Orai1 open state that involve conformational changes in these regions [38,43,47]. In the pore rotation model, hydrophobic side chains of V102 and F99 extending into the pore near the outer mouth of the channel create a barrier to water entry and ion permeation in the closed state [36,43]. STIM1 binding changes the pore accessibility of cysteines introduced at F99 or G98 in opposite ways, consistent with a local ~20^{o} rotation of TM1 helices that moves the F99 side chains out of the lumen [43] (Figure 3E, *left*). Molecular dynamics simulations illustrate how this reduction of pore hydrophobicity would lower the energy barrier for water entry to allow Ca^{2+} to enter the pore [43]. Interestingly, a coupled rotation of F99 and the selectivity filter at E106 (two helical turns above F99) could serve to configure the Ca^{2+} binding site to optimize selectivity (Figure 3E, *right*), helping to explain the tight coupling of gating and ion selectivity in CRAC channels.

A second gating model based on dilation of the polybasic pore region is based on the crystal structure of a constitutively active dOrai mutant. As discussed above, the H134A mutation in human Orai1 opens the channel while retaining almost normal Ca^{2+} selectivity in the absence of STIM1 [32,47]. Comparison of the homologous dOrai H206A structure with that of the closed WT dOrai revealed two major changes. First, the M4ext helices are fully extended rather than crossed in paired coiled-coil interactions (Figure 3D). Second, the inner pore of the H206A channel is highly dilated (by ~ 10 Å) due to a rigid body rotation of all four TM helices away from the pore axis, which would remove a conduction block imposed by anion binding seen between the three rings of closely apposed basic residues in the closed state (Figure 3C, D). Iodide localized in the open pore structure suggests that free intracellular anions may ease the passage of Ca^{2+} through this region by screening positive charges lining the inner pore [38,48]. A second closed-state structure with straightened M4 extensions showed that breaking the M4ext coiled-coils is not sufficient for opening, but importantly is required to allow enough room for the TM helices to move outward and reach the open state. Hou et al [38] suggest that the crossed M4ext helices serve as latches and enable M4-M3 interactions to stabilize the closed state, and these must be released before STIM1 can bind and open the channel. Interestingly, the latched configuration requires the helical bend at P288 (human P245) and the SHK hinge (Figure 3D), which may help to

explain the activating effects of mutations at these locations [38] (see above). A third gating model based on MD simulations and cysteine crosslinking of the H134A Orai1 mutant suggests that gating involves a more modest dilation of both the hydrophobic and basic sections of the pore, and a rotation of R91 side chains toward the pore perimeter [47].

How can these several gating models be reconciled? While the dOrai H206A structure lacks sufficient resolution to localize side chains and permit detailed comparisons, some aspects of the models are compatible. For example, Yeung et al [32] reported rotation of F99 out of the pore in the H134A mutant hOrai1 channel, along with a small (~2 A) pore dilation in this region, and Hou et al [38] observed a downward shift in the location of Ba^{2+} bound to the selectivity filter in the open channel, which could result from dilation and/or rotation at this site. However, the large pore dilation observed in the dOrai H206A structure is unique, and it is important to consider whether it accurately mimics the physiological STIM1-bound open state. Multiple factors could exaggerate the dilation, including the absence of bound STIM1 combined with stabilization of fully extended M4ext helices by contacts in the crystal lattice. An atomic-resolution structure of the full length STIM1-Orai1 complex will be indispensable in fully resolving the native open state of Orai1.

Beyond activation: negative regulation of SOCE

CRAC channels are also negatively regulated in several significant ways to control the extent of Ca^{2+} influx. Alternative splicing of STIM2 inserts 8 residues into the CC2 of CAD/SOAR, transforming STIM2 from an activator into an inhibitor of Orai1 [49,50]. Biochemical studies thus far show that the insert reduces the helicity and enhances the exposed hydrophobicity of CAD/SOAR, which may underlie its inhibitory action [51]. Redox regulation of Orai1 has been traced to oxidation of C195 at the extracellular end of TM3 [52], which has been proposed to hydrogen bond with S239 at the top of TM4 to prevent channel opening [53]. Fast Ca^{2+}-dependent inactivation (CDI) of Orai1 depends on functional interactions of an acidic inactivation domain in STIM1 [54–56] with W76 in the cytosolic extension of the pore [57], raising the possibility of STIM1-TM1 interaction. For slow CDI [58], recent evidence suggests that Ca^{2+}-calmodulin binds to the C-terminal end of CC2 in CAD (L390, F391), to displace STIM1 from Orai1 and allow channel closure [59]. These several modes of CRAC channel regulation have not been as extensively studied as activation, and direct structural studies will undoubtedly help clarify their mechanisms.

