MINIREVIEW / MINISYNTHÈSE

Partial unfolding and oligomerization of stromal interaction molecules as an initiation mechanism of store operated calcium entry[1]

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Abstract: Spatiotemporally discrete cytoplasmic Ca^{2+} fluctuations are fundamental eukaryotic signals in myriad physiological and pathophysiological functions. Store-operated Ca^{2+} entry is the process whereby a decrease in endoplasmic reticulum (ER) luminal Ca^{2+} levels activates Ca^{2+} release activated calcium (CRAC) channels on the plasma membrane (PM), providing a sustained Ca^{2+} elevation to the cytoplasm and ultimately replenishing the ER lumen Ca^{2+} supply. Stromal interaction molecules (STIMs) are the Ca^{2+} sensors of the ER lumen, which macromolecularly couple depleted ER Ca^{2+} to the assembly and opening of PM CRAC channels. The considerable stability difference caused by Ca^{2+} loading and depletion within the luminal portion of STIMs modulates intramolecular cytoplasmic domain interactions essential to the assembly of PM CRAC channels. Thus, the action of the entire complex is tightly regulated through the Ca^{2+} sensitivity of luminal STIM domains. Recent structural and biochemical studies suggest that partial unfolding – coupled oligomerization of STIMs is a crucial step in CRAC channel activation. Based on these and other published data, this minireview discusses what is currently known about the molecular mechanism of ER Ca^{2+} sensing by STIMs.

Key words: stromal interaction molecule, store operated calcium entry, calcium release activated calcium, protein unfolding.

Résumé : Les fluctuations spatio-temporelles du Ca^{2+} intracellulaire constituent des signaux fondamentaux dans une myriade de fonctions physiologiques et pathophysiologiques chez les eucaryotes. L'influx capacitif du Ca^{2+} (« store-operated calcium entry ») est un processus par lequel une diminution des niveaux de Ca^{2+} dans la lumière du réticulum endoplasmique (RE) active les canaux CRAC (« Ca^{2+}release activated calcium ») de la membrane plasmique, résultant en une élévation prolongée de Ca^{2+} dans le cytoplasme et ultimement, en une reconstitution des réserves de Ca^{2+} dans la lumière du RE. Les STIM (« stromal interaction molecules ») sont des senseurs de Ca^{2+} de la lumière du RE qui couplent de façon macromoléculaire le RE épuisé en Ca^{2+} à l'assemblage et à l'ouverture des canaux CRAC. La différence de stabilité considérable causée par le chargement et la déplétion de Ca^{2+} à l'intérieur de la partie luminale des STIM module les interactions intramoléculaires des domaines cytoplasmiques essentielles à l'assemblage des canaux CRAC de la membrane plasmique. Ainsi, l'action du complexe entier est intimement modulée par la sensibilité au Ca^{2+} des domaines luminaux des STIM. Des études structurales et biochimiques récentes suggèrent que l'oligomérisation des STIM couplée à un dépliement partiel est une étape clé de l'activation des canaux CRAC. Cette mini-revue présente des éléments connus du mécanisme moléculaire de la détection de Ca^{2+}du RE par les STIM, à partir de ces travaux et d'autres données publiées.

Mots-clés : STIM), entrée de calcium capacitive, canaux CRAC, dépliement des protéines.

[Traduit par la Rédaction]

Introduction

T-cell receptor engagement with antigen-presenting cells leads to an intracellular protein phosphorylation cascade that ultimately activates phospholipase Cγ1. Alternatively, phospolipase β can be activated through G-protein coupled chemokine receptor stimulation. The phospholipases catalyze the production of inositol-1,4,5-trisphosphate (IP_3), which binds to the endoplasmic reticulum (ER) resident IP_3 receptor, inducing the release of Ca^{2+} from the luminal

