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Structural elements of stromal interaction molecule function

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ABSTRACT

Stromal interaction molecule (STIM)-1 and -2 are multi-domain, single-pass transmembrane proteins involved in sensing changes in compartmentalized calcium (Ca^{2+}) levels and transducing this cellular signal to Orai1 channel proteins. Our understanding of the molecular mechanisms underlying STIM signaling has been dramatically improved through available X-ray crystal and solution NMR structures. This high-resolution structural data has revealed that intricate intramolecular and intermolecular protein-protein interactions are involved in converting STIMs from the quiescent to activation-competent states. This review article summarizes the current high resolution structural data on specific EF-hand, sterile α motif and coiled-coil interactions which drive STIM function in the activation of Orai1 channels. Further, the work discusses the effects of post-translational modifications on the structure and function of STIMs. Future structural studies on larger STIM:Orai complexes will be critical to fully defining the molecular bases for STIM function and how post-translational modifications influence these mechanisms.

Stromal interaction molecules (STIM) 1 and 2 are endoplasmic reticulum (ER) membrane-inserted, single pass transmembrane (TM) proteins [1,2] which structurally respond to fluctuations in ER luminal calcium (Ca^{2+}) levels [3,4]. Upon Ca^{2+} depletion, human STIM1 undergoes a conformational change which culminates in its oligomerization and translocation to ER-plasma membrane (PM) junctions [5–8]. Translocated STIM1 subsequently mediates the opening of Orai1-constituted Ca^{2+} release activated Ca^{2+} (CRAC) channels [9–13] in a cellular process known as store operated calcium entry (SOCE) [14–16]. SOCE induces a more sustained influx of Ca^{2+} into the cytoplasm of cells, leading to downstream intracellular Ca^{2+} signaling cascades [14,17–19]. On the other hand, the homologous STIM2 protein is more intimately involved in cellular Ca^{2+} homeostasis, maintaining basal Ca^{2+} levels in the ER lumen and cytoplasm [3,20,21].

Since the identification of the principal protein components involved in mediating SOCE over a decade ago, considerable progress has been made in elucidating the structural elements underlying their function. This review focuses on the mechanisms of STIM function based on available high resolution structural information. Additionally, the work discusses how post-translational modifications, which are found in the regions of STIM with a well-defined role in mediating SOCE, may fine-tune these structural mechanisms.

1. STIM luminal domain structure and function

Human STIM1 is translated into a 685 amino acid polypeptide, while its homolog, STIM2, is translated into longer 746–841 amino acid peptides in multiple isoforms (Fig. 1). The N-terminal region of STIMs contains an ER signal peptide, canonical and non-canonical EF-hand domains, and a sterile α-motif (SAM) domain. The EF-hand and SAM domains reside in the ER lumen, upstream of a single ER-inserted TM domain. Together, the EF-hand and SAM domains constitute a region of high sequence conservation while the section interceding the N-terminal ER signal peptide and canonical EF-hand is highly variable between human and other higher to lower order STIM homologues among eukaryotes [22,23]. The EF-hand in conjunction with the SAM domain (*i.e.* EF-SAM) constitutes the Ca^{2+} sensing machinery of STIM proteins [21]. Despite the overall high sequence similarity between the human STIM1 and STIM2 luminal N-terminal domains [24,25], noteworthy structural differences in the EF-SAM domain exist, resulting in distinct stability, activation, and Ca^{2+}-sensitivity characteristics between STIM homologs [21,26–28].

It was initially proposed that a single EF-hand motif was the sole Ca^{2+} sensing unit of STIM based on primary sequence analyses; however, following the elucidation of the human STIM1 EF-SAM domain structure, it is now known that two EF-hand motifs exist within STIM proteins: a canonical as well as a non-canonical EF-hand [27]. Together, these EF-hand motifs form an EF-hand domain characterized by two