Conclusions/Future Perspectives

Precise regulation of STIM1 and Orai1 activity is critical for human health, and the bistability of STIM1 activation, multi-site stabilization of the Orai1 closed state, nonlinear activation of Orai by STIM1 binding, and negative regulation all contribute to the fine control of SOCE. While the physical bases of these mechanisms are beginning to emerge, there are as yet very few atomic-level structures of STIM1 and Orai1 in their various states individually or in combination, and definitive solutions to many of these mechanistic questions will ultimately require more extensive and direct structural information. A central goal is to delineate the complete sequence of conformational changes that drive STIM1 activation as well as its binding and activation of Orai1. In this regard, structures of the full-

length STIM1-Orai1 complex will be particularly informative, in describing the clustered arrangement of STIM1 around the channel, its binding interface with Orai1, and the physiologically relevant open state of the channel. One additional hope is that detailed structural information will reveal intermediate states as potential druggable targets for treating a variety of SOCE-associated diseases.

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residues that are involved in transmitting the STIM1 gating signal to the pore. The also show that H134S opens the pore in a similar manner as STIM1 binding (through pore rotation), and that it may function as a steric brake to prevent activation in the absence of STIM1.

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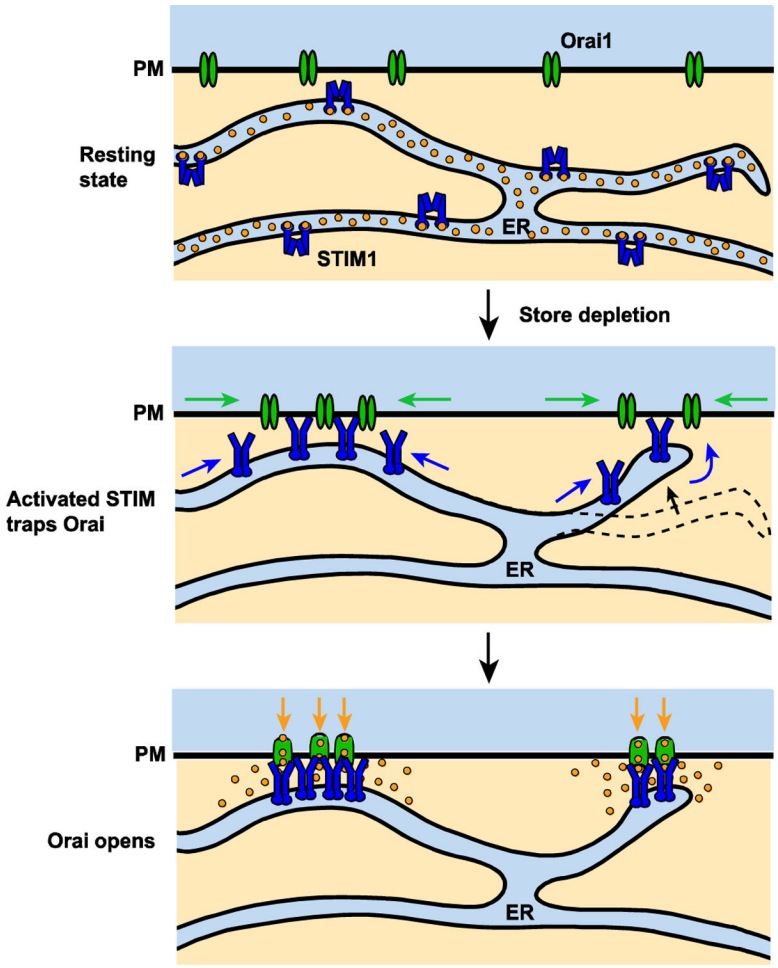

Figure 1. An overview of SOCE choreography.