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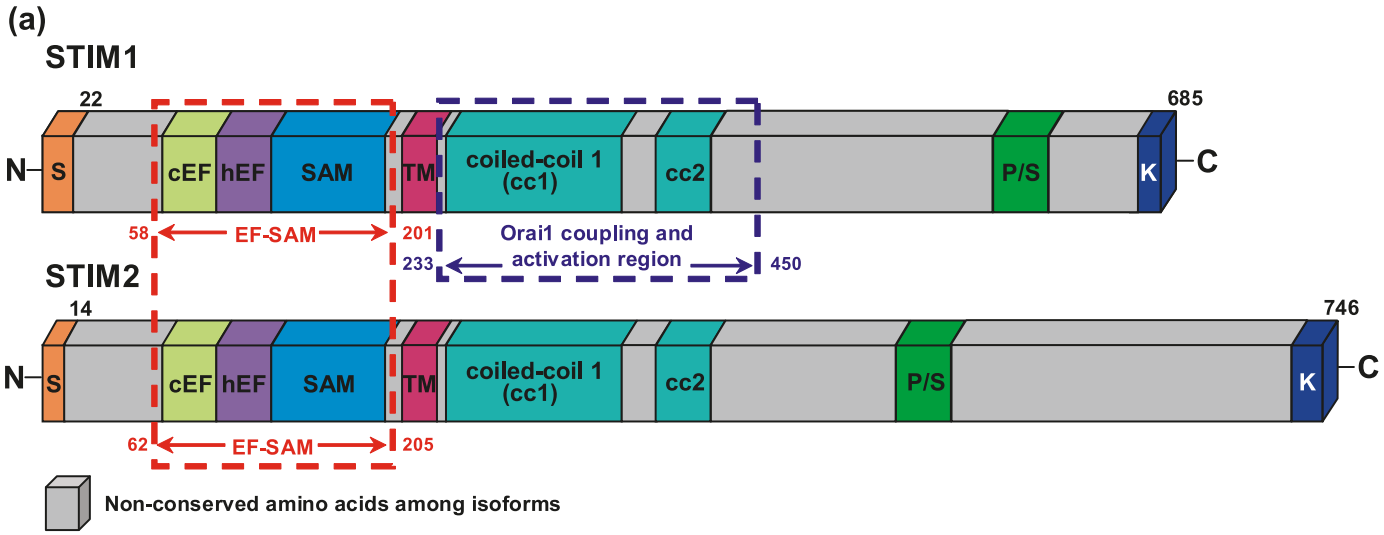
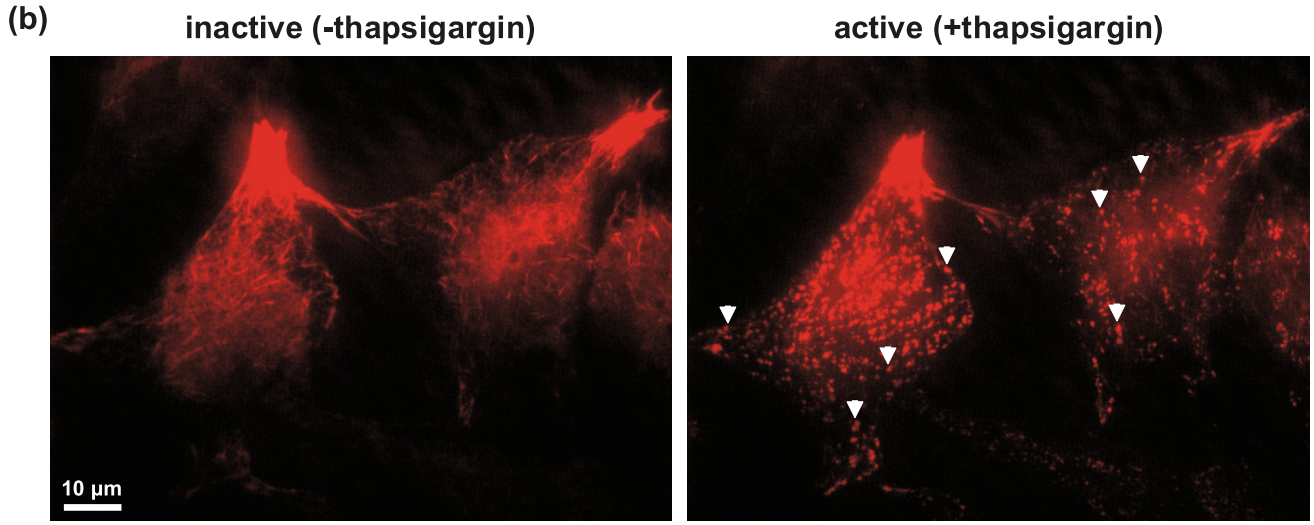
Fig. 1. STIM domain architecture and cellular localization. (A) Comparison of human STIM domain architecture. The most conserved regions among all isoforms are the luminal EF-hand and SAM domains, and the cytosolic coiled-coil regions. The luminal and cytosolic domains are separated by a single transmembrane segment (TM). S, ER signal peptide; cEF, canonical EF-hand motif; hEF, hidden EF-hand motif; P/S, Pro/Ser-rich segment; K, Lys-rich segment. (B) Localization of STIM1 in HeLa cells assessed by total internal reflective fluorescence microscopy. The HeLa cells are expressing N-terminally cherry fluorescent protein (cherryFP)-fused STIM1. With ER stores replete of Ca^{2+}, the inactive cherryFP–STIM1 shows a pervasive and tubular-like distribution (left panel). Upon passive depletion of ER Ca^{2+} using thapsigargin, the cherryFP–STIM1 forms distinct puncta (e.g., white arrowheads) throughout the cell (right panel).