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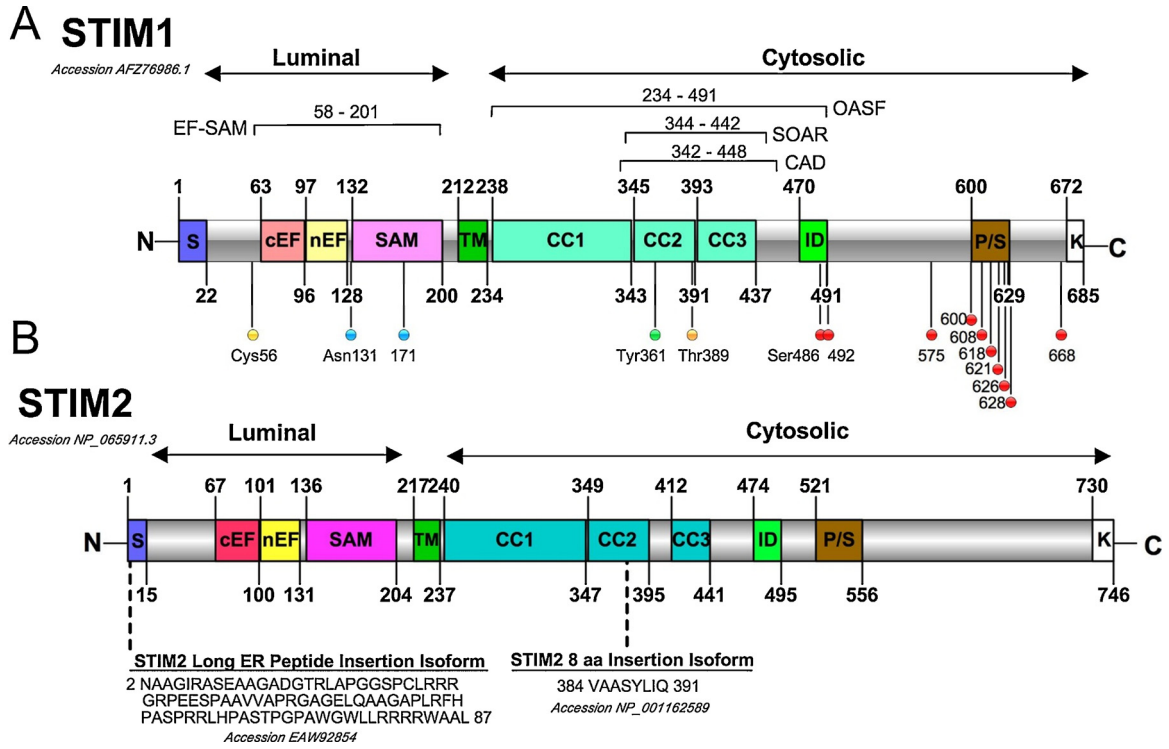

Fig. 1. Domain architecture of human STIM1 and STIM2 proteins. *A*. Key structural features on the human STIM1 domain architectures. Residue ranges are shown on top or bottom of the domain architecture. Domain boundaries and names of the various fragments used in the structure-function studies of STIMs are shown on top. The relative locations of detected post-translational modifications are shown below the diagram (yellow, *S*-glutathionylation; blue, *N*-glycosylation; green, Tyr-phosphorylation; orange, Thr-phosphorylation; red, Ser-phosphorylation). ***B.*** Key structural features on the human STIM2 domain architectures. Residue ranges are shown on top or bottom of the domain architecture. Differences in sequence associated with the STIM2 isoforms are indicated by broken lines specifying the amino acid insertions. In *A* and *B*, the cellular localization of the region of STIM is indicated on top of the diagrams. S, signal peptide; cEF, canonical EF-hand motif; nEF, non-canonical EF-hand motif; SAM, sterile α motif; TM, transmembrane; CC1, 2, 3, coiled-coil-1, -2, -3; ID, inhibitory domain; P/S, proline-serine-rich region; K, polybasic region.

