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Orai Channel Pore Properties and Gating by STIM: Implications from the Orai Crystal Structure

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Abstract

The Orai channels are unusual, yet prominent, calcium (Ca^{2+}) signal mediators in most cell types. Orai proteins are structurally unique, having little sequence homology with other ion channels. They are also functionally unique with exceedingly high selectivity for Ca^{2+} , mediating both short-term Ca^{2+} homeostasis and long-term Ca^{2+} signals important for transcriptional control. Operating in the plasma membrane (PM), Orai channel regulation is unprecedented among ion channels; channel gating occurs through an elaborate intermembrane coupling with stromal interaction molecule (STIM) proteins in the endoplasmic reticulum (ER). STIM proteins function as sensors of Ca^{2+} stored in the ER lumen and translocate into ER-PM junctions to tether and activate Orai channels when ER Ca^{2+} concentration decreases. Crystallization studies reveal an unexpected hexameric structure for the Orai channel and provide important insights into the pore architecture, the structural basis of its unusual cation selectivity, and how channel gating occurs through its coupling with STIM proteins.

Calcium (Ca^{2+}) signals crucial in the control of numerous cellular functions are mediated by the opening of several distinct and tightly regulated Ca^{2+} channels. Among these, the family of highly Ca^{2+} -selective Orai channels represents some newly discovered paradigms in channel structure and gating (1–3). These plasma membrane (PM)-localized “store-operated” channels were identified in 2006 (4–6) as a new class of channels with four transmembrane domains. Their gating is through a unique process of intermembrane coupling with the endoplasmic reticulum (ER)-resident stromal interaction molecule (STIM) proteins, which function as sensors of Ca^{2+} stored within the ER (3). As the ER Ca^{2+} concentration decreases, STIM proteins aggregate and translocate into discrete PM-associated junctions, where they physically couple with and activate Orai channels (1–3), leading to Ca^{2+} signals that control longer-term responses including transcription, cell division, and growth (3, 7). Crystallization of the active Orai-interacting domain of STIM (8) has given structural insights into its mechanism of coupling and activation of Orai (3, 8). Hou *et al.* (9) solved the crystal structure of a large component of the *Drosophila* Orai channel, providing a crucial new perspective on the architecture and operation of this unusual channel. Indeed, this represents the first crystal structure determination of a Ca^{2+} -selective channel to include the pore-forming helices.

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Most notable from the new crystallographic data is that the Orai channel comprises a hexameric arrangement of Orai subunits around a central pore lined by the M1 transmembrane helices (Fig. 1). This stoichiometry is confirmed by cross-linking and light-scattering data (9). Earlier biochemical approaches suggesting a tetrameric configuration (10–13) may have underestimated the stoichiometry, although the characterization of a functionally expressed tetrameric Orai1 concatemer is less easily explainable (14). A possible transitional dimeric state suggested previously (10, 13) may find support from the new crystallographic hexameric structure, which comprises three pairs of Orai subunits, the proteins within each pair interacting through their M4-extension helices (Fig. 1).