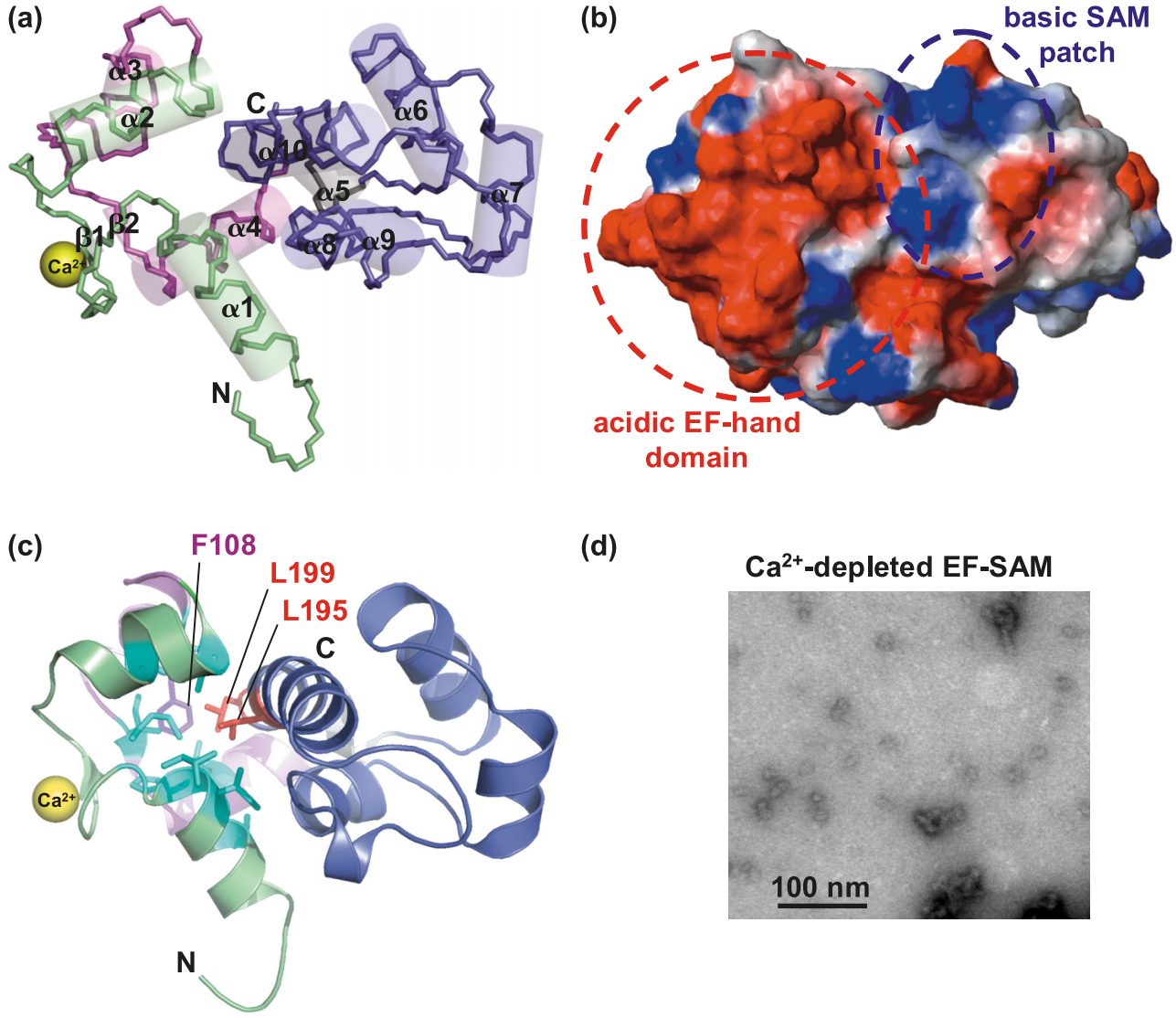




stores into the cytoplasm. Since the ER extends through a large volumetric proportion of eukaryotic cells contiguously from the nuclear envelope to the plasma membrane (PM), a significant source of Ca^{2+} is compartmentalized within the lumen of this organelle. More importantly, a steep concentration gradient exists between the ER lumen and cytoplasm that permits movement of Ca^{2+} down the gradient at a low energetic cost to the cell. This gradient is created as the cytosol of non-excitable cells maintains Ca^{2+} levels ranging from 10^{-7} to 10^{-6} mol·L^{-1}, whereas the ER lumen contains free Ca^{2+} at concentrations ranging from 10^{-4} to 10^{-3} mol·L^{-1}. The sub-micromolar free Ca^{2+} levels of the cytosol are well-adapted to eukaryotic Ca^{2+} signaling, in which only relatively small fluctuations in local Ca^{2+} concentrations directly or indirectly affect proteins that regulate myriad physiological and pathophysiological processes such as vision, memory, fertilization, proliferation, transcription, contraction, and the immune response (Berridge et al. 2003).

Cytosolic Ca^{2+} sourced from intracellular stores is transient due to the finite organellar supply, steadfast binding of Ca^{2+} to buffer and sensor proteins, and rapid redistribution of Ca^{2+} into other compartments via pumps and exchangers. With numerous fundamental Ca^{2+}-dependent processes taking place within the ER, such as protein folding, degradation, chaperonal activity, vesicle trafficking, and lipid and steroid biosynthesis (Berridge 2002), it is imperative that luminal Ca^{2+} levels do not become detrimentally low and stores are quickly replenished. Eukaryotes have evolved store-operated Ca^{2+} entry (SOCE) as a critical Ca^{2+} entry pathway for sustaining prolonged cytosolic elevations in Ca^{2+} and refilling ER luminal stores. SOCE takes advantage of the virtually inexhaustible supply of extracellular Ca^{2+}

