Published in final edited form as:

Curr Opin Physiol. 2020 October ; 17: 106–114. doi:10.1016/j.cophys.2020.07.018.

The Intricate Coupling Between STIM Proteins and Orai Channels

James H. Baraniak Jr.¹, Yandong Zhou^{1,a}, Robert M. Nwokonko¹, Donald L. Gill^{1,a}

¹Department of Cellular and Molecular Physiology, The Pennsylvania State University College of Medicine, Hershey, PA 17033

Abstract

Store-operated Ca^{2+} entry signals are critical for cellular regulation. This intricate signaling pathway involves coupling of proteins in two different membranes: the ER-resident Ca^{2+} -sensing STIM proteins and the highly Ca^{2+} -selective PM Orai channels. The molecular nature of the STIM-Orai coupling interface in ER-PM junctions and consequent Orai channel gating, are processes under intense study. We describe recent developments in determining the mechanism of Orai activation through the key STIM-Orai Activating Region (SOAR) of STIM1. We describe the unexpected unimolecular coupling of STIM with Orai and explain the observed variable stoichiometry of STIM-Orai interactions. We also define the discrete C-terminal regions in Orai channels that initially latch onto STIM proteins and mediate allosteric activation of the channel. A critical “nexus” region closely associated with the STIM-activated C-terminus of Orai1, propagates the STIM-binding signal through the four tightly-associated transmembrane helices of Orai1, finally to modify the pore-forming helices and effect channel opening.

Introduction

Store-operated calcium entry (SOCE) plays an integral role in an array of eukaryotic cellular functions ranging from proliferation to apoptosis (Nwokonko et al., 2017; Prakriya and Lewis, 2015; Putney et al., 2017; Zhou et al., 2017). This dynamic process involves two interconnected protein families, the ER-resident STIM proteins and the plasma membrane (PM) Orai Ca^{2+} channels. The single transmembrane-spanning STIM proteins (STIM1 and STIM2) are able to sense ER luminal Ca^{2+} changes through their N-terminal EF hand motifs (Stathopulos et al., 2006; Zhang et al., 2005). The depletion of ER Ca^{2+} stores triggers a scissor-like conformational change in the naturally dimerized STIM1 molecule allowing its polybasic C-terminus to associate with acidic phospholipids within the PM (Ma et al., 2015; Prakriya and Lewis, 2015; Shim et al., 2015; Soboloff et al., 2012). This interaction of STIM1 with the PM occurs within pre-existing ER-PM junctions (Orci et al., 2009). The unfolded STIM1 protein is able to easily span the approximately 15 nm gap between ER and PM within the junctions. Upon being retained in junctions, STIM1 is able to interact with

^a To whom correspondence should be addressed: dongill@psu.edu or zhouyd@psu.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

and activate Orai Ca^{2+} channels in the PM. The coupling and activation of Orai Ca^{2+} channels via STIM is important for both the homeostatic replenishment of ER Ca^{2+} stores and the generation of ER-PM junction-localized Ca^{2+} microsignals that mediate control over cellular transcription, motility, and secretion (Amcheslavsky et al., 2015; Derler et al., 2016; Prakriya and Lewis, 2015; Soboloff et al., 2011; Zhou et al., 2015a). Although it is clear that STIM1 directly binds to and activates Orai channels, the molecular nature of this intricate coupling interface has derived considerable speculation and uncertainty. One of the primary goals for our work has been to better understand the STIM-Orai coupling interface and concomitant channel gating. This has powerful ramifications toward elucidating the SOCE process that underlies essential physiological processes and a number of disease states (Hogan et al., 2010; Lacruz and Feske, 2015; Lewis, 2011; Rothberg et al., 2013; Soboloff et al., 2011; Soboloff et al., 2012; Yang et al., 2009). In this review we discuss some of the more significant advances we and others have made in the area of STIM-Orai coupling.

The Structure of STIM proteins and their Orai-Binding Locus

There are two major subtypes of the ER-resident STIM protein, STIM1 and STIM2, each of which exist under resting conditions in a folded, dimerized conformation. The STIM proteins serve a vital Ca^{2+} sensing role which is attributed to an N-terminal assembly of two EF hand domains and a sterile α -motif (SAM) domain that reside in the ER lumen (Liou et al., 2005; Soboloff et al., 2011; Soboloff et al., 2006; Stathopoulos et al., 2006; Zhang et al., 2005). ER Ca^{2+} store-depletion triggers a remarkably dynamic conformational change within the STIM molecule which causes it to unfold and become tethered to the PM through its poly-lysine rich region near the C-terminus (Fig. 1A). Unfolding of STIM exposes a critical and highly conserved ~100 amino acid binding domain, known as the STIM-Orai Activating Region (SOAR) (Yuan et al., 2009) or the Channel Activating Domain (CAD) (Park et al., 2009). When expressed alone, the SOAR domain has the exceptional intrinsic capacity to bind to and activate Orai channels (Park et al., 2009; Yuan et al., 2009). X-ray crystallographic analysis of SOAR from human STIM1 reveals that it exists in a dimerized state, with each SOAR monomer consisting of four α -helical domains (S α 1-S α 4) (Yang et al., 2012) (Fig. 1B). How this domain within STIM1 is able to couple with Orai became a fundamental question that a number of labs, including our own, have tried to solve.