The crystallographic data for the single *Drosophila* Orai channel provide much new information and support some of the Orai pore architecture suggested by biochemical and functional approaches (12, 15–17). A distinctive feature of the Orai channel is its selectivity filter, which is formed by a ring of glutamate side chains (E106 in human Orai1; Fig. 1). Indeed, retraction of the carboxyl group by just one methylene in the E106D (Glu¹⁰⁶→Asp) mutant profoundly lowers ion selectivity (15, 18–20). The crystal structure shows that this filter narrows the pore entrance to just 6 Å, indicating that a permeating Ca²⁺ ion would shed most of its water as it enters the pore, and may coordinate directly with the side-chain oxygens (Fig. 1B). Beyond the selectivity filter, the pore opens to a wider cavity, 12 Å in diameter, lined by three rings of hydrophobic side chains from valine, phenylalanine, and leucine (Val¹⁰², Phe⁹⁹, and Leu⁹⁵ in human Orai1). Mutation of these highly conserved residues [for example, V102C (Val¹⁰²→Cys)] profoundly alters pore selectivity and conductance (16). After Ca²⁺ sheds its hydration shell within the glutamate “cage” of the selectivity filter ring, it enters the water-containing hydrophobic cavity, where it rapidly rehydrates and presumably remains hydrated for the remainder of its journey through the pore. The single-ion selectivity filter contrasts with the multi-ion selectivity filters of, for example, potassium (K⁺) channels (21). Whether this single Ca²⁺-binding region alone can explain the high selectivity of the Orai channel is uncertain. In L-type Ca²⁺ channels, a single Ca²⁺ ion likely binds tightly to a selectivity filter, and conduction is facilitated through a “knock-on” mechanism in which a second entering Ca²⁺ ion displaces the first into an adjacent binding site by electrostatic repulsion (22). Although a second discrete binding site was not detected in the Orai crystal structure, its existence perhaps immediately beneath the selectivity filter might explain how micromolar concentrations of external Ca²⁺ block Na⁺ ion permeation, but millimolar concentrations of Ca²⁺ are required for Ca²⁺ to pass through Orai channels (23). Whereas Orai channels, like L-type channels, are highly selective for Ca²⁺, Orai channels have a much lower Ca²⁺ conductance than L-type calcium channels and are crucial for STIM-mediated local and prolonged Ca²⁺ signals, for controlling Ca²⁺ oscillations, and for “tweaking” Ca²⁺ homeostatic control in many cell types (1–3, 24). Thus, Orai channels serve a very different role than the more “global” cellular Ca²⁺-signaling function of L-type channels, and this is likely reflected in their different pore properties.

Beyond the selectivity filter and hydrophobic cavity, the Orai crystal structure suggests a pore mechanism that severely limits the passage of Ca²⁺ ions under resting conditions. Slight flexion of this region may provide the basis for STIM-induced channel gating.

Functional studies had predicted that Gly⁹⁸ in human Orai1 forms a flexible hinge region (17). Thus, the pore-lining region intracellular to the glycine-hinge might flex or twist to reduce the impedance of Ca²⁺ flow, once Ca²⁺ has breached the selectivity filter. The Orai1 crystal structure confirms the plausibility of this idea and suggests how gating might be achieved. Unexpectedly for a cation channel, the crystal structure reveals positively charged residues lining the intracellular portion of the pore: Arg¹⁵⁵, Lys¹⁵⁹, and Lys¹⁶³, which correspond to Arg⁸³, Lys⁸⁷, and Arg⁹¹ in human Orai1 (Fig. 1B). The lining of the pore by three consecutive and relatively narrow (~6 Å diameter) rings of six positive charges suggests that in the closed state, Ca²⁺ flow is impeded either by an electrostatic barrier, by the binding of one or more larger anions (perhaps phosphates), or by both mechanisms. In either case, this barrier must be released to permit ion permeation through the channel. The crystal structure reveals that the M1 pore-forming helices project 14 amino acids farther into the cytosol than previously predicted from sequence analysis (Fig. 1, A and B). We refer to this cytosolic helical region as the M1 extension. Moreover, these additional four helical turns protrude considerably beyond the rest of the Orai molecule, suggesting that they could interact with STIM proteins. It appears likely that channel activation occurs through a force at the cytoplasmic end of the pore-lining helices, presumably from the binding of STIM proteins, inducing a movement to stabilize the open state (Fig. 1B).