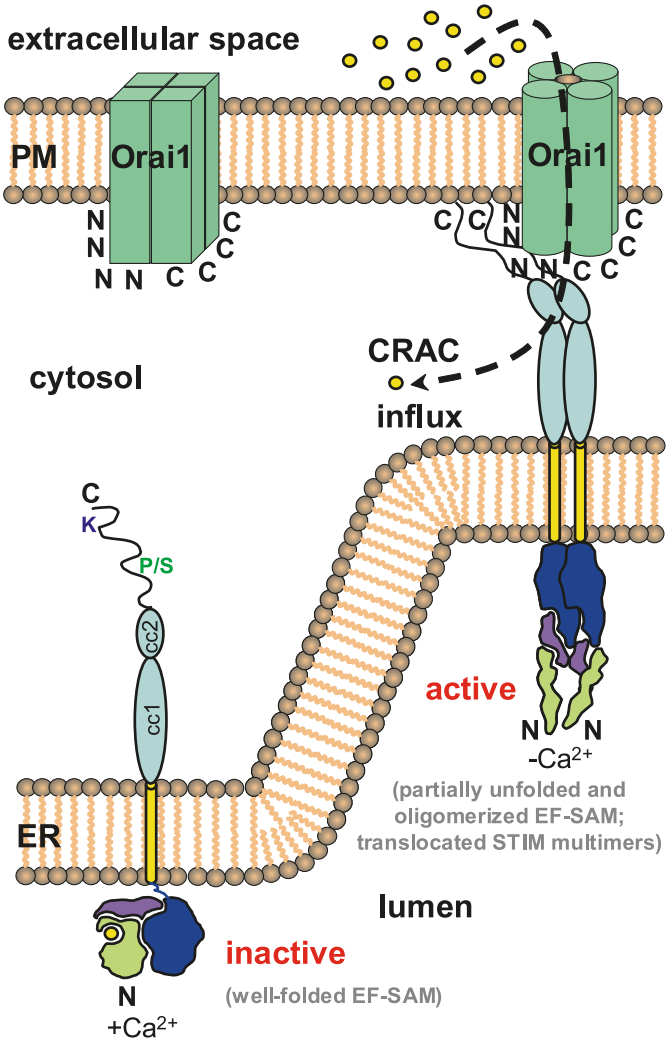
Fig. 2. Structural characteristics of EF–SAM. (A) Atomic resolution structural motifs of Ca^{2+}-loaded STIM1 EF–SAM. The backbone atoms are continually traced with a solid line, whereas the secondary structure components are shown as transparent cylinders labeled α1, α2, α3, α4, α5, α6, α7, α8, α9, and α10 for helices, and arrowheads labeled β1 and β2 for sheets. The Ca^{2+} ion is illustrated as a sphere and amino and carboxy termini are denoted with N and C, respectively. (B) Electrostatic surface of Ca^{2+}-loaded STIM1 EF–SAM. Acifid EF-hand and basic SAM patches are encircled by broken lines. (C) Imperative hydrophobic interactions in Ca^{2+}-loaded STIM1 EF–SAM. The prominent SAM domain hydrophobic protrusion side chains are bounded by side chains oriented from the EF-hand domain, maintaining an intimate intramolecular interaction. Destabilization of the interaction by mutation at F108 or Leu195 causes Ca^{2+}-insensitive partial unfolding-coupled oligomerization. (D) Electron micrograph of Ca^{2+}-depleted STIM1 EF–SAM. Polydisperse EF–SAM oligomers in the Ca^{2+}-depleted state are visualized with 2% *w*/*v* uranyl acetate and transmission electron microscopy (original magnification = 71 000×). The particle size of the monomeric Ca^{2+}-loaded STIM1 EF–SAM is beyond the resolution of the technique. The RCSB pdbID for STIM1 EF–SAM is 2k60.pdb.



maintained at ~10^{-3} mol·L^{-1} by opening PM channels that are selectively permeable to Ca^{2+} in response to ER luminal Ca^{2+} store depletion, thereby facilitating persistent Ca^{2+} flux down the steep gradient to the cytosol (Putney 1986; Putney 2007). Prolonged increases in cytosolic Ca^{2+} are critical for eliciting numerous functional responses such as transcriptional activation and for refilling the ER luminal Ca^{2+} stores.

In lymphocytes, SOCE through PM Ca^{2+} release activated Ca^{2+} (CRAC) channels is the primary means of augmenting cytosolic Ca^{2+} vital for T-cell activation, proliferation, and other immune cell effector functions. After more than two decades of uncertainty regarding a precise molecular identity, the ER Ca^{2+}-sensing and Ca^{2+}-selective PM channel components of CRAC entry have been elucidated with the stromal interaction molecules (STIMs) (Liou et al. 2005; Roos et al. 2005; Zhang et al. 2005) and the Orai pore subunits (Feske et al. 2006; Prakriya et al. 2006; Vig et al. 2006*a*; Vig et al. 2006*b*; Yeromin et al. 2006; Zhang et al. 2006), respectively. Co-overexpression of STIM1 with Orai1 in cell culture results in enormous amplification of the inward rectifying current generated by the cytoplasmic influx of Ca^{2+} through open PM CRAC channels (Mercer et