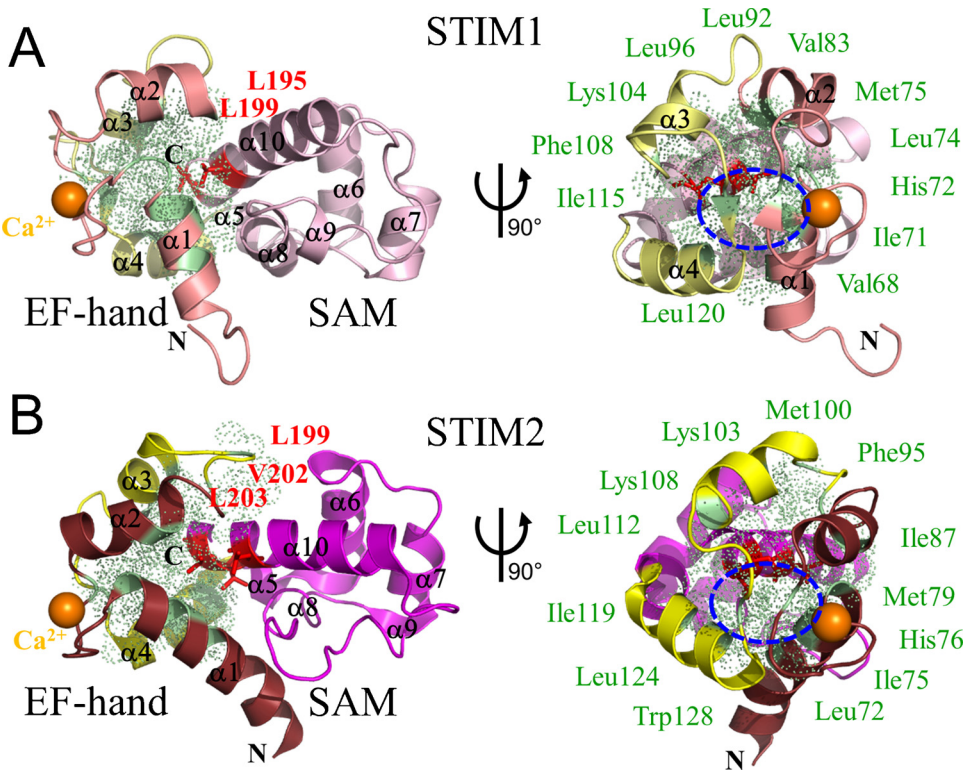

Fig. 2. High resolution structures of Ca^{2+}-loaded human STIM1 and STIM2 EF-SAM domains. *A*. Solution NMR structure of STIM1 EF-SAM. ***B.*** Solution NMR structure of STIM2 EF-SAM. In *A* and *B*, EF-SAM secondary structure components are labeled (left). The density occupied by the residues making up the hydrophobic pockets is shown with green dots. The residues making up the EF-hand hydrophobic pockets are labeled (right). The α10 SAM anchor residues are shown as red sticks. Ca^{2+} ions are depicted as orange spheres. The regions of cEF and nEF main-chain hydrogen bonding stabilizing the loops are indicated by dashed blue circles. cEF, nEF and SAM domains are coloured to match Fig. 1. STIM1 and STIM2 EF-SAM coordinates are from 2K60 and 2L5Y, respectively.

helix-loop-helix motifs. The canonical EF-hand 1 is made up of α1-loop1-α2, whereas the non-canonical EF-hand 2 is composed of the α3-loop2-α4 structural units [27] (Fig. 2A). Canonical EF-hand loop1 coordinates a single Ca^{2+} ion, while non-canonical EF-hand loop2 exerts structurally stabilizing effects on loop1 through backbone hydrogen (H) bonding (Fig. 2A). The non-canonical EF-hand and its vital role in stabilizing the canonical Ca^{2+} binding EF-hand is structurally conserved between STIM1 and STIM2, even though it does not appear to have an ability to coordinate Ca^{2+} [28]. Therefore, while two EF-hand motifs exist, only one Ca^{2+} ion is coordinated with measurable affinity within the EF-SAM domain of STIM proteins.

Typical of other EF-hands found in nature, Ca^{2+} coordination causes the canonical EF-hand domains to assume an "open" conformation where the entering and exiting helices of each EF-hand motif are in a semi-perpendicular orientation relative to one another. This "open" conformation exposes hydrophobic residues from both EF-hand motifs in the formation of a concave non-polar cleft (Fig. 2A) [29,30]. Remarkably, the non-canonical STIM EF-hands adopt an "open" conformation in harmonization with the canonical EF-hands even in the absence of Ca^{2+} coordination, indicative of the intimate cooperativity in structure, folding and function.

When the Ca^{2+} ion dissociates from the canonical EF-hand, a destabilization coupled with a partial unfolding of the EF-SAM domain initiates luminal domain oligomerization prior to cytosolic domain conformational changes within STIM (see below) and subsequent to SOCE activation [6,21,26,27,31]. The structural mechanism underlying the initiating oligomerization event involves the concerted folding of the EF-hand together with the SAM domains. The two EF-hand motifs are connected to the SAM domain through a short helix (*i.e.* α5) in sequence. The SAM domain contains five α-helices (*i.e.* α6- α 10), folding into a five-helix bundle (Fig. 2A). The terminal α10 helix of the STIM1 SAM domain contains two hydrophobic anchor residues (*i.e.* L195 and L199) which pack into the EF-hand domain hydrophobic cleft. While both EF-hand and SAM domains are common components of myriad other proteins in higher and lower order eukaryotes, the intimate interaction between the EF-hand and SAM domain which occurs in the presence of Ca^{2+} is relatively unique in nature [1,32,33].

The tertiary structure of the Ca^{2+}-bound EF-SAM domain creates a surface electrostatic potential that is primarily acidic in nature [27,28]. The EF-hand domain contributes a majority of the acidic surface electrostatic potential through an abundance of anionic residues. It is tempting to speculate that the acidic surface potential is involved in longer-range electrostatic guidance of Ca^{2+} ions.