In order to investigate this coupling mechanism, we developed a series of concatenated SOAR dimers of STIM1 (SOAR1) and STIM2 (SOAR2) (Wang et al., 2014; Zhou et al., 2015b). Despite having over 80% sequence homology, SOAR1 had a 2.5-fold higher FRET interaction with Orai1 than its SOAR2 counterpart (Wang et al., 2014). Figuring out the structural differences between these two homologous regions was crucial in better understanding the binding locus of the STIM molecule. We used a novel set of SOAR1-SOAR2 chimeras, each comprising different combinations of the four α -helical segments. We discovered that the second α -helical sequence, S α 2, was critically important in binding with Orai1. Furthermore, we were able to discriminate a key binding locus within this α -helix, the F394 phenylalanine residue that imparts the strong agonist characteristic of SOAR1 (Wang et al., 2014) (Fig. 1B). Alteration of this residue in STIM1 to the smaller hydrophobic leucine residue found in STIM2 resulted in partial agonist behavior (F394L), while its binding to and activation of Orai channels was further abrogated upon mutating the

F394 residue to a similarly size yet polar histidine residue (F394H) (Fig. 1C) (Wang et al., 2014). Mutations in the F394 did not alter the resting state of STIM, nor its capacity to enter into ER-PM junctions. Our work strongly implicated the distinctive binding and gating locus of the two STIM molecules, and revealed the crucial nature of the apical region of SOAR in tuning Orai gating (Wang et al., 2014; Yang et al., 2012). This was an important step in further understanding how STIM proteins bind and gate Orai channels, and gave critical clues to understanding how STIM proteins are able to cluster a number of Orai channels together.

The Unimolecular Coupling Model

The stoichiometric nature of STIM coupling with Orai channels has been a perplexing question with some surprising variable conclusions (Hoover and Lewis, 2011; Li et al., 2011; Scrimgeour et al., 2009). Biochemical and electrophysiological approaches have been interpreted to indicate that maximal activation occurs at a 2:1 ratio of STIM1:Orai1 subunits. But, this is complicated by NMR studies involving the use of partial SOAR fragments and partial Orai C-terminal regions, which indicated a stoichiometric ratio closer to 1:1 (Stathopoulos et al., 2013). This 1:1 ratio would suggest that each hexameric Orai channel needs only three STIM dimers to bind in a bimolecular fashion (Fig. 1D, left). We used a rather different approach to help solve the stoichiometric nature of STIM coupling to Orai using fluorescently-tagged concatenated SOAR dimers (Fig. 1C) and measuring a combination of both functional channel activation and FRET with Orai channels. We were able to introduce the non-agonist F394H mutation in either one or both of the SOAR monomers and co-express them with Orai1 (Zhou et al., 2015b) (Fig. 1C). We observed that F394H heterodimer mutants had normal localization with Orai in the PM. Surprisingly, these hetero-mutant dimers did not prevent or decrease SOCE. Thus, we revealed that the heterodimers still activate the Orai channel identically with their wildtype counterparts. This was further recapitulated by FRET measurement with Orai, which were not hampered by a single monomeric alteration in the concatenated SOAR dimer (Zhou et al., 2015b). These results were quite unexpected since the bimolecular interaction model would predict that such an alteration in just one of the SOAR subunits in the dimer would result in a malfunctioning SOAR construct. These findings militate against the prevailing bimolecular model (Fahrner et al., 2014; Nwokonko et al., 2017; Stathopoulos et al., 2013), and demonstrate rather strongly that the SOAR units within STIM molecules couple with the Orai channels in a unimolecular fashion (Fig. 1D, right panel). This model would also be compatible with the variability of STIM-Orai stoichiometry that was observed in the earlier work (Li et al., 2011).

Under this unimolecular model, it is proposed that the STIM dimer can function as a bridge between hexameric Orai channels, thus facilitating oligomerization of a multitude of Orai channels (Fig. 1E). When STIM proteins are present in junctions in excess amounts relative to Orai channels (for example, when stores are fully emptied), each hexameric Orai channel may have a full complement of 6 STIM dimers (a total of 12 individual STIM proteins) giving a STIM-Orai stoichiometry of 2:1. Under conditions in which STIM proteins are limited and there is an excess of Orai channels, our model envisages formation of a lattice structure with STIM dimers crosslinking between Orai channel hexamers (Fig. 1E). As this

cross-linked lattice grows, the resulting ratio of STIM and Orai would become closer to 1:1. These results are also compatible with freeze-fracture electron microscopic studies performed on cells in which stores had been depleted. In this case, the lateral distance between Orai channels in the plasma membrane closely approximated to the distance measured between STIM molecules in the ER (Perni et al., 2015). We consider that the ability of STIM to ultimately coordinate oligomerization and hence control the density of Orai channels. This is crucial in the homeostatic refilling of Ca^{2+} stores during receptor-induced activation, and further modulates the propagation of transient Ca^{2+} spikes that have powerful control over cellular activity (Kar et al., 2011; Kar and Parekh, 2015; Zhou et al., 2018; Zhou et al., 2015a).