Fig. 3. Model of STIM1–Orai1 CRAC activation. STIM1 maintains a monomeric state concurrent with a Ca^{2+}-bound and well-folded luminal EF–SAM region. Upon Ca^{2+} depletion, EF–SAM is destabilized and undergoes partial unfolding – coupled oligomerization, re-establishing cytosolic intermolecular coiled-coil interactions. STIM oligomers translocate to ER–PM junctions, recruiting, coupling, and activating Orai1 CRAC channels via sequentially conserved coiled-coil domains. The minimal CRAC complex includes an Orai1 tetramer coupled to a STIM1 dimer.



al. 2006; Peinelt et al. 2006; Soboloff et al. 2006*b*; Zhang et al. 2006). Further, pedigree analysis has linked a form of inheritable severe combined immunodeficiency disease (SCID) to a missense mutation in the Orai1 protein (Feske et al. 2006), and familial nonsense mutations within STIM1 result in a clinical phenotype comparable to SCID (Picard et al. 2009), in which CRAC entry is abrogated in cells from these patients and lymphocyte activation is severely compromised. The intrusive nature of these and other pathophysiologies combined with the functional effect of these proteins in cell culture underscores the necessity and sufficiency of both STIM and Orai proteins in CRAC entry and eukaryotic function. The present minireview will focus on the structural, biochemical, and biophysical characterization of the luminal portion of STIMs, which has yielded important data on the sensing initiation mechanism of this fundamental Ca^{2+} signaling pathway.

Domain architecture of STIMs

Inhibiting and interfering ribonucleic acid (RNA) studies identified STIMs as the ER-resident component of CRAC entry, as knockdown of these proteins in cell culture significantly depresses SOCE (Liou et al. 2005; Roos et al. 2005; Zhang et al. 2005). STIMs are single-pass, type I transmembrane proteins located on the ER or plasma membranes such that the soluble C-terminal regions are oriented within the cytosol (Fig. 1A). Mammals express two isoforms (i.e., STIM1 and STIM2) with only a small fraction of STIM1 being targeted to the PM, probably due to glycosylation of Asn131 and Asn171 residues located on the soluble N-terminal side of the protein (Manji et al. 2000; Spassova et al. 2006; Williams et al. 2001; Williams et al. 2002; Zhang et al. 2005). Hence, STIM2 and the majority of STIM1 reside on the ER with the lumen-oriented regions encoding EF-hand motifs and sterile α-motif (SAM) domains prior to the 21 residue transmembrane segments. The cytosolic C-terminal segments translate predicted coiled-coil domains, Pro/Ser and Lys-rich regions. The primary sequence-based domains are highly conserved between homologues and among species; however, significant variability exists proximal to the EF-hand motifs, towards the N-terminus and proximal to the coiled-coil domains, towards the C-terminus (Cai and Cookson 2007) (Fig. 1A).

Depletion of ER luminal Ca^{2+} by phospholipase activation, sarco-endoplasmic reticulum Ca^{2+}-ATPase pump inhibition, or ethylene glycol tetraacetic acid (EGTA) treatment of cells causes a STIM1 relocalization from a pervasive ER distribution to distinct cluster sites at ER–PM junctions (Liou et al. 2005; Zhang et al. 2005) (Fig. 1B). The Ca^{2+}-depleted STIM1 clustering facilitates recruitment of Orai1 to the same sites creating puncta, obligatory for CRAC channel assembly (Luik et al. 2006; Várnai et al. 2007; Wu et al. 2006; Xu et al. 2006). Although the cytosolic regions of STIMs play an important role in targeting the molecule to sites in close apposition to the PM and interactions with channel pore subunits (Baba et al. 2006; Huang et al. 2006; Li et al. 2007; Liou et al. 2007) (vide infra), the N-terminal domains are essential for the conformational and functional sensitivity to Ca^{2+} that STIMs exhibit. This sensitivity is exemplified in cell culture experiments that mutate luminal domain canonical EF-hand motif Ca^{2+}-coordinating residues causing constitutive ER Ca^{2+}-insenstive STIM1 puncta and CRAC entry (Liou et al. 2005; Mercer et al. 2006; Spassova et al. 2006; Zhang et al. 2005). Additionally, STIM1 with a deleted SAM domain is unable to form inducible puncta in live cells (Baba et al. 2006).

Live cell fluorescence-resonance energy transfer experiments demonstrated that CRAC activation occurs as a multi-step process, where accretion of STIM1 at ER–PM junctions and subsequent channel formation is preceded by a preliminary homotypic STIM1 oligomerization step (Liou et al. 2007). The importance of the preliminary STIM1 oligomerization phase was accentuated in a cell culture study that replaced the prominent luminal STIM domains (i.e., the EF-hand together with the SAM domain (EF–SAM)) with Ca^{2+}-independent, but rapamycin- or rapamycin analogue-

dependent protein oligomerization domains (i.e., FK506- and rapamycin-binding protein (FKBP12) or FKBP-rapamycin binding (FRB) domain of mammalian target of rapamycin). Treatment with rapamycin oligomerized the fusion STIM molecules in live cells, forming puncta and activating CRAC channels without depletion of ER Ca^{2+} stores (Luik et al. 2008). These data stressed the importance of EF–SAM in molecularly linking Ca^{2+}-depletion with oligomerization of full-length STIM1 for subsequent translocation to ER-PM junctions and recruitment of Orai1 subunits in the formation of STIM1–Orai1 CRAC complexes.