While the STIM EF-SAM sequence is highly conserved with STIM2, some notable differences exist which contribute to distinctions in structure and function. First, the largest helix (*i.e.* α10) in the SAM domain of STIM2 contains an additional hydrophobic residue (*i.e.* Val201). This Val201 packs into the STIM2 EF-hand hydrophobic cleft (Fig. 2B). The STIM2 EF-hand domain also has more residues making up the cleft which interacts with the α10 helix compared to STIM1 [28]. Finally, the STIM2 SAM domain structure reveals more residues packed into the non-polar core. Thus, the enhanced hydrophobic cleft size, increased number of hydrophobic residues packing within the SAM domain core and augmented number of SAM domain α10 anchor residues interacting with the EF-hand cleft all contribute to an enhanced STIM2 EF-SAM domain stability in the presence of Ca^{2+} compared to STIM1 (Fig. 2B). This stability difference is further complemented by a strong potential for ionic bond formation between the Asp200 in the SAM α10 helix which is in close proximity of the Lys103 located on the EF-hand. This structural feature appears unique to STIM2 as the Lys103 aligns with a His99 in STIM1 [28]. Nevertheless, it is interesting to speculate that the His99 of STIM1 may confer a pH sensitivity to the EF-SAM domain, where decreases in pH may protonate the His99, and thereby promote an ionic bond with the Asp196 of the STIM1 SAM domain.

No high-resolution structural data is currently available on the Ca^{2+}-depleted conformation of the STIM luminal domains. Nevertheless, biophysical and low-resolution analyses show that both STIM1 and STIM2 EF-SAM domains dimerize and/or oligomerize upon Ca^{2+} depletion [26–28,33]. This oligomerized state is in contrast to the strict monomers detected for the EF-SAM domains in isolation. Critically, the Ca^{2+}-depletion dependent oligomerization event results in a change in the orientation of the dimerized TM domains of STIM molecules [34,35], propagating downstream conformational changes through the cytosolic domains required for coupling to Orai1 and activation of SOCE [36–40]. The structural mechanisms underlying these conformational changes in Orai1 channel activation are discussed in more detail below.

2. STIM cytosolic domain structure and function

The C-terminal cytosolic segments of STIMs facilitate the translocation of STIM oligomers to PM-ER junctions where they can directly bind to Orai1 and activate CRAC channels [5,41–45]. The cytosolic architecture is composed of three putative coiled-coil (CC) domains downstream of the single-pass TM helix; there are also two distally located Ser/Pro and Lys-rich domains near the carboxyl terminus (Fig. 1). Previous studies have shown that STIMs lacking the Ca^{2+} sensing luminal N-terminal region are able to induce SOCE independent of the ER Ca^{2+}-sensing mechanism [43–46], indicating that the cytosolic domains contain the structural machinery required to elicit Orai1-mediated Ca^{2+} entry. While the entire cytosolic region of STIM1 in isolation can activate Orai1 [37,38,46,47], specific domains regulate interactions with the PM [6,48–50], oligomerization [37,40], maintaining the quiescent conformation [35,36,38], binding to Orai1 and gating the channel [44,51–53].

The Orai-activating STIM fragment (OASF; residues 234–450/474) contains the structures needed to activate SOCE. OASF encompasses the three CC domains (*i.e.* CC1, CC2, and CC3) [38] (Fig. 1). Contained within the OASF fragment are the STIM-Orai activating region (SOAR; residues 344–442) [45] and the CRAC-activating domain (CAD; residues 342–448) [44] which include the CC2 and CC3 domains. In the absence of ER Ca^{2+} store depletion, CC2-CC3 (*i.e.* CAD/SOAR) expressed in cells can maximally activate Orai channels [43–45]. While these two CC domains are requisite for activation of Orai currents, a unique function of the CC1 domain exists. Co-clustering of CC1 (residues 238–343) alongside OASF inhibits Orai activity [54,55]. Thus, CC1 is vital to maintaining the OASF region in a quiescent state [36], while the minimal requirements for the generation of Orai1 currents are located within the CC2 and CC3 structural domains.

Insights into the high resolution structure of human STIM1 SOAR have begun teasing out the molecular basis for cytosolic STIM1 domain function. The crystal structure of human SOAR, containing a triple mutation to make crystallization tractable, is comprised of four α-helices. CC2 forms a long extended α -helix, while CC3 is broken into three smaller helices (Fig. 3). Specifically, CC3 forms two short α-helices which position the third longer α-helix in an antiparallel orientation relative to CC2 [56]. Thus, the four helices form an "R"-shaped subunit with both the N- and C-termini adjacent to one another [56]. Two "R" shaped monomers are arranged into a "V"-shaped dimer (Fig. 3). The dimer structure is promoted by hydrophobic interactions, H-bonding and aromatic stacking interactions. At the apex of the "R" shaped structure are a series of basic and other residues which play an important role in binding to Orai1 as mutation disrupts co-localization with Orai1 and SOCE activation [39,56–59].