Orai Channel Structural Analysis Implicates a C-terminal Binding Site

Whereas our prior work has defined some of the structural requirements of STIM protein coupling with Orai, the reciprocal binding interface of Orai has remained less certain. The three major mammalian Orai subtypes, Orai1, Orai2, and Orai3, each comprise a cytosolic N-terminus, four transmembrane domains (TM1–4), and a cytosolic C-terminal extension (M4x). Crystallographic analysis of the *Drosophila melanogaster* Orai ortholog, dOrai, reveals a hexameric structure (Hou et al., 2012; Rothberg et al., 2013). The hexameric arrangement of the Orai1 channel was also determined from functional studies (Cai et al., 2016; Yen et al., 2016). The TM1 helices from each of the six Orai subunits form the highly selective Ca^{2+} pore with six-fold symmetry. The six-fold symmetry persists through most of the dOrai channel structure, but transitions into a threefold symmetry toward the peripheral M4x regions. The M4x region has been implicated by a number of studies as a STIM binding site (Muik et al., 2008; Park et al., 2009; Zhou et al., 2016). In addition, STIM1 has been suggested to interact with other Orai cytosolic domains, in particular, the N-terminus and intracellular TM2-TM3 loop (Frischauf et al., 2009; Frischauf et al., 2011; Gudlur et al., 2014; Park et al., 2009). It is suggested that all three of these cytosolic regions may contribute to the binding with STIM. This brings into question, what structural requirements of Orai are most critical in dictating this coupling process?

Upon closer investigation of the crystal structure, there is suspected to be a “trimer of dimers” involving the cytosolic M4x regions (Hou et al., 2012). Based on this model, two neighboring M4x regions are held together in a symmetrical, anti-parallel fashion by two hydrophobic residues (I316, L319 for dOrai) (Fig. 2A). These hydrophobic residues are a highly conserved feature between dOrai and all three mammalian Orai subtypes (Fig. 2B). In the mammalian Orai1 and Orai3 subtypes, both of these residues are leucines, whereas Orai2 has an isoleucine and leucine. The molecular nature and role of these hydrophobic amino acids is an area of much interest. Earlier work suggested the importance of the coiled-coil nature of the M4x helices – mutation of the two hydrophobic residues was thought to disrupt the helices and prevent the interaction with STIM1 (Muik et al., 2009; Muik et al., 2008). From the crystallographic data, although these M4x helices were held together by the hydrophobic residues, it was thought they might undergo rearrangement or possible unfolding to allow STIM1 to bind (Hou et al., 2012). Other studies suggested that the M4x helices might only undergo modest rearrangement upon STIM1 interaction (McNally et al., 2013; Navarro-Borelly et al., 2008; Tirado-Lee et al., 2015). Thus, a model was derived that

the hydrophobic M4x residues stabilize a dimerized STIM1 binding pocket (Zhou et al., 2016). More recently, based on NMR data derived from the interaction of one of the helices from SOAR together with the isolated M4x peptide from Orai1, it was concluded that the two conjoined M4x peptides form a binding pocket that was able to bind to the $\alpha 1$ helix from STIM1 (Fahrner et al., 2014; Stathopoulos et al., 2013). However, this model was based on a SOAR-derived peptide that, unlike SOAR, had no biological function and would require the complete unfolding of SOAR to expose the residues thought to be interacting with Orai1 M4x. Indeed, more recent analyses are showing that the dimeric SOAR structure does not undergo unfolding, but remains a tightly-structured unit that is the same whether in the folded inactive STIM1 molecule or in the unfolded active state within junctions (Zhou et al., 2015b).

More recent structural studies on Orai from the labs of Long and Shen have attempted to understand the structural basis of Orai channel gating (Hou et al., 2018; Liu et al., 2019). These studies used two different mutations that cause constitutive Orai channel opening, in both cases indicating that the activated Orai channel is associated with drastically unlatched M4x regions. This is in agreement with and further supported by our recent work involving concatenated Orai1 proteins (Cai et al., 2018). In this study, we used a 36 amino acid linker to join two Orai1 proteins together. These concatenated Orai1 dimers allowed us to observe the structural characteristics of the analogous hydrophobic residues, L273 and L276, within the proposed M4x binding pocket (Fig. 2B). We introduced a charged mutation at L273 (L273D) which should obstruct the helices ability to form a dimerized pair. Heterodimeric L273D mutants were still able to interact with STIM1, and able to be activated albeit at a greatly diminished rate (Cai et al., 2018). Further complicating the dimerized-M4x STIM binding pocket hypothesis, is that it is based on an earlier crystal structure of dOrai that was Zn^{2+} -stabilized through distal His residues at the C-terminus of the M4x (H330, H334) (Hou et al., 2012; Zhou et al., 2019). This Zn^{2+} -stabilized trimeric assembly may have locked the far end of the M4x peptides into the 3-fold symmetrical, antiparallel dimerized configuration observed. Thus, whether the paired association between adjacent M4x peptides occurs in native Orai channels is currently unproven.

These results and challenges posed against the prevailing dimerized STIM binding pocket model may provide an interesting new understanding of the STIM-binding site with Orai channels. This led us to ponder if the M4x region may need to unlatch to allow STIM binding to gate the channel. Indeed, we questioned whether the hydrophobic residues are actually essential for maintaining a dimerized pocket, or whether instead they are directly involved in the interaction with STIM1. Using mutated peptide constructs we are currently trying to solve whether the M4x regions exist as a STIM1 binding pocket or act as individual binding sites. We developed novel PM-tethered fluorescently tagged peptides with which we can investigate the M4x region of each of the major Orai subtypes (Fig. 2B–C). The results of these studies are helping us determine the elusive molecular nature of the Orai binding interface for STIM and providing information on some extraordinary differences in the function of the M4x helices in the different Orai channel subtypes.