Atomic structure of the Ca^{2+}-sensing region of STIM1

To fully appreciate the molecular mechanism of STIM1 Ca^{2+} sensing and functional coupling to CRAC channels, higher resolution information must be considered. A recombinant EF–SAM segment of STIM1 has provided a wealth of data regarding the Ca^{2+}-sensing mechanism of the luminal region of STIM proteins. Using solution nuclear magnetic resonance (NMR), the nuclear Overhauser effect (NOE)-determined, Ca^{2+}-loaded, EF–SAM atomic resolution structure exhibits a single and compact tertiary fold, despite the modular nature of the gene consisting of EF-hand and SAM homology sequences in tandem. The mutually dependent organization of the EF-hand and SAM protein domains in 3D space reveals that the luminal STIM domains are stabilized through intramolecular interactions, also intimating a cooperativity in function.

The structural topology of EF–SAM is primarily α-helical (i.e., 10 helices in total) with an additional small β-sheet (i.e., 2 strands in total) within the fold (Stathopulos et al. 2008) (Fig. 2A). The canonical EF-hand motif, which is identifiable by sequence homology with other EF-hand motifs, is made up of α1–β1–α2 structural components, whereas the SAM domain folds into a 5-helix bundle, α6–α7–α8–α9–α10, common to other SAM domains (Qiao and Bowie 2005). The 3D EF–SAM structure exposes a very critical feature of STIMs not apparent from primary sequence analyses; the luminal portion of STIMs contains a second, non-canonical EF-hand motif, α3–β2–α4. This sequentially hidden EF-hand motif is non-functional in Ca^{2+} binding (vide infra); however, Ile115 within the loop establishes two hydrogen bonds with Val83 from the canonical EF-hand loop, creating the small β-sheet and stabilizing the EF-hand pair. A short linker helix, α5, connects the EF-hand pair with the SAM domain (Fig. 2A). The tertiary distribution of EF–SAM residues forms a primarily acidic surface at neutral pH; however, a small basic patch of residues is evident on the SAM face of the protein (Stathopulos et al. 2008) (Fig. 2B). The negative potential may be involved in electrostatic guidance of Ca^{2+} to the canonical binding loop, whereas the positive region on the SAM domain might promote other biomolecular interactions such as with membrane lipids (Barrera et al. 2003).

Both the canonical and hidden EF-hand motifs of STIM1 adopt an open conformation with interhelical angles of ~80° and 97°, respectively. Open helical angles, defined as entering and exiting helices that are oriented >70° relative to one another, are found in numerous Ca^{2+}-loaded EF-hand containing proteins, exposing several hydrophobic side chains for interactions with targets (Barrera et al. 2003; Yap et al. 1999; Yap et al. 2002). The canonical EF-hand angle is remarkably similar to the leading EF-hand motif within the C-terminal domain of bovine calmodulin (CaM) at 80° vs. 81°, respectively. Together, the bovine CaM C-terminal EF-hand pair exhibit a relatively small backbone root-mean-squared deviation (RMSD) of 3.2 Å when aligned with the EF–SAM pair in 3D space. The STIM1 SAM domain is structurally similar to many other SAM domains such as the EphB2 receptor (i.e., RMSD = 2.1 Å).

Although the EF-hand and SAM domains are structurally typical of numerous other proteins, the intimate intramolecular association observed for EF–SAM is distinctive and the first such example in nature. The domains within EF–SAM form a single compact entity as a result of vigorous non-polar contacts between the EF-hand pair and the SAM domain. The open EF-hand pair residues Val68, Ile71, His72, Leu74, Met75, Leu92, Leu96, Lys104, Phe108, Ile115, and Leu120 form a cleft, which interacts with prominent hydrophobic SAM residues from the α10 helix including Leu195 and Leu199 (Fig. 2C). This intramolecular EF–SAM interaction is akin to the intermolecular target recognition mechanism employed by CaM (Stathopulos et al. 2008).