Considerable evidence indicates that the C-terminal cytosolic segment of Orai1 (the M4 extension, amino acids 263 to 301 in human Orai1) is important for interacting with STIM1 (1–3, 25, 26). We refer to this part of Orai1 as the “tethering” site for SOAR, the STIM–Orai-activating region of STIM (Fig. 2A). This association may be mediated by electrostatic interactions between acidic residues in the helical M4 extension of Orai and a segment of basic residues strategically exposed within the short-interacting segment of STIM1 (3, 8, 27, 28). Human Orai1 has a second STIM1-binding site within its N-terminal region comprising amino acids 70 to 91 (25), which includes the entire 14–amino acid cytosolic M1-extension sequence. This region of Orai1 is essential for STIM1-mediated activation (25, 29), and we refer to it as the “gating” site (Fig. 2A). In human Orai1, residue Lys⁸⁵ in this region protrudes away from the pore, and mutation of Lys⁸⁵ prevents STIM1-mediated Orai1 activation without altering its ability to interact with Orai1 (30). The crystallographic data support the prediction of Park *et al.* (25) that binding of STIM1 across the cytosolic M1- and M4-extended helices may gate the channel. We may now extend this model to predict that the STIM1 bridging between M1 and M4 provides a force that allows the M1 helix to flex and move apart, enabling anions to dissociate from the basic pore-lining residues and lowering a major barrier to Ca²⁺ flux (Fig. 1B and Fig. 2A). The crystal structure shows the feasibility of this model, revealing the spatial availability of the two STIM-interacting regions and allowing predictions of the possible Orai-gating site on STIM1.

This *Drosophila* Orai structural data also sheds light on the stoichiometry and molecular configuration of the STIM-Orai interaction. STIM1 molecules at rest are likely dimers (31) that associate into large aggregates as they interact with densely packed Orai channels within ER-PM junctions (1–3). Structural analysis of the isolated SOAR fragment reveals a dimeric structure (8), and the coupling stoichiometry appears to be two STIM subunits per Orai subunit (32, 33). There is good evidence that STIM molecules are functional dimers in

their interaction with Orai channels (3, 8). The surprising revelation of a hexameric Orai channel leads to some predictions regarding the STIM-coupling configuration (Fig. 2B). Although the channel pore has six-fold symmetry, only three of the outer M4 extended helices, which contain the SOAR-tethering site (8, 25–28), are oriented toward the cytoplasm. Thus, Orai displays only three-fold symmetry overall (9). In the *Drosophila* Orai crystal structure, the cytosolic M4-extension helices of two adjacent Orai subunits interact reciprocally through the conserved hydrophobic Ile³¹⁶ and Leu³¹⁹ residues (Leu²⁷³ and Leu²⁷⁶ in human Orai1), as shown in Figs. 1A and 2A. One prediction of the STIM-Orai interaction is that one STIM dimer binds to each of the three pairs of Orai subunits within a single-channel assembly (Fig. 2, A and B). However, this configuration would not be consistent with a coupling stoichiometry of one STIM dimer per Orai subunit (32, 33). Alternatively, Hou *et al.* (9) hypothesized that during activation, a STIM-induced interaction with the M4-extension helices of Orai might disrupt the Orai interhelical hydrophobic interactions and allow all six of the M4-extension helices to extend into the cytosol and bind STIM (Fig. 2C). In this case, STIM proteins would induce a six-fold symmetry in binding sites on Orai, and each STIM dimer could interact with a single M4-extension helix, satisfying the 2:1 ratio between STIM and Orai proteins. However, militating against this model is the observation that mutation of either of the conserved leucine interhelical-binding residues in Orai1 to serine (L273S) or aspartic acid (L276D) results in both failure of STIM1 to activate Orai1 channels and loss of the Orai1-STIM1 interaction (26, 34, 35). Thus, if simple M4 unfolding were a prerequisite for STIM interaction, the mutations might be expected to enhance, rather than block, STIM-Orai coupling by allowing exposure of the acidic residues on M4 thought to mediate the electrostatic coupling with STIM (3, 8, 27, 28). Perhaps the Leu²⁷³ and Leu²⁷⁶ residues might themselves be mediating both interhelical M4-M4 and intermolecular M4-STIM interactions, or perhaps the helical disruption of M4 by these mutations prevents the correct orientation of the M4 acidic residues needed for the electrostatic STIM interaction. If the unfolded M4 model of activation is correct, then the resulting sixfold STIM-binding symmetry would predict a model in which six dimers of STIM1 would interact with the hexameric Orai channel (Fig. 2C, bottom).