Although SOAR includes both CC2 and CC3 domains, the regions needed to maximally activate Orai1 currents, the structural interplay between CC1, CC2 and CC3 are fundamental to switching STIM1 between a quiescent conformation and the activated state necessary to bind and gate Orai1 channels [35,36,38,39]. A solution NMR structure of the human CC1-CC2 region encompassing residues 312–387 provided insights into the interplay between these CC regions as well as the

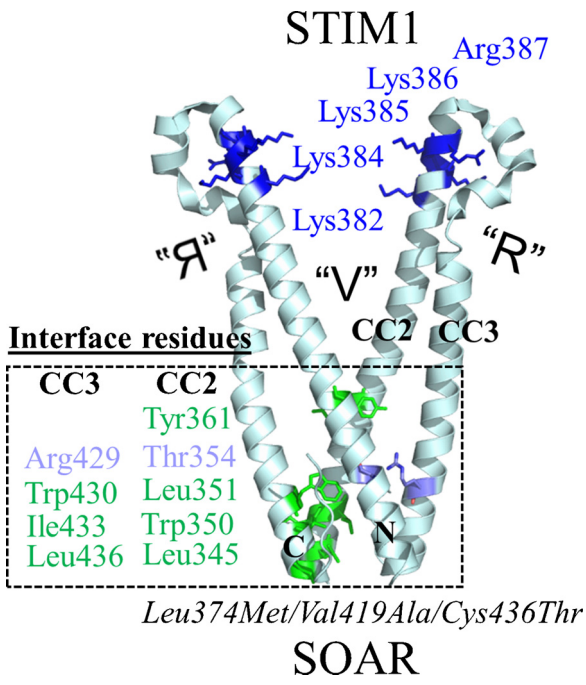

Fig. 3. High resolution structure of the human STIM1 Leu374Met/Val419Ala/Cys436Thr SOAR domain.The relative orientations of CC2 and CC3 making up the "R"-shaped monomers are indicated on the X-ray crystal structure of the SOAR domain. The manner in which two "R"-shaped monomers assemble into a "V" shaped dimer is shown. Basic residues on CC2 involved in binding to Orai1 are shown as blue sticks. The polar (light blue), non-polar (green) and Tyr-stacking (green) interactions stabilizing the dimer are shown as sticks. The dashed black box surrounds the list of specific residues and the region involved in dimerization. The coordinates are from 3TEQ.

coupling mechanism with Orai1 subunits [39]. The structure of human CC1-CC2 is comprised of two extended α-helices. The two α -helices within each monomer are connected by a short loop; further, they are arranged in an anti-parallel manner, thereby forming "U" shaped subunits (Fig. 4A). In solution, these subunits form a homodimer after assembly in an antiparallel manner. There are three protein-protein interaction interfaces which stabilize this conformation of the dimer. First, canonical coiled-coil interactions can be observed between CC1 helices on each subunit. Second, coiled-coil interactions occur between CC2 helices, also in a canonical fashion. Finally, the loop region of each monomer contacts the C-terminal region of the partner CC2 helix (Fig. 4A).

The dimer conformation observed for the CC1-CC2 solution structure appears primed for interaction with human Orai1 based on the solution NMR structure of the CC1-CC2 dimer in complex with two Orai1 C-terminal helices (*i.e.* residues 272–292) [39]. Specifically, the antiparallel orientation of the CC2 helices creates a STIM-Orai association pocket (SOAP) after a separation of these helices (Fig. 4B). Separation can occur without disruption of the dimer due to maintenance of the CC1 intermolecular interactions between CC1-CC2 subunits. The importance of these intermolecular CC1 interactions is evident from mutations which stabilize the CC1 domain and spontaneously activate Orai1 currents; in contrast, mutations disturbing the CC1:CC1' interaction suppress Orai1 channel activity [39]. The SOAP region reveals that both hydrophobic and electrostatic interactions are involved in coupling with the Orai1 C-terminal helices (Fig. 4B). The SOAP confers an electropositive surface potential which is complementary to the highly acidic nature of the Orai1 C-terminal helices. Further, both the SOAP and the Orai1 C-terminal helices contain a series of hydrophobic residues which are also important for promoting the heteromeric interaction. Thus, both electrostatic and non-polar interactions are vital for STIM1 coupling to Orai1. Importantly, the STIM1:Orai1 complex structure indicates a mechanism of Orai1 channel activation that is driven by a dynamic movement of the cytosolic C-terminal Orai1 helices when binding into the SOAP, since the *Drosophila melanogaster* Orai C-terminal helix conformations exhibited in the crystal structure are analogous, but not identical, to the human complex structure [39,60].