STIM1 Remotely Gates the Orai Channel

A central question for our lab is to better understand the molecular nature of the STIM-Orai coupling process and transduction of that signal into channel activation. This finely tuned process by which STIM gates the Orai channel is under much scrutiny. Earlier studies suggest that there is a two-site gating process by which STIM couples and pulls at both the N-terminal TM1-extension (M1x) and M4x region of the Orai protein (Gudlur et al., 2014; McNally et al., 2013; Palty et al., 2015; Zheng et al., 2013). While there is a notably strong interaction of STIM1 with Orai1's M4x region, a number of studies indicate that STIM1 undergoes weak interactions at the cytosolic M1x (Derler et al., 2013; Gudlur et al., 2014; Lis et al., 2010; McNally et al., 2013; Park et al., 2009). However, these studies do not completely substantiate the role of the M1x in direct contributions to STIM1-binding and channel gating.

Our recent studies reveal a critical five amino acid region, known as the “nexus,” which links the TM3 and TM4 helices of Orai. The nexus consists of a “hinge” domain (Orai1: S263, H264, K265) and a “hinge plate” domain (Orai1: L261 and V262). Earlier studies to alter the flexion of the hinge by introducing a proline or diamide crosslinking of cysteines within the hinge domain, were shown to prevent STIM1 binding and channel activation (Tirado-Lee et al., 2015; Zhou et al., 2016). The “hinge plate” was less understood, but may act as a hydrophobic conduit between the TM4 and TM3 helices of Orai1. Specifically, the L261 residue in the TM4 helix may form hydrophobic interactions with the L174 residue of TM3. We proposed that STIM1 binding with the M4x peptide may remotely gate the activity of Orai via a crucial series of events (Fig. 3). First, the act of STIM1 binding to the M4x region induces ‘unlatching’ which is ‘sensed’ and further propagated by conformational changes in the adjacent nexus region. The nexus would then act as a critical mediator since displacement at this junction would impact TM4 association with TM3. This concerted force that started at the M4x is proposed to be transmitted through to the TM3, followed by the TM2, and ultimately the TM1 pore itself. This dynamic process would finally result in dilation of the TM1 pore helix by conformational realigning of Orai1's extracellular selectivity filter, the E106 residue, and/or an adjacent pore-lining V102 residue (Gudlur et al., 2014) (Fig. 3).

In order to test this model, we introduced mutations within the nexus region that simulate the conformational changes that are induced upon STIM1 binding to the M4x (Residues 261–265, LVSHK). Upon introduction of the ANSGA mutation in Orai1, we found that it was constitutively active and had properties exactly consistent with STIM1-activated Orai1. Furthermore, addition of the L273D M4x mutation did not alter the constitutive activity of this ANSGA model nor was it capable of binding with STIM1 (Zhou et al., 2016). This constitutive activity was blocked by Orai channel pore mutations (E106A, R91W, K87A, and R83A), thus demonstrating that the ANSGA mutation doesn't directly impact the pore and its structural activation is upstream from that of the M4x region. These results corroborate strongly with our model that STIM1 acts as an allosteric regulator for Orai channel activation. This model has been further substantiated by a recent study from Prakriya's lab, which demonstrate the importance of the surrounding TM3/TM2 ring in gating of the TM1 pore (Yeung et al., 2020).

Our ANSGA model is also a powerful tool in better understanding the role of the Orai1 N-terminus. We introduced mutations in three M1x residues that were previously noted as critical for binding STIM1 and gating (L81, S82, and K85) (Gudlur et al., 2014). Triple mutational modification (L81E, S82A, and K85E), double mutants (L81A, S82A), and even single point mutants (L81A, K85E) completely abolish the Orai1-ANSGA channel activity in the same fashion as they would block STIM1-induced activated wildtype Orai (Zhou et al., 2016). However, none of these mutants can diminish the constitutive channel activity of pore mutations such as the Orai1-V102C mutation. This strongly indicates that these three M1x residues are not directly be involved in channel gating.

These results do not negate the potential of M1x's supportive role in STIM1 binding adjunctive to the primary M4x interaction (McNally et al., 2013). In order to help address this concern, we introduced a key M1x mutation, K85E, in our concatenated Orai dimer channel model noted above (Cai et al., 2018). E-FRET measurements of K85E bearing hetero- or homodimer Orai mutants showed diminished interaction with STIM1 (Cai et al., 2018). Either combination of Orai1 concatenated dimers containing the K85E still had SOCE activity, albeit at a diminished quantity (Cai et al., 2018). These results combined with our earlier work on the ANSGA model strongly implicate the M1x role in binding and channel configuration. Further studies are needed to fully address the molecular nature of the M1x region for Orai function.

Concluding Remarks

The fundamental question of how STIM proteins binds to activate Orai channels is one that we have focused on to answer. Understanding the molecular nature of the STIM-Orai coupling interface and the eventual transduction of this signal toward channel activity is critical for further comprehending the dynamics of SOCE that mediates so many crucial cellular functions. We have used a combination of novel molecular models and tools to help solve this question. Our work with SOAR concatemers and chimeras helped elucidate the distinct binding locus for STIM. We were then able to use a key single mutation that resides in this exposed apical region, F394H, to reveal the nature of STIM coupling with Orai (Wang et al., 2014). This unimolecular coupling model argues against the prevailing bimolecular model, and addresses key understanding of the variable stoichiometry that was shown in previous studies. This work is key to knowing exactly how STIM acts as a cross-linker between multiple Orai channels, and results in oligomerization that appears critical to regulating the generation of local Ca^{2+} signals (Zhou et al., 2018).