Clearly, atomic structures of both the Orai channel (9) and the key SOAR domain of the STIM protein (8) usher in an exciting new era of discovery on the molecular mechanisms for store-operated Ca²⁺ signaling. Presently, we can only guess at the intricate molecular coupling mechanisms between STIM and Orai. Solving the structure of the two proteins cocrystallized will allow us to finally understand this complex intermembrane signaling process.

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Fig. 1. Structure of the Orai Ca²⁺ channel. **(A)** Schematic depictions of the six subunits of the single *Drosophila* Orai channel from the crystal structure (Protein Data Bank accession no. 4HKS) showing two adjacent subunits (red and purple), and the other four subunits (gray). Transmembrane helices M1, M2, M3, and M4 and the cytoplasmic M1- and M4-extensions (M1-ext; M4-ext) are labeled. Side chains shown are Glu¹⁷⁸ (Glu¹⁰⁶ in hOrai1), forming the selectivity filter; Ile³¹⁶, Leu³¹⁹ (Leu²⁷³, Leu²⁷⁶ in hOrai1), mediating M4-extension association. **(B)** Two pore-lining M1 helices from opposing Orai subunits illustrating the

permeation pathway and possible STIM-induced gating mechanism. Left: Orai channel in its closed state: Green shading shows relative hydration of Ca^{2+} , and the water within the hydrophobic pore section; anions (“A⁻”) are shown bound to side chains in the pore section lined with basic residues (*Drosophila* Orai pore-lining residues are numbered; corresponding residues from human Orai are in parentheses). Right: Hypothetical open state of the Orai channel: The interaction of STIM with the Orai channel may induce a force (yellow arrows) that flexes the M1 helices, widening the pore section lined with basic side chains. Subsequent dissociation of anions allows Ca^{2+} to permeate through the pore. Abbreviations for the amino acid residues are as follows: E, Glu; F, Phe; K, Lys; L, Leu; R, Arg; V, Val; W, Trp; and Y, Tyr.

Fig. 2.

Hypothetical STIM-Orai coupling models. (A) Possible binding of one SOAR dimer with one of the three pairs of Orai subunits in the hexameric channel. A “tethering” interaction involves binding between basic residues of the SOAR Sa1 helix and acidic residues on the M4-extension (M4-ext) of Orai. SOAR may also couple with the cytosolic M1-extension denoted as the “gating” interaction. By bridging across the M1- and M4-extension helices from adjacent Orai subunits, STIM may induce flexion (yellow arrow) of the M1 helix to open the pore. Binding between M4-extension helices of adjacent Orai subunits is through Ile³¹⁶-Leu³¹⁹ hydrophobic interactions. Sa1-4, SOAR α helices 1 through 4. (B and C). Two hypothetical configurations of the interaction of SOAR dimers (arrows) with the hexameric Orai channel (viewed from the cytosolic side). (B) Top: The published crystal structure for Orai, with three-fold symmetry in the M4 helices due to asymmetric interactions between two adjacent M4-ext helices. Bottom: Theoretical arrangement by which three SOAR dimers (blue) bind to one hexameric Orai channel, one SOAR dimer binding across each of the three available extended M4-ext helices shown above [same interaction depicted in (A)]. (C) Top: Hypothetical “unfolded” structure for the hexameric Orai channel in which the hydrophobic Ile³¹⁶-Leu³¹⁹ interactions between adjacent M4-ext helices are broken and all six M4-ext regions are configured symmetrically. Bottom: Theoretical arrangement by which six SOAR dimers bind to the six available M4-ext helices in the Orai “unfolded” hexamer structure shown above.