In resting cells with high ER [Ca^{2+}], STIM1 and Orai1 diffuse in the ER and PM, respectively (*top*). Upon ER Ca^{2+} depletion, STIM1 becomes activated and accumulates at ER-plasma membrane junctions to bind and trap Orai1 (*middle*). STIM1 binding opens Orai1 channels and allows extracellular Ca^{2+} to flow into the cell (*bottom*).

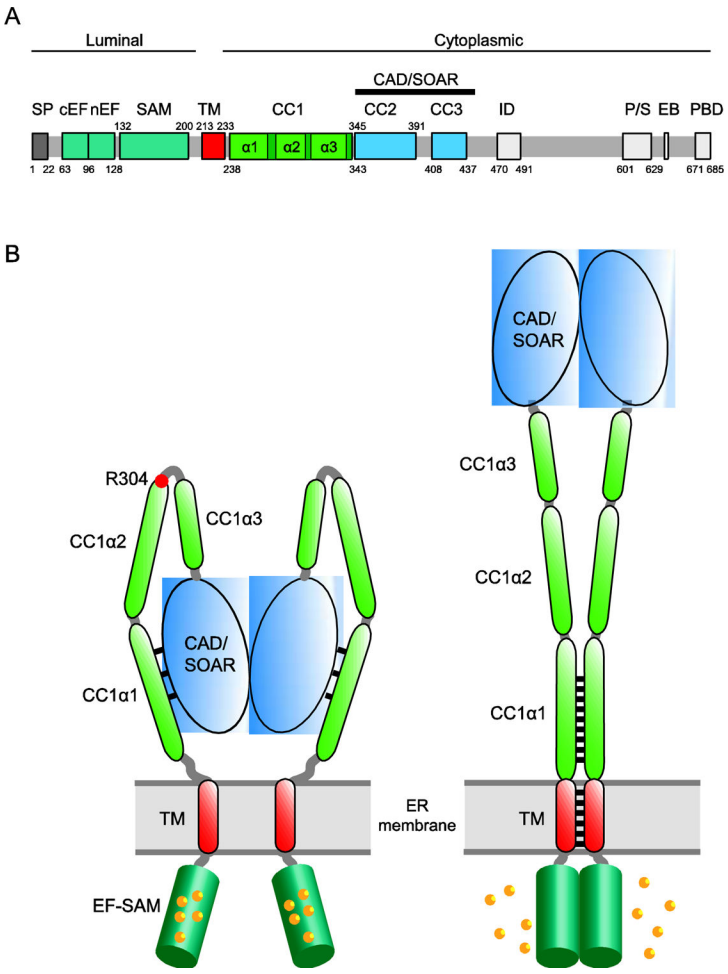

Figure 2. The initial events of STIM1 activation.

A. Domain organization of STIM1. Colored regions indicate structural and functional domains (SP, signal peptide; cEF, canonical EF hand; nEF, noncanonical EF hand; SAM, sterile alpha motif; TM, transmembrane domain; CC1–3, putative coiled-coil domains 1–3; CAD, CRAC activation domain (aa 342–448) or SOAR, STIM-Orai activating region (aa 344–442); ID, inactivation domain; P/S, proline/serine-rich domain; EB, EB1 binding domain; PBD, polybasic domain. B. Cartoon showing STIM1 activation by ER Ca^{2+} depletion. In the resting state (*left*), the luminal EF-SAM domains are bound with 5–6 Ca^{2+} and separated. The cytosolic domain is in a compact conformation in which CC1α1 binds to CAD/SOAR and keeps it close to the ER membrane. Following store depletion (*right*), Ca^{2+} release from the EF-SAM domain triggers its dimerization which brings the TM domains together to form a coiled-coil. The rearrangement dissociates CC1α1 from CAD/SOAR to extend the coiled-coil (represented by black lines) and move CAD/SOAR towards the PM. The affinity and number of Ca^{2+} ions released may help explain the $[Ca^{2+}]_{ER}$ sensitivity and high cooperativity of STIM1 and Orai1 activation in vivo ($K_{1/2}$ ~200 μM, Hill coefficient of 4–8; [6,60]).