Whereas the STIM coupling interface has been notably elucidated, it is also imperative for us to better understand the structural binding requirements of the Orai protein. We proposed that the primary site of interaction for STIM is the cytosolic C-terminal extension, the M4x helix. The nature of how these M4x helices interact directly with STIM is of much interest to us. We are developing tools to help understand whether these M4x helices are kept as a dimerized STIM binding pocket or can unlatch into independent binding sites. Whether or not our work corroborates with the recent crystal and cryo-EM structures is an exciting endeavor we are currently undertaking (Hou et al., 2018; Liu et al., 2019).

How this STIM-Orai coupling is transduced into channel activation is another area of our focus. A number of studies suggest that both the N-terminus and C-terminus are crucial for gating in a two-site process (Gudlur et al., 2014; McNally et al., 2013; Palty et al., 2015; Zheng et al., 2013). Using the novel ANSGA mutational model in the nexus region of Orai1, we were able to demonstrate that the STIM molecule can remotely gate the Orai channel at the M4x region (Zhou et al., 2016). While this work doesn't negate the N-terminal role in channel integrity or adjunctive binding, it clearly implicates the prime role that the M4x has in binding and allosterically controlling Orai.

From this work we can more clearly delineate the crucial chain of events necessary for binding and gating of the Orai channel by STIM. Under our current model, we proposed that the M4x region acts as a prime interaction site for STIM. Whether the M4x helices in native Orai channels are associated with each other in the resting state is unclear. What is becoming clear is that these M4x peptides can bind to SOAR individually without the need to be bound to each other. Likely, the binding of a SOAR unit to the Orai M4x helix causes their unlatching that results in a conformational change in the nexus region. The altered nexus region may act as a coupling trigger between the TM4 and the TM3 helices. This propagates additional structural alterations through the TM2 helix and ultimately pore-opening by a configurational change in the neighboring TM1 pore helix itself (Fig. 3). While there is much work needed, our current model suggests a rather simple and direct peptide-peptide interaction between the SOAR Sα2 helix and the Orai-M4x helix is sufficient to trigger a complex allosteric change that is propagated through the Orai channel to effect gating of the pore.

Acknowledgements

This work was supported by National Institutes of Health Grants R35 GM131916, R01 GM120783, and R01 GM109279.

References

- Amcheslavsky A, Wood ML, Yeromin AV, Parker I, Freitas JA, Tobias DJ, and Cahalan MD (2015). Molecular Biophysics of Orai Store-Operated Ca Channels. *Biophys J* 108, 237–246. [PubMed: 25606672]
- Cai X, Nwokonko RM, Loktionova NA, Abdulqadir R, Baraniak JH Jr., Wang Y, Trebak M, Zhou Y, and Gill DL (2018). Pore properties of Orai1 calcium channel dimers and their activation by the STIM1 ER calcium sensor. *J Biol Chem* 293, 12962–12974. [PubMed: 29954946] * Paper shows the importance of combining monomers of Orai and the effects of changing the M4x peptide.
- Cai X, Zhou Y, Nwokonko RM, Loktionova NA, Wang X, Xin P, Trebak M, Wang Y, and Gill DL (2016). The Orai1 store-operated calcium channel functions as a hexamer. *J Biol Chem* 291, 25764–25775. [PubMed: 27780862]
- Derler I, Jardin I, and Romanin C (2016). Molecular mechanisms of STIM/Orai communication. *Am J Physiol Cell Physiol* 310, C643–C662. [PubMed: 26825122]
- Derler I, Plenk P, Fahrner M, Muik M, Jardin I, Schindl R, Gruber HJ, Groschner K, and 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. [PubMed: 23943619]
- Fahrner M, Muik M, Schindl R, Butorac C, Stathopoulos P, Zheng L, Jardin I, Ikura M, and Romanin C (2014). A coiled-coil clamp controls both conformation and clustering of stromal interaction molecule 1 (STIM1). *J Biol Chem* 289, 33231–33244. [PubMed: 25342749]