The CC1 domain appears to play multiple important structural roles in the mechanism of STIM1 function. A double mutant crystal structure of residues 237–310 shows that this region of STIM1 can form a long and extended helix of ~13 nm (Fig. 4C), which must be vital to bridging the physical gap between the ER and PM membranes where STIM1 and Orai1 are inserted, respectively [61]. Nevertheless, the structural interactions of CC1 with CC2-CC3 (*i.e.* SOAR) have also been shown to maintain the entire STIM1 cytosolic domain in a compact conformation [35,36,38,62]. Further, dissection of the STIM1 cytosolic domain components and interactions assessments in mammalian cells have revealed that CC1 and CC3 exhibit the strongest interaction between all CC domains and represent the basis for the structural clamp which prevents the exposure of the CAD/SOAR region [36]. Thus, CC1 *i*) interacts with CC3 to clamp the STIM1 cytosolic domains in a quiescent state, *ii*) adopts an extended helical conformation to bridge the ER-PM gap and *iii*) self-interacts to promote this extension and present the Orai1 binding site (*i.e.* SOAP) close to the PM.

It is important to note that regions downstream of the CC3 domain also play a role in inhibiting STIM1 activity and SOCE. The 14-amino acid sequence located immediately C-terminal to CC3 (*i.e.* residues 449–462) in STIM1 inhibits clustering and activation of STIM1. STIM1 constructs containing these additional 14 amino acids C-terminal to the CAD/SOAR domain (residues 1–462) inhibit oligomerization, whereas constructs without these 14 amino acids (residues 1-448) do not prevent oligomerization and adopt an extended conformation, as assessed using fluorescence resonance energy transfer microscopy [47]. Previous work also identified a region between residues 474–485 (*i.e.* "ID", see Fig. 1) which is enriched with negatively charged amino acids and may associate with Ca^{2+} to inhibit the STIM1-Orai1 interaction [37,63].

Thus, the STIM cytosolic C-terminal region controls both the activation state of STIM and Orai channel activity through a dynamic interplay of multiple specific structural interactions.

3. Post-translational modifications to STIM structure and function

Proteins which mediate cell signaling cascades are often able to adopt multiple structural conformations which promote distinct functions. Post-translational modifications can play a pivotal role in controlling both intra- and inter-molecular interactions associated with biological function [64]. The CAD/SOAR domain of STIMs can undergo phosphorylation events which impact both structure and function (Table 1). STIMs mediate both store-dependent CRAC channel as well as store-independent arachidonic acid regulated Ca^{2+}-selective (ARC) channel activations [65,66]. However, the underlying mechanism by which STIMs differentially activate these two processes was unknown until the discovery that phosphorylation of Thr389 by protein kinase A (PKA) induces structural changes that leads to the selective activation of ARC channels [67]. Using molecular dynamics simulations and optical spectroscopy, it was demonstrated that phosphorylation of Thr389 in STIM1 induces a helical extension of the C-terminal region of the CC2 domain, resulting in an increased stability of the helix [59]. It is this structural conformation mediated by Thr389 phosphorylation that establishes STIM1 specificity in activating ARC channels over CRAC channels.

Tyrosine phosphorylation of STIM1 has been identified and may be required for STIM1 to couple to Orai1 in platelets [68]. Furthermore, STIM1 phosphorylation has been detected at Tyr361 of the CAD/SOAR region in endothelial cells (Table 1). This phosphorylation is mediated by proline rich kinase 2 (Pyk2) following ER Ca^{2+} store depletion [69]. Interestingly, when this phosphorylation site was conservatively