Destabilization and partial unfolding – coupled oligomerization of STIM1 EF–SAM

In the absence of the SAM domain, expression of the STIM1 EF-hand pair results in a constitutively unfolded polypeptide. Furthermore, expression of the SAM domain alone yields little-to-no protein. However, the domains expressed in tandem mutually and cooperatively fold into a stable and compact state in the presence of Ca^{2+} (Stathopulos et al. 2006; Stathopulos et al. 2008). Upon chelation of Ca^{2+} from EF–SAM, the protein undergoes a marked loss in α-helicity and tertiary structure, as assessed by circular dichroism (CD) and other biophysical techniques. The closed EF-hand conformation observed for apo CaM with interhelical angles of ~45° (Yap et al. 1999; Yap et al. 2002) may be incompatible in a tandem EF-hand–SAM protein resulting in significant destabilization of the EF–SAM entity (Stathopulos et al. 2008). The Ca^{2+} depletion induced partial unfolding is coupled with an increase in quaternary structure, transitioning from a monomeric holo Ca^{2+}-loaded and well-folded state to a polydisperse oligomeric apo Ca^{2+}-depleted and partially unfolded state (Stathopulos et al. 2006). There is evidence for dimers and higher order oligomers, but not monomers, in destabilized solutions of Ca^{2+}-depleted EF–SAM (Fig. 2D). The Ca^{2+} depletion induced loss in structure proceeds through both the EF-hand and SAM domains, augmenting the surface exposed hydrophobicity of the protein (Stathopulos et al. 2006; Stathopulos et al. 2008). The Ca^{2+}-dependent differences in EF–SAM structure translate into marked distinctions in stability. The midpoint of heat denaturation (T_m) for STIM1 EF–SAM is augmented by ~26 °C in the presence of Ca^{2+}, whereas the midpoint of urea unfolding is increased by ~1.5 $mol \cdot L^{-1}$. Protein unfolding is cooperative for Ca^{2+}-depleted EF–SAM, as assessed by thermal and chemical denaturation experiments (Stathopulos et al. 2006), implying that apo EF–SAM is not random coil, but preserves some residual tertiary structure.

The monomer-to-oligomer transition is reversible, as binding of Ca^{2+} restores the compact and monomeric EF–

SAM fold. Binding assays reveal a temperature-dependent Ca^{2+} equilibrium dissociation constant (K_d) for STIM1 EF–SAM at between ~0.2 and 0.6 mmol·L^{-1} with a stoichiometry of 1 (Stathopulos et al. 2006). The number of binding sites is coherent with the single functional Ca^{2+} binding loop, whereas the low affinity is consistent with the estimated concentration of ER Ca^{2+} at which puncta formation and CRAC entry is half maximally activated (i.e., ~0.2 mmol·L^{-1}) (Brandman et al. 2007; Luik et al. 2008) and reconcilable with the generally high Ca^{2+} levels of the ER lumen (Feske 2007).

Considering the functional significance of full-length homotypic STIM1 oligomerization in the activation of CRAC established within live cells (see Introduction), the sequence conservation of EF–SAM among phylogeny, the reversible Ca^{2+}-depletion dependent monomer-to-oligomer transition of recombinant EF–SAM, the physiologically reconcilable Ca^{2+} affinity of EF–SAM, the necessity for co-stabilization of the STIM1 EF-hand together with the SAM domain, and the destabilization and partial unfolding – coupled oligomerization observed for Ca^{2+}-depleted EF–SAM, it follows that the stability of the intimate EF-hand:SAM domain interaction is central to the sensory function of STIM proteins. Consistent with this notion, mutagenic disruption of the EF-hand:SAM interaction, by introduction of charged side chains into the EF-hand hydrophobic cleft with Phe108Asp/Gly110Asp or into the SAM protrusion with Leu195Arg, constitutively oligomerizes the protein in a partial unfolding – coupled process. Full-length STIM1 harboring these same point mutations persistently activates PM CRAC channels in live cells independent of ER luminal Ca^{2+} (Stathopulos et al. 2008). A Ca^{2+}-binding disruption mutation (i.e., Glu87Ala) demonstrates a similar structural phenotype recombinantly in vitro and functional phenotype with full-length STIM1 in live cells as observed with the inter-domain disruption mutants (Stathopulos et al. 2008).

Isoform-specific stability, unfolding, and oligomerization characteristics

The sensory function of human STIM2 appears more convoluted and distinct compared with STIM1 based on current available data. Published reports show that STIM2 knockdown diminishes CRAC entry (Liou et al. 2005), that STIM2 is an inhibitor of STIM1-mediated CRAC activity (Mercer et al. 2006; Soboloff et al. 2006*a*; Soboloff et al. 2006*b*), and that STIM2 activates CRAC channels in an ER Ca^{2+} store-dependent and -independent manner (Parvez et al. 2008). Additionally, a role for STIM2 in the maintenance of basal cytosolic and luminal Ca^{2+} is reported based on the observation that STIM2 oligomerizes and couples to Orai1 at higher ER Ca^{2+} levels (i.e., ~0.4 mmol·L^{-1}) compared with STIM1 (Brandman et al. 2007). Furthermore, T-cells lacking STIM2 do not show the level of impairment in CRAC entry observed for STIM1, yet attenuate cytokine production and translocation of nuclear transcription factor to a comparable extent as STIM1 in the same cell type (Oh-Hora et al. 2008).

STIM1 and STIM2 recombinant EF–SAM proteins also exhibit notable distinctions in vitro. First, STIM2 EF–SAM is more stable than STIM1 EF–SAM in both the Ca^{2+}-loaded (T_m ~50 vs. 45 °C, respectively) and -depleted states (T_m ~36 vs. 21 °C, respectively) (Zheng et al. 2008). The ~5 °C augmentation in thermal stability observed for apo STIM2 EF–SAM translates into the maintenance of a monomeric conformation at 4 °C, unlike apo STIM1 EF–SAM, which partially unfolds to form dimers and oligomers at similarly low temperatures (Stathopulos et al. 2006; Zheng et al. 2008). Nevertheless, oligomerization of apo STIM2 EF–SAM coupled with partial unfolding occurs at higher ambient temperatures. Furthermore, a time and concentration dependence on the kinetics of oligomerization is observed for Ca^{2+}-depleted STIM2 EF–SAM, which is not seen with the persistently oligomerized apo STIM1 EF–SAM under the same experimental setup (Stathopulos et al. 2009; Zheng et al. 2008). The kinetic differences are conserved when starting from the Ca^{2+}-loaded context, where STIM1 EF–SAM immediately begins partial unfolding-coupled oligomerization, whereas STIM2 remains more resistant to homotypic associations upon chelation of Ca^{2+} (Stathopulos et al. 2009).