- Frischauf I, Muik M, Derler I, Bergsmann J, Fahrner M, Schindl R, Groschner K, and Romanin C (2009). Molecular Determinants of the Coupling between STIM1 and Orai Channels: DIFFERENTIAL ACTIVATION OF Orai 1–3 CHANNELS BY A STIM1 COILED-COIL MUTANT. *J Biol Chem* 284, 21696–21706. [PubMed: 19506081]
- Frischauf I, Schindl R, Bergsmann J, Derler I, Fahrner M, Muik M, Fritsch R, Lackner B, Groschner K, and Romanin C (2011). Cooperativeness of Orai cytosolic domains tunes subtype-specific gating. *J Biol Chem* 286, 8577–8584. [PubMed: 21220423]
- Gudlur A, Quintana A, Zhou Y, Hirve N, Mahapatra S, and Hogan PG (2014). STIM1 triggers a gating rearrangement at the extracellular mouth of the ORAI1 channel. *Nat Commun* 5, 5164. [PubMed: 25296861]
- Hogan PG, Lewis RS, and Rao A (2010). Molecular basis of calcium signaling in lymphocytes: STIM and ORAI. *Annu Rev Immunol* 28, 491–533. [PubMed: 20307213]
- Hoover PJ, and Lewis RS (2011). Stoichiometric requirements for trapping and gating of Ca²⁺ release-activated Ca²⁺ (CRAC) channels by stromal interaction molecule 1 (STIM1). *Proc Natl Acad Sci U S A* 108, 13299–13304. [PubMed: 21788510]
- Hou X, Burstein SR, and Long SB (2018). Structures reveal opening of the store-operated calcium channel Orai. *Elife* 7.* Interesting recent determination of the possible structure of the Orai open channel
- Hou X, Pedi L, Diver MM, and Long SB (2012). Crystal structure of the calcium release-activated calcium channel Orai. *Science* 338, 1308–1313. [PubMed: 23180775] ** Original structural determination of dOrai. A landmark paper.
- Kar P, Nelson C, and Parekh AB (2011). Selective activation of the transcription factor NFAT1 by calcium microdomains near Ca²⁺ release-activated Ca²⁺ (CRAC) channels. *J Biol Chem* 286, 14795–14803. [PubMed: 21325277]
- Kar P, and Parekh AB (2015). Distinct Spatial Ca²⁺ Signatures Selectively Activate Different NFAT Transcription Factor Isoforms. *Mol Cell* 58, 232–243. [PubMed: 25818645]
- Lacruz RS, and Feske S (2015). Diseases caused by mutations in ORAI1 and STIM1. *Ann N Y Acad Sci* 1356, 45–79. [PubMed: 26469693]
- Lewis RS (2011). Store-operated calcium channels: new perspectives on mechanism and function. *Cold Spring Harb Perspect Biol* 3, 1–24.
- Li Z, Liu L, Deng Y, Ji W, Du W, Xu P, Chen L, and Xu T (2011). Graded activation of CRAC channel by binding of different numbers of STIM1 to Orai1 subunits. *Cell Res* 21, 305–315. [PubMed: 20838418]
- Liou J, Kim ML, Heo WD, Jones JT, Myers JW, Ferrell JE Jr., and Meyer T (2005). STIM is a Ca²⁺ sensor essential for Ca²⁺-store-depletion-triggered Ca²⁺ influx. *Curr Biol* 15, 1235–1241. [PubMed: 16005298]
- Lis A, Zierler S, Peinelt C, Fleig A, and 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. [PubMed: 21115697]
- Liu X, Wu G, Yu Y, Chen X, Ji R, Lu J, Li X, Zhang X, Yang X, and Shen Y (2019). Molecular understanding of calcium permeation through the open Orai channel. *PLoS Biol* 17, e3000096. [PubMed: 31009446] * A recent very interesting analysis of the possible structure of the open Orai channel
- Ma G, Wei M, He L, Liu C, Wu B, Zhang SL, Jing J, Liang X, Senes A, Tan P, et al. (2015). Inside-out Ca(2+) signalling prompted by STIM1 conformational switch. *Nat Commun* 6, 7826. [PubMed: 26184105]
- McNally BA, Somasundaram A, Jairaman A, Yamashita M, and 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. [PubMed: 23613525]
- Muik M, Fahrner M, Derler I, Schindl R, Bergsmann J, Frischauf I, Groschner K, and Romanin C (2009). A cytosolic homomerization and a modulatory domain within STIM1 C-terminus determine coupling to ORAI1 channels. *J Biol Chem* 284, 8421–8426. [PubMed: 19189966]

- Muik M, Frischauf I, Derler I, Fahrner M, Bergsmann J, Eder P, Schindl R, Hesch C, Polzinger B, Fritsch R, et al. (2008). Dynamic coupling of the putative coiled-coil domain of ORAI1 with STIM1 mediates ORAI1 channel activation. *J Biol Chem* 283, 8014–8022. [PubMed: 18187424]
- Navarro-Borelly L, Somasundaram A, Yamashita M, Ren D, Miller RJ, and Prakriya M (2008). STIM1-ORAI1 interactions and ORAI1 conformational changes revealed by live-cell FRET microscopy. *J Physiol* 586, 5383–5401. [PubMed: 18832420]
- Nwokonko RM, Cai XY, Loktionova NA, Wang YJ, Zhou YD, and Gill DL (2017). The STIM-Orai Pathway: Conformational Coupling Between STIM and Orai in the Activation of Store-Operated Ca²⁺ Entry. *Store-Operated Ca²⁺ Entry (Soce) Pathways: Emerging Signaling Concepts in Human (Patho)Physiology*, 2nd Edition 993, 83–98.
- Orci L, Ravazzola M, Le Coadic M, Shen WW, Demaurex N, & Cosson P (2009). From the Cover: STIM1-induced precortical and cortical subdomains of the endoplasmic reticulum. *Proceedings of the National Academy of Sciences of the United States of America*, 106(46), 19358–19362. 10.1073/pnas.0911280106. [PubMed: 19906989]
- Palty R, Stanley C, and Isacoff EY (2015). Critical role for Orai1 C-terminal domain and TM4 in CRAC channel gating. *Cell Res* 25, 963–980. [PubMed: 26138675]
- Park CY, Hoover PJ, Mullins FM, Bachhawat P, Covington ED, Raunser S, Walz T, Garcia KC, Dolmetsch RE, and Lewis RS (2009). STIM1 clusters and activates CRAC channels via direct binding of a cytosolic domain to Orai1. *Cell* 136, 876–890. [PubMed: 19249086] * One of the first papers to show the action of the SOAR fragment from STIM1
- Perni S, Dynes JL, Yeromin AV, Cahalan MD, and Franzini-Armstrong C (2015). Nanoscale patterning of STIM1 and Orai1 during store-operated Ca²⁺ entry. *Proc Natl Acad Sci U S A* 112, E5533–5542. [PubMed: 26351694]
- Prakriya M, and Lewis RS (2015). Store-Operated Calcium Channels. *Physiol Rev* 95, 1383–1436. [PubMed: 26400989] ** Important review on store-operated calcium signaling and the structure/function of STIM and Orai channels.
- Putney JW, Steinckwich-Besancon N, Numaga-Tomita T, Davis FM, Desai PN, D'Agostin DM, Wu S, and Bird GS (2017). The functions of store-operated calcium channels. *Biochim Biophys Acta Mol Cell Res* 1864, 900–906. [PubMed: 27913208]
- Rothberg BS, Wang Y, and Gill DL (2013). Orai channel pore properties and gating by STIM: implications from the Orai crystal structure. *Sci Signal* 6, pe9. [PubMed: 23512988]
- Scrimgeour N, Litjens T, Ma L, Barritt GJ, and Rychkov GY (2009). Properties of Orai1 mediated store-operated current depend on the expression levels of STIM1 and Orai1 proteins. *J Physiol* 587, 2903–2918. [PubMed: 19403622]
- Shim AH, Tirado-Lee L, and Prakriya M (2015). Structural and functional mechanisms of CRAC channel regulation. *J Mol Biol* 427, 77–93. [PubMed: 25284754]
- Soboloff J, Madesh M, and Gill DL (2011). Sensing Cellular Stress through STIM Proteins. *Nature Chem Biol* 7, 488–492. [PubMed: 21769090]
- Soboloff J, Rothberg BS, Madesh M, and Gill DL (2012). STIM proteins: dynamiccalcium signal transducers. *Nat Rev Mol Cell Biol* 13, 549–565. [PubMed: 22914293] * Complete review on the action of STIM proteins.
- Soboloff J, Spassova MA, Hewavitharana T, He LP, Xu W, Johnstone LS, Dziadek MA, and Gill DL (2006). STIM2 is an inhibitor of STIM1-mediated store-operated Ca²⁺ Entry. *Curr Biol* 16, 1465–1470. [PubMed: 16860747]
- Stathopulos PB, Li GY, Plevin MJ, Ames JB Ikura M Stored Ca²⁺ + depletion-induced oligomerization of stromal interaction molecule 1 (STIM1) via the EF-SAM region: An initiation mechanism for capacitive Ca²⁺ + entry. *The Journal of Biological Chemistry*, 281 (47) (2006), pp. 35855–35862 [PubMed: 17020874]
- Stathopulos PB, Schindl R, Fahrner M, Zheng L, Gasmi-Seabrook GM, Muik M, Romanin C, and Ikura M (2013). STIM1/Orai1 coiled-coil interplay in the regulation of store-operated calcium entry. *Nat Commun* 4, 2963. [PubMed: 24351972] * This paper shows the NMR structure for peptides from STIM and Orai. Although an impressive structural analysis the work uses non-functional fragments and may not be applicable to real STIM and Orai interactions.