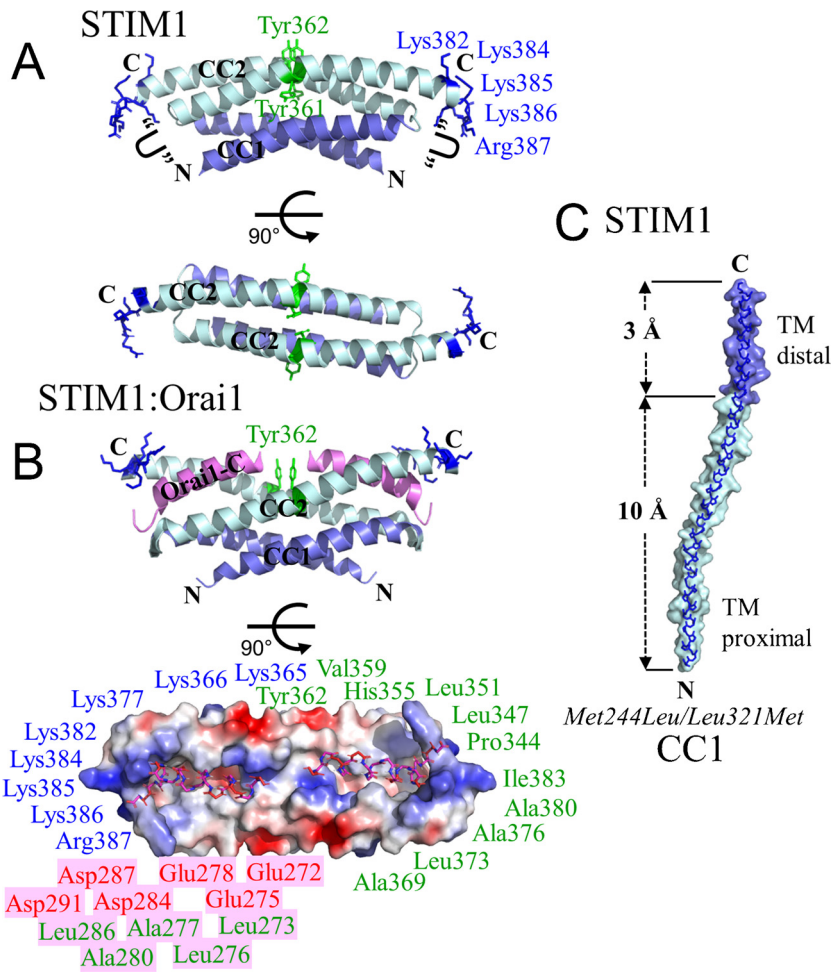

Fig. 4. High resolution structures of the human STIM1 CC1 and CC1-CC2 fragments. *A.* Solution NMR structure of the human STIM1 CC1-CC2 dimer. The relative location of CC1 (slate blue cartoon ribbons) and CC2 (cyan cartoon ribbons) making up the "U"-shaped monomers are labeled. Two "U" shaped monomers assemble in an antiparallel fashion to create the dimer. ***B.*** Solution NMR structure of the human STIM1 CC1-CC2 dimer in complex with two human Orai1 C-terminal peptides (magenta cartoon ribbons). The electrostatic surface potential of the CC1-CC2 dimer is shown below [-2 (red) to + 2 (blue) kT/e] with the two Orai1 C-terminal peptides (main-chain stick representations) packing into the SOAP. The basic (blue) and hydrophobic residues (green) making up the SOAP are labeled. The complementary acidic (red) and non-polar residues (green) of the Orai1 C-terminal peptides are labeled on magenta background text. In *A* and *B*, basic residues on CC2 involved in binding to Orai1 are shown as blue sticks. The central Tyr interactions are depicted as green sticks. ***C.*** X-Ray crystal structure of the human STIM1 Met244Leu/Leu321Met CC1 region. The elongated helical structure is illustrates as a main-chain ribbon (blue) on the surface representation. The distances traversed by this extended CC1 are shown. In *A, B* and *C*, the corresponding CC1 regions are coloured slate blue to match. The coordinates for CC1-CC2, CC1-CC2:Orai1 and CC1 are 2MAJ, 2 MA K and 4O9B, respectively.

mutated (*i.e.* Tyr361Phe), STIM1 was able to oligomerize, but unable to recruit Orai1 subunits, thus preventing activation of the SOCE pathway [69]. It is noteworthy that Tyr361 forms self-stacking interactions in the CAD/SOAR crystal and CC1-CC2 NMR dimer interfaces. Thus, Tyr361 phosphorylation may affect the dimer architecture of the critical CAD/SOAR region, and thereby interactions with Orai1.

Outside CAD/SOAR, STIM1 is phosphorylated by extracellular-signal-regulated kinase 1 and 2 (ERK1/2) at Ser575, Ser608 and Ser621, as observed in HEK293 cells [70] (Table 1). When ERK1/2 phosphorylation sites were mutated in STIM1 (*i.e.* Ser575Ala/Ser608Ala/Ser621Ala), the apparent binding affinity of Orai1 was reduced, thereby suppressing SOCE in HEK293 cells [70]. Ser486 and Ser668 have also been identified as mitosis-specific phosphorylation sites [71] (Table 1). Mitotic cells co-expressing Orai1 and fluorescently tagged STIM1 Ser486Ala/Ser668Ala displayed higher levels of SOCE when compared to the same mitotic cells expressing wildtype STIM1 [71]. Thus, site-specific phosphorylation of STIM1 can either promote [Tyr361, (Ser575, Ser608, Ser621)] or inhibit (Thr389, Ser486, Ser668) SOCE, thereby adding another layer of regulation to STIM1 function.