Nevertheless, the EF–SAM proteins share some important similarities in that the Ca^{2+}-affinity of STIM2 is of the same magnitude as STIM1, where the EF-hand domains coordinate only a single Ca^{2+} ion, complementary to the high ER luminal Ca^{2+} concentrations (Zheng et al. 2008). More importantly, both STIM EF–SAM domains have an inherent ability to form homotypic oligomers in the Ca^{2+}-depleted state, consistent with an initiation function in CRAC channel activation for both mammalian homologues.

The differences elucidated in EF–SAM oligomerization dynamics likely afford STIM proteins one level of isoform-specific regulation. Further tweaking may be a function of non-conserved residues beyond EF–SAM within the lumen. In fact, recombinant human STIM proteins that include the full complement of luminal residues are stabilized by ~15 °C in both the Ca^{2+}-loaded and -depleted states (Stathopulos et al. 2009). These extraneous residues appear to have a marked effect on the CRAC activation kinetics in live cells, as STIM1 protein fusions containing STIM2 N-terminal residues exhibit slower CRAC activation kinetics similar to wild-type STIM2, while STIM2 chimeras harboring the STIM1 N-terminal residues enhance CRAC entry as per wild-type STIM1 (Zhou et al. 2009). Biophysical analyses suggest that the added residues contribute little-to-no additional structure to EF–SAM, as the proteins undergo a similar Ca^{2+}-depletion dependent structural transformation when destabilized in vitro (Stathopulos et al. 2009). Higher resolution data is needed to assess the precise structural and thermodynamic contribution of isoform-dependent N- and C-terminal residues to the oligomerization function of STIM proteins.

Orai1–STIM activation coupling

Although atomic resolution data is completely lacking on the cytosolic domains of STIM proteins, live cell data have mapped critical regions required for STIM1–Orai1 coupling and CRAC activation. The residues are encoded through the STIM C-terminal coiled-coil domains and are highly conserved among isoforms. Three separate investigations identified STIM1 residues 233–450 (Muik et al. 2009), 342–448 (Park et al. 2009), and 344–443 (Yuan et al. 2009) as critical amino acid stretches for induction of Orai1 channels.

The functional stoichiometric approximation of the minimal CRAC complex includes a STIM1 dimer together with an Orai1 tetramer (Ji et al. 2008). Moreover, biochemical analyses show that the 233–450 and 344–443 STIM1 recombinant proteins are dimeric in vitro (Muik et al. 2009; Yuan et al. 2009) and disruption of the dimeric nature precludes CRAC activation in cell culture (Muik et al. 2009). Deletion of the cytosolic C-terminal residues from Orai1 prevents STIM1–Orai1 clustering and CRAC activation (Li et al. 2007; Muik et al. 2008; Park et al. 2009; Yuan et al. 2009), whereas elimination of cytosolic Orai1 N-terminal residues inhibits CRAC activity, but not co-clustering of STIM1–Orai1 (Li et al. 2007). Therefore, STIM1 likely interacts directly with both the N- and C-terminal domains of Orai1 in the formation and activation of CRAC channels.

Model of CRAC channel activation

The current biophysical, biochemical, and live cell data depict a model of CRAC initiation and activation that involves a rearrangement of the entire STIM molecule, tightly coordinated by the Ca^{2+}-dependent stability of the luminal domains (Fig. 3). Under high resting ER Ca^{2+} levels EF–SAM exists as a stable, well-folded, globular monomer. Upon depletion of ER luminal Ca^{2+}, the EF–SAM region of STIM is drastically destabilized, forming higher-order dimers and oligomers in a partial unfolding – coupled process. Intermolecular association of EF–SAM entities institutes the inherent C-terminal domain interactions, which is followed by translocation of homotypically associated STIM1 to ER–PM junctions. Subsequently, STIMs recruit PM Orai1 to the same junctions where puncta are sites of direct Orai1–STIM1 heterotypic interaction and CRAC channel formation. These CRAC complexes reversibly dissipate upon stabilization of EF–SAM through Ca^{2+} binding.

There are several poignant unanswered structural questions regarding the STIM-mediated CRAC initiation mechanism. How does partially unfolded and destabilized EF–SAM lead to oligomerization? How does this oligomerization promote or re-establish cytoplasmic homotypic interactions? Additionally, how does oligomerized STIM couple to and activate Orai1-composed Ca^{2+} channels? Elucidation of an atomic resolution Ca^{2+}-depleted EF–SAM structure in addition to structural information on the cytosolic STIM domains will greatly enhance our understanding of the molecular mechanisms governing SOCE and CRAC entry.

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