- Tirado-Lee L, Yamashita M, and Prakriya M (2015). Conformational Changes in the Orai1 C-Terminus Evoked by STIM1 Binding. *PLoS One* 10, e0128622. [PubMed: 26035642]
- Wang X, Wang Y, Zhou Y, Hendron E, Mancarella S, Andrade MD, Rothberg BS, Soboloff J, and Gill DL (2014). Distinct Orai-coupling domains in STIM1 and STIM2 define the Orai-activating site. *Nat Commun* 5, 3183. [PubMed: 24492416] * An important paper identifying the critical F394 residue in STIM1
- Yang S, Zhang JJ, and Huang XY (2009). Orai1 and STIM1 are critical for breast tumor cell migration and metastasis. *Cancer Cell* 15, 124–134. [PubMed: 19185847]
- Yang X, Jin H, Cai X, Li S, and Shen Y (2012). Structural and mechanistic insights into the activation of Stromal interaction molecule 1 (STIM1). *Proc Natl Acad Sci U S A* 109, 5657–5662. [PubMed: 22451904] * The first paper to identify the structure of the SOAR unit from STIM1.
- Yen M, Lokteva LA, and Lewis RS (2016). Functional Analysis of Orai1 Concatemers Supports a Hexameric Stoichiometry for the CRAC Channel. *Biophys J* 111, 1897–1907. [PubMed: 27806271]
- Yeung PS, Yamashita M, and Prakriya M (2020). Molecular basis of allosteric Orai1 channel activation by STIM1. *J Physiol* 598, 1707–1723. [PubMed: 30950063]
- Yuan JP, Zeng W, Dorwart MR, Choi YJ, Worley PF, and Muallem S (2009). SOAR and the polybasic STIM1 domains gate and regulate Orai channels. *Nat Cell Biol* 11, 337–343. [PubMed: 19182790] * One of the first papers to show the action of the SOAR fragment from STIM1.
- Zhang SL, Yu Y, Roos J, Kozak JA, Deerinck TJ, Ellisman MH, Stauderman KA, Cahalan MD STIM1 is a Ca²⁺ sensor that activates CRAC channels and migrates from the Ca²⁺ store to the plasma membrane. *Nature*, 437 (7060) (2005), pp. 902–905. [PubMed: 16208375]
- Zheng H, Zhou MH, Hu C, Kuo E, Peng X, Hu J, Kuo L, and 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. [PubMed: 23447534]
- Zhou Y, Cai X, Loktionova NA, Wang X, Nwokonko RM, Wang X, Wang Y, Rothberg BS, Trebak M, and Gill DL (2016). The STIM1-binding site nexus remotely controls Orai1 channel gating. *Nat Commun* 7, 13725. [PubMed: 27929067] ** An important study to define the nexus region in Orai1 critical to transmission of STIM1 binding to initiate gating of the Orai1 channel
- Zhou Y, Cai X, Nwokonko RM, Loktionova NA, Wang Y, and Gill DL (2017). The STIM-Orai coupling interface and gating of the Orai1 channel. *Cell Calcium* 61, 1–7. [PubMed: 27720443]
- Zhou Y, Nwokonko RM, Baraniak JH Jr., Trebak M, Lee KPK, and Gill DL (2019). The remote allosteric control of Orai channel gating. *PLoS Biol* 17, e3000413. [PubMed: 31469825]
- Zhou Y, Nwokonko RM, Cai X, Loktionova NA, Abdulqadir R, Xin P, Niemeyer BA, Wang Y, Trebak M, and Gill DL (2018). Cross-linking of Orai1 channels by STIM proteins. *Proc Natl Acad Sci U S A* 115, E3398–E3407. [PubMed: 29581306] * This paper reveals the importance of STIM-mediated clustering of the Orai channel and has special relevance to understanding the propagation of store-operated calcium signals.
- Zhou Y, Trebak M, and Gill DL (2015a). Calcium signals tune the fidelity of transcriptional responses. *Mol Cell* 58, 197–199. [PubMed: 25884365]
- Zhou Y, Wang X, Wang X, Loktionova NA, Cai X, Nwokonko RM, Vrana E, Wang Y, Rothberg BS, and Gill DL (2015b). STIM1 dimers undergo unimolecular coupling to activate Orai1 channels. *Nat Commun* 6, 8395. [PubMed: 26399906] ** This paper was the first to identify the unimolecular coupling model for STIM-induced activation of the Orai channel.