Cysteine *S*-glutathionylation is the covalent and reversible attachment of a tripeptide glutathione moiety to a cysteine thiol. *S*-Glutathionylation is commonly promoted under oxidative stress and is implicated in redox signaling [72,73]. In response to oxidative stress, STIM1 undergoes *S*-glutathionylation near the conserved luminal Ca^{2+} sensing domain (Table 1). Specifically, after subjecting DT40 cells to oxidative stress, STIM1 was found to be *S*-glutathionylated at Cys56; moreover, the modification at this position constitutively activated Ca^{2+} entry *via* CRAC channels, independent of ER luminal Ca^{2+} concentrations [74]. While STIM1 is typically activated when intracellular ER Ca^{2+} levels are depleted, *S*-glutathionylation of Cys56 decreased the affinity of the immediately downstream canonical EF-hand for Ca^{2+}, thereby leading to STIM1 activation and SOCE even under replete ER

Table 1
Summary of detected post-translation modifications on STIM1.

modification	location on STIM1[a]	localization in the cell	effect on SOCE	references
S-glutathionylation	Cys56 – N-terminal variable	ER lumen	enhances	[74]
N-glycosylation	Asn131 – SAM domain	ER lumen and extracellular space	enhances	[1,2,66,79–82]
N-glycosylation	Asn171 – SAM domain	ER lumen and extracellular space	enhances	[1,2,66,79–82]
O-glycosylation	Ser/Thr – P/S	cytosol	suppresses	[84]
phosphorylation	Tyr361 – SOAR/CAD	cytosol	enhances	[68,69]
phosphorylation	Thr389 – SOAR/CAD	cytosol	suppresses	[59,67]
phosphorylation	Ser486 – OASF, Ser668 – P/S	cytosol	suppresses	[71]
phosphorylation	Ser575, Ser608, Ser621 – P/S	cytosol	enhances	[70]

[a] See Fig. 1A for relative location in sequence space and on STIM1 domain architecture.

luminal Ca^{2+} conditions [74]. Given the ability of this Cys residue, conserved among vertebrates, to undergo modification under oxidative conditions, it is tempting to speculate that free thiols in this luminal region of STIM1 may undergo other modifications under various environmental stresses (*e.g. S*-nitrosylation *via* nitric oxide).

N-linked glycosylation is the covalent attachment of an oligosaccharide to the amide nitrogen of an asparagine residue *via* a series of enzymatic reactions in the ER and Golgi. *N*-glycosylation is distinct from *O*-glycosylation which occurs on the side chain hydroxyl groups of Ser or Thr residues [75]. A minor fraction of STIM1 is located on the PM with the EF-SAM domain directed into the extracellular space [1,2,76–78]. This cell surface targeting is mediated by *N*-glycosylation of Asn131 and Asn171 [1,2,66,79–81]. Both these Asn residues are located within the EF-SAM domain, and recent solution NMR approaches have shed light into the structural mechanisms of regulation *via* modifications at these sites [82,83]. Site-selective covalent attachment of glucose at Asn131 and Asn171 in isolated STIM1 EF-SAM resulted in a reduced Ca^{2+} binding affinity, concomitant loss in stability and enhanced oligomerization propensity of the domain; moreover, the two simultaneous modifications were found to cause structural changes which pervaded through the EF-SAM core [82]. Consistent with these structural and biophysical alterations to EF-SAM, blocking *N*-glycosylation in full-length STIM1 *via* Asn131Gln/Asn171Gln resulted in diminished SOCE in HEK293 cells [82]. Interestingly, STIM1 can also *O*-link N-acetylglucosamine, resulting in suppressed SOCE [84]. The likely target of this modification is the STIM1 cytosolic domains, given that *O*-glycosylation typically takes place in the cytosol or nucleus and the cytosolic region of STIM contains a PEST sequence (*i.e.* rich in Pro, Glu, Ser, Thr) [85] which has high *O*-linked modification propensity [84].

4. Concluding remarks

Current high resolution structural data on STIM1 and STIM2 have unravelled key mechanisms underlying the function of these Ca^{2+} sensors which are essential for activating PM Ca^{2+} channels. While most of the structural work has focused on STIM1, the mechanistic analogies as well as distinctions in specific STIM2 Ca^{2+} sensing function has been revealed by solution NMR. Namely, hydrophobic and charged interaction reinforcements within STIM2 EF-SAM enhance the stability of the auto-inhibited conformation compared to STIM1, endowing this homologue with a suppressed propensity for oligomerization. Structural work on the CC domains of human STIM1 and lower order orthologues has established that an intricate interplay between CC1, CC2 and CC3 are involved in cycling the cytosolic domains from a quiescent conformation to an active state, primed for interaction with PM Orai1 proteins. The fundamental mechanisms underlying STIM function are fine-tuned by numerous post-translational modifications on both sides of the single TM domain. *N*-Glycosylation and *S*-glutathionylation affect Ca^{2+} binding affinity and stability of STIM1 EF-SAM; further, Tyr/Ser/Thr phosphorylation can either enhance or inhibit the coupling of the STIM1 to Orai1, depending on the specific residue modification within the STIM1 cytosolic domains