STIM and Orai: Dynamic Intermembrane Coupling to Control Cellular Calcium Signals

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Ca2+ signals controlling a vast array of cell functions involve both Ca2+ store release and external Ca2+ entry. These two events are coordinated through a dynamic intermembrane coupling between two distinct membrane proteins, STIM and Orai. STIM proteins are endoplasmic reticulum (ER) luminal Ca2+ sensors that undergo a profound redistribution into discrete junctional ER domains closely juxtaposed with the plasma membrane (PM). Orai proteins are PM Ca2+ channels that migrate and become tethered by STIM within the ER-PM junctions, where they mediate exceedingly selective Ca2+ entry. We describe a new understanding of the nature of the proteins and how they function to mediate this remarkable intermembrane signaling process controlling Ca2+ signals.

Store-operated Channels: Role and Significance

Cellular Ca2+ homeostasis and Ca2+ signaling are closely entwined processes. Cytosplasmic Ca2+ is tightly controlled at ~100 nM; elevations to 300–500 nM constitute powerful signals controlling a spectrum of cell functions ranging from short-term contractile, secretory, or metabolic responses to longer term regulation of transcription, growth, and cell division (2). The ER3 has a special role in Ca2+ signaling, accumulating high (~500 μM) luminal free Ca2+ levels. The luminal Ca2+ serves two roles: maintaining a correct protein folding environment (2) and serving as the major source of Ca2+ for signaling (1). Cell-surface receptors coupled to phospholipase C and inositol 1,4,5-trisphosphate production induce rapid Ca2+ signals by releasing ER-stored Ca2+ through inositol 1,4,5-trisphosphate receptors. This triggers a second Ca2+ signaling pathway through activation of SOCs (3–5). These PM Ca2+ channels are activated by decreased ER luminal Ca2+, involving an intricate ER-PM coupling process. SOCs carry a small but highly Ca2+-selective current termed icrAc (3–5). This movement of Ca2+ ions can be viewed as a tightly regulated “trickle” of Ca2+ into cells (6), crucial in mediating longer term control of both cytoplasmic and ER luminal Ca2+ (5, 7, 8). Since the first description of SOCs (9), the ER-PM coupling has been considered to involve direct protein interactions occurring at close junctions between the ER and PM (10, 11). The function of the newly discovered STIM and Orai proteins fulfills this prediction.

STIM and Orai: The Machinery of SOCs

Recent high-throughput RNA interference screens identified two protein families as being essential for SOC activation: STIM in the ER (12, 13) and Orai in the PM (14–16). STIM proteins are highly dynamic membrane proteins located mostly in the ER and are able to sense luminal Ca2+ changes and undergo rapid translocation into discrete junctional areas of the ER, closely juxtaposed with the PM (7). Orai proteins are PM Ca2+ channels that translocate within the PM to the same ER junctions and become activated through coupling with STIM proteins (7). Although the function of SOCs has been best recognized in hematopoietic cells (3–5), STIM and Orai proteins are widely expressed among tissues (17, 18), representing potentially crucial pharmacological targets for controlling an array of cell functions.

STIM Proteins: Dynamic SOC Intermediaries

The discovery of STIM1 transformed the store-operated hypothesis into an authentic mechanistic paradigm (12, 13). STIM1 was originally identified as a surface membrane protein in stromal cells (19). Highly homologous STIM proteins are expressed in species ranging from Drosophila (12) to Caenorhabditis elegans (20). Vertebrates also express a second gene product, STIM2 (17). The ubiquitously expressed STIM1 and STIM2 proteins are highly similar, varying only at the extreme N and C termini (Fig. 1). Both STIM1 and STIM2 are predominately in the ER (21–23). Although some STIM1 is also in the PM, where it can influence SOC activation (23, 24), it functions primarily in the ER, coupling to activate SOCs by transfer into ER-PM junctions (7). Store depletion is reported to increase PM STIM1 (25, 26); however, SOC activation does not require PM insertion of STIM1 (23, 27). STIM1 is normally widely distributed through the ER (13, 28, 29) but rapidly oligomerizes and moves into PM junctional regions seconds after emptying stores (13, 29). The N-terminal Ca2+-sensing domain of STIM1 is a tightly clustered group of short α-helices comprising EF-hand and SAM domains. The cytoplasmic C-terminal region contains more extensive α-helical regions sufficiently long to span much of the ER-PM junctional gap, estimated to be 10–20 nm (13, 25, 28–30), and couple with PM Orai channels.