Figure 1:

Representation of the STIM-Orai coupling process. (A) The STIM1 protein exists as a folded dimer, consisting of a luminal N-terminal Ca^{2+} -sensing domain and a dynamic cytoplasmic Orai-activating domain. ER Ca^{2+} store depletion triggers a dynamic series of events including unfolding of the C-terminal domain of the STIM1 molecule followed by translocation into ER-PM junctions due to interactions between the exposed K-rich C-terminus and acidic phospholipids in the PM. Unfolding and activation of STIM1 upon Ca^{2+} dissociation exposes its STIM-Orai activating region (SOAR) which is able to bind with and activate overlying Orai channels. (B) Crystal-derive structure of the SOAR domain showing the two symmetrical Orai-binding sites. (C) Our novel SOAR concatemers modified at the F394 Orai-binding locus. Mutational modification of this key residue into a similarly size yet polar histidine (F394H) results in a powerful tool to assess STIM-Orai coupling. The heterodimeric F394H mutants were still able to bind and activate Orai. (D) The earlier prevailing model was for a bimolecular for STIM-Orai interaction, requiring both STIM1 subunits to interact with adjacent Orai subunits. Our results showed this not to be the case since only a monomeric subunit is require to bind and activate Orai. Thus, STIM couples to Orai in a unimolecular fashion. (E) Model to show that the unimolecular interaction of STIM with Orai allows STIM proteins to cross-link and cluster Orai channels. The model

depicts SOAR dimers (red) and Orai hexamers (purple). The model explains the variable stoichiometry observed for STIM-Orai interactions. Thus, with excess Orai channels, limited cross-linking leads to a STIM-Orai stoichiometry of 2:1 (left) whereas excess STIM favors clustering with STIM1-Orai stoichiometry approaching 1:1.

Figure 2:

Crystal structure of the *Drosophila melanogaster* Orai ortholog (dOrai1). (A) Analysis of the structure revealed a hexameric Orai channel, bearing a mostly six-fold symmetry with the dOrai TM1 helix forming the channel pore. The structure indicated that toward the channel periphery the symmetry becomes 3-fold with a ‘trimer of dimers’ in which pairs of cytosolic TM4-extensions (M4x) are formed. It is proposed that these M4x dimers are held together in an anti-parallel configuration through hydrophobic interactions of Leu (or Ile) residues which are a conserved feature between dOrai and the three major mammalian Orai subtypes (B). Based on this model, one M4x region within each pair exists in a straight configuration, whereas the adjacent M4x region is bent nearly 180°, allowing the anti-parallel interaction between the two M4x helices. Whether these hydrophobic residues are essential for stabilization of a dimerized STIM1 binding pocket or may act as independent binding sites is currently being assessed using the construct shown in (C). This construct comprises a PM-tethered, fluorescently tagged M4x peptide that can be used to determine the molecular nature of the M4x binding to SOAR and STIM. Using these, we can also assess important functional differences in the M4x peptides derived from each of the three major mammalian Orai subtypes.

Figure 3:

Proposed STIM-induced allosteric activation of the Orai1 channel. Dimers of Orai1 TM4-extensions are proposed to be held together via hydrophobic interactions between L273 and L276. One TM4-extension (TM4-ext-A, pink) exists in a straight configuration, whereas the neighboring TM4-extension (TM4-ext-B, purple) is bent almost 180°. The association of the two TM4-ext may be necessary of binding of the SOAR moiety exposed within activated STIM proteins. However, it is likely that STIM-binding instigates unlatching of the TM4-extension helices (1–2). This unlatching would propagate further upstream allosteric modification of the Orai protein, likely through flexion of the adjacent “nexus” region (Orai1: 261–265) (3). Displacement of the nexus, specifically the L261 residue, initiates a coupling trigger with the neighboring L174 residue of TM3 (4). This allosteric displacement is likely propagated through the TM3 and TM2 helices, and finally to the pore-lining TM1 helix (5). Realignment of critical pore-lining residues of the TM1 helix results in channel opening (6).