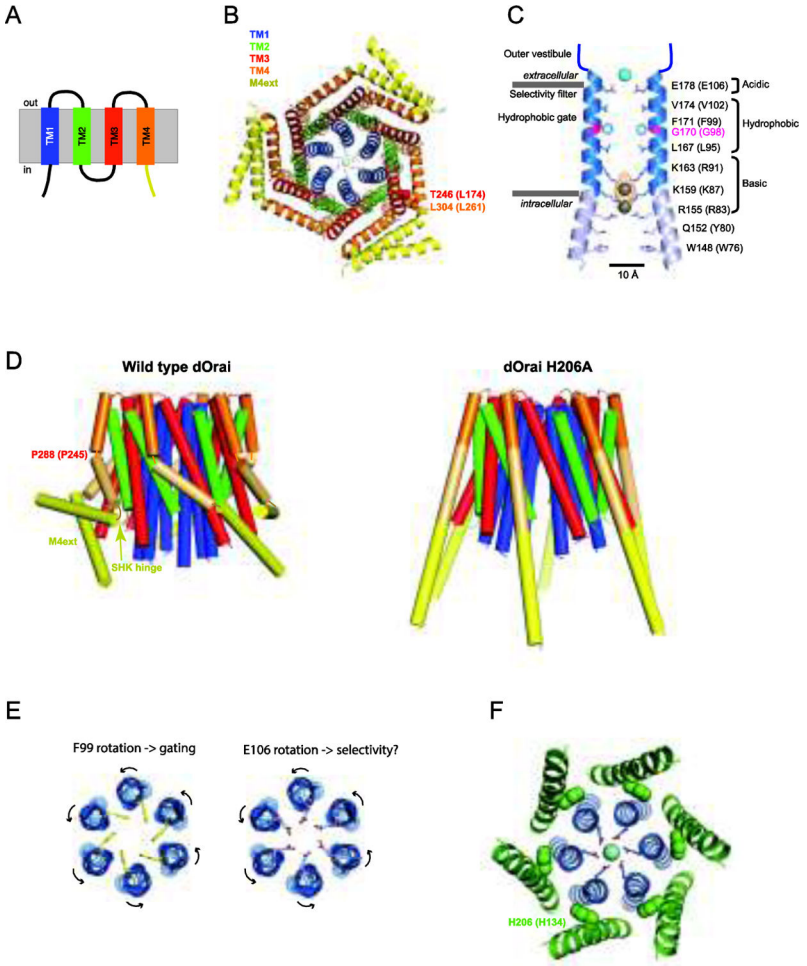

Figure 3. Structural aspects of Orai function.

A. Transmembrane topology of Orai1 showing 4 TM helices and the M4ext (*yellow*). B. Crystal structure of dOrai as viewed from the cytosolic side (4HKR.pdb). The closed channel shows six TM1 helices lining the pore, interlocking TM2 and TM3 helices, and peripheral TM4 helices ending in crossed M4ext pairs in the cytosol. C. A side view of two TM1 helices lining the dOrai pore. Pore-lining residues of dOrai are shown (human Orai1 equivalents in parentheses). Ba^{2+} is shown above the selectivity filter, and the anion density is shown in yellow with Fe atoms modeled into the structure of the inner pore. Adapted from [22]. D. Side view structures of WT and H206A dOrai channels. In the open mutant (*right*), the bends at P288 (human P245) and the SHK hinge straighten, separating the crossed M4ext pairs and allowing dilation of the inner pore (4HKR.pdb, 6BBF.pdb). E. (*left*) Top view of TM1 helices showing opening of the hydrophobic dOrai gate through rotation of F99 side chains out of the pore lumen. (*right*) A similar rotation of the selectivity filter at E106 may account for increased Ca^{2+} selectivity from STIM1 binding. F. Top view of dOrai TM1 (*blue*) and TM2 (*green*) helices displaying the close proximity of H206 (human H134) side chains in TM2 to the TM1 pore helix.