Molecular Basis of ER Luminal Ca2+ Sensing

The luminal N terminus of STIM1 includes two EF-hand motifs: a “canonical” cEF-hand (13, 25) with a Kd for Ca2+ in the 0.2–0.6 μM range (31) immediately adjacent to a hEF-hand that
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FIGURE 1. STIM protein domain structures. Upper, domain comparison of STIM1 and STIM2, including signal peptides (SP), a pair of highly conserved cysteines (CC), canonical cEF- and hEF-hands, SAMs, Asn-linked glycosylation sites (hexagons), TMs, coiled-coil regions (CC1 and CC2), proline-rich domains (P), and polybasic-rich domains (K). The minimal region of STIM1 known to be required for coupling to Orai1 is also shown (positions 344–442). Lower, diagrammatic representation of domain topology of STIM1.

STIM2: Similar but Different

Although STIM2 has a structure very similar to STIM1 (Fig. 1), its function has some notable distinctions with important physiological implications. Most cells express both STIM1 and STIM2, although STIM1 levels are generally higher (17, 40). When overexpressed, STIM2 has a strong negative effect on endogenous SOC activity (8, 22, 38), contrasting with the small enhancing effect of STIM1 (22, 24). Moreover, STIM2 mediates slower Orai1 channel activation than comparably expressed STIM1 (41). STIM2 knockdown has a greater effect than STIM1 on basal cytosolic and ER luminal Ca\textsuperscript{2+} levels, suggesting STIM2 functions as a feedback regulator controlling basal Ca\textsuperscript{2+} (8). When Ca\textsuperscript{2+} stores are released gradually with external EGTA, movement of STIM2 into puncta and SOC activation occur with minimal store release, whereas more store release is required for STIM1 activation (8). Indeed, STIM2 overexpression results in high constitutive Ca\textsuperscript{2+} entry and CRAC channel activity (38, 42), likely reflecting its sensitivity to small luminal Ca\textsuperscript{2+} changes. The greater sensitivity of STIM2 to store depletion could be related to EF-hand structures. Mutation of three residues in the STIM1 cEF-hand to resemble STIM2 resulted in a more store-sensitive version of STIM1, although the reciprocal exchange mutation did not make a less sensitive STIM2 (8). Direct measurement of Ca\textsuperscript{2+} binding to the EF-SAM domain of STIM2 revealed a K\textsubscript{d} of 0.5 mM, not much different from the K\textsubscript{d} of STIM1 (33, 43). Importantly, it was revealed that, compared with STIM1, the STIM2 EF-SAM domain undergoes a 3-fold slower unfolding and 70-fold slower rate of aggregation upon Ca\textsuperscript{2+} withdrawal (33). Moreover, the short STIM subtype-specific flexible N-terminal sequences upstream of the EF-SAM domains confer large stability changes on the EF-SAM domain, greatly altering the Ca\textsuperscript{2+} dissociation-induced destabilization that results in STIM protein activation (33). Thus, the much slower rate of Ca\textsuperscript{2+} dissociation-induced STIM2 unfolding and aggregation may account for the slower kinetics of SOC activation by STIM2 (41) as well as the negative dominance of overexpressed STIM2 on SOC activation (22). Although STIM2 appears to be sensitized to tiny changes in ER Ca\textsuperscript{2+}, the slow unfolding and activation of STIM2 may serve as an important regulatory control to prevent otherwise uncontrolled activation of SOCs (44).

Orai Proteins Are SOCs Carrying I\textsubscript{CRAC}

The extraordinary characteristics of the CRAC current, the signature of SOC function, predicted a novel channel protein. Despite numerous reports of TRPC and other transient receptor potential channels mediating Ca\textsuperscript{2+} entry or currents modified by store release, none had CRAC current characteristics. This changed with identification of the three-member Orai family of tetramembrane-spanning proteins (14–16). Their discovery arose from a combination of genome-wide RNA interference screening (14–16) and modified linkage analysis revealing that a mutation in Orai1 (R91W) caused a rare but SCID that ablates T-cell Ca\textsuperscript{2+} entry (14). This Orai1 mutation eliminated I\textsubscript{CRAC} (14–16); wild-type Orai1 expression restored CRAC channel activity in cells from SCID patients (14). The
Orai1 protein fulfills all the criteria of being the store-operated channel moiety itself, carrying the highly Ca\(^{2+}\) selective CRAC current (16, 38, 45–49). The combined overexpression of STIM1 with Orai1 results in massive levels of authentic CRAC channel activity (16, 38, 45–49). There are three closely related STIM1 with Orai1 results in massive levels of authentic CRAC current (16, 38, 45–49). The combined overexpression of Ca\(^{2+}\) channel moiety is a tetramer (56–58). Although dimer-to-tetramer transition was reported during Orai1 activation (59), it appears more likely that Orai channels have a constant tetrameric stoichiometry (57, 58).

**STIM-Orai Coupling Environment**

The coupling between STIM and Orai proteins involves a remarkable dynamic convergence. Both STIM and Orai translocate within their respective membranes into discrete, tightly coupled ER-PM junctions (Fig. 2). Aggregated regions of ER STIM proteins observed following store depletion, referred to as “puncta” (13), are areas of STIM proteins undergoing aggregation and accumulation, although not necessarily linked to Orai in ER-PM junctions (28, 36, 57, 60, 61). These regions appear to be largely pre-existing ER-PM junctional domains (29, 36) within which accumulated STIM proteins capture and activate Orai channels. The junctions may dramatically increase in number and size with time (23, 29). Direct interaction between STIM and Orai channels occurring in puncta is now established. Although STIM-Orai pulldown was initially not detected (18), there are now numerous reports revealing direct biochemical association between the proteins (37, 47, 49, 57) and/or Förster resonance energy transfer between appropriately labeled derivatives (37, 42, 62, 63). These interactions are supported by numerous studies revealing that STIM and Orai proteins are superimposedly clustered in puncta following store depletion (35, 42, 57–61, 64). Oligomerization between STIM1 molecules is required for accumulation of STIM1 aggregates within puncta. STIM1 with its entire N-terminal domain replaced by a rapalogue-inducible cross-linking domain underwent oligomerization, accumulation in puncta, and coupling to activate CRAC channels (30). However, STIM1 simply missing its entire N terminus (thus with no Ca\(^{2+}\) sensing or oligomerization through the N terminus) still aggregated and moved into puncta to activate CRAC channels (35). Thus, the STIM1 C terminus itself is able to undergo aggregation, a result supported by several studies (27, 34, 35, 57). The elaborate N-ter-
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Molecular Mechanism of STIM-Orai Coupling

The molecular characteristics of the STIM-Orai interaction are becoming clearer. Within the Orai1 molecule, the C-terminal cytoplasmic coiled-coil domain appears key for interaction with and activation by STIM1 (37, 65). The Orai1 L273S mutation, which disrupts this coiled-coil region, blocks STIM1 interaction and channel activation and dominates in blocking wild-type Orai1 (37). The N terminus of Orai1 can be truncated up to residue 73 with little effect on STIM1 coupling (35), but truncation of the following 15 amino acids prevents channel activation while still allowing interaction with STIM1, mimicking the R91W mutation present in SCID patients (37). For STIM1, expression of soluble C-terminal fragments can fully activate Orai1 channels without store emptying (27, 35, 42, 59, 65, 66). Remarkably, a short stretch of just over 100 amino acids including the second coiled-coil domain (Fig. 1) is sufficient for Orai1 activation (57, 65). Thus, SOAR (65) and CAD (57) are potent, full activators of Orai1. The STIM1 CAD fragment undergoes biochemical interactions with the isolated C-terminal domain 254–301 of Orai1 (57) as well as the N-terminal fragment 70–91 (57), suggesting that the interaction with STIM1 involves both termini of Orai1. C-terminal fragments of STIM cause clustering and activation of PM Orai channels (42, 57–59). However, STIM-induced Orai1 clustering is not sufficient for Orai1 channel activation. Thus, CAD with eight C-terminal residues (positions 441–448) removed still binds and clusters Orai1 yet cannot activate channel activity (57). Moreover, although the two STIM1 truncations 1–448 and 1–440 both enter puncta upon store depletion and cluster Orai1, only construct 1–448 activates Orai1 channel activity (57). Because the SOAR fragment (positions 344–442) activates Orai1 (65), the two missing residues (positions 441 and 442) in the non-activating fragment 340–440 (57) may play a crucial role in gating Orai1. Both CAD and SOAR exist as multimers (57, 65). In solution, CAD is predominantly a tetramer (57) and causes Orai1 channel particles to cluster in extended multimeric arrays (57). Thus, STIM1 multimers induce a strong cross-linking event resulting in large clustered arrays of Orai channels. The lysine-rich C-terminal extremity (K-region) of STIM1 plays an interesting ancillary function in SOC activation. Although known to play a role (28, 67), the K-region is not essential for SOC coupling (37, 42, 57, 65). Indeed, Drosophila STIM is devoid of this region and still activates SOCs. The K-region appears to enhance PM targeting of STIM1. Hence, although whole STIM1 expressed alone readily forms PM-associated puncta, K-region-deficient STIM1 requires coexpression of Orai1 for punctum formation (57). Thus, the K-region appears to tether oligomeric STIM1 within junctions by binding to an as yet unidentified PM target. The exposed CAD/SOAR domains within STIM are able to trap and conformationally activate Orai channels. Although expressed CAD or SOAR spontaneously binds to and activates Orai channels (57, 65), larger soluble STIM1 C-terminal fragments are poorly active and remain cytoplasmic (42, 57). Hence, the C terminus of STIM1 appears to require unfolding to expose active CAD/SOAR. Interestingly, 2-aminoethoxydiphenyl borate induces rapid binding between soluble whole STIM1 or STIM2 C termini and PM Orai1, causing clustering and full Orai1 channel activation, apparently mimicking the STIM-unfolding and successful Orai-docking events (42).

Overall, STIM proteins are remarkably sensitive and dynamic ER Ca\(^{2+}\)_sensors, coupling to exceedingly Ca\(^{2+}\)-selective PM Orai channels. STIM-Orai coupling is facilitated through ER-PM junctions acting as conduits of close approach between the two membranes. The coordinated function of these two proteins mediates crucial control over cytosolic and luminal Ca\(^{2+}\)_homeostasis and the generation of both rapid and long-term Ca\(^{2+}\)_signals. Understanding the molecular details of the STIM-Orai transmembrane interaction has great significance as a universally important signaling mechanism.

REFERENCES

1. Berridge, M. J., Bootman, M. D., and Roderick, H. L. (2003) Nat. Rev. Mol. Cell Biol. 4, 517–529
2. Zhang, K., and Kaufman, R. J. (2008) Nature 454, 455–462
3. Parekh, A. B., and Penner, R. (1997) Physiol. Rev. 77, 901–930
4. Venkatachalam, K., van Rossum, D. B., Patterson, R. L. Ma, H. T., and Gill, D. L. (2002) Nat. Cell Biol. 4, E263–E272
5. Parekh, A. B., and Putney, J. W., Jr. (2005) Physiol. Rev. 85, 757–810
6. Gill, D. L., Spassova, M. A., and Soboloff, J. (2006) Science 313, 183–184
7. Lewis, R. S. (2007) Nature 446, 284–287
8. Brandman, O., Liou, J., Park, W. S., and Meyer, T. (2007) Cell 131, 1327–1339
9. Putney, J. W., Jr. (1986) Cell Calcium 7, 1–12
10. Berridge, M. J. (1995) Biochem. J. 312, 1–11
11. Patterson, R. L., van Rossum, D. B., and Gill, D. L. (1999) Cell 98, 487–499
12. Roos, J., DiGregorio, P. J., Yeromin, A. V., Ohlsen, K., Lioudyno, M., Zhang, S., Safrina, O., Kozak, J. A., Wagner, S. L., Cahalan, M. D., Velichebi, G., and Stauderman, K. A. (2005) J. Cell Biol. 169, 435–445
13. Liou, J., Kim, M. L., Heo, W. D., Jones, J. T., Myers, J. W., Ferrell, J. E., Jr., and Meyer, T. (2005) Curr. Biol. 15, 1235–1241
14. Feske, S., Gwack, Y., Praktikey, M., Srikanti, S., Puppel, S. H., Tanasa, B., Hogan, P. G., Lewis, R. S., Daly, M., and Rao, A. (2006) Nature 441, 179–185
15. Vig, M., Penielt, C., Beck, A., Kooma, D. L., Rabah, D., Kohnan-Huberson, M., Kraft, S., Turner, H., Fleig, A., Penner, R., and Kinet, J. P. (2006) Science 312, 1220–1223
16. Zhang, S. L., Yeromin, A. V., Zhang, X. H., Yu, Y., Safrina, O., Penna, A., Roos, J., Stauderman, K. A., and Cahalan, M. D. (2006) Proc. Natl. Acad. Sci. U.S.A. 103, 9357–9362
17. Williams, R. T., Manji, S. S., Parker, N. J., Hancock, M. S., Van Stekelenburg, L., Eid, J. P., Senior, P. V., Kazenwadel, J. S., Shandala, T., Saint, R., Smith, P. J., and Dziadek, M. A. (2001) Biochem. J. 357, 673–685
18. Gwack, Y., Srikanti, S., Feske, S., Cruz-Guilloty, F., Oh-hora, M., Neems, D. S., Hogan, P. G., and Rao, A. (2007) J. Biol. Chem. 282, 16232–16243
19. Oritani, K., and Kincade, P. W. (1996) J. Cell Biol. 134, 771–782
20. Strange, K., Yan, X., Lorin-Nebel, C., and Xing, J. (2007) Cell Calcium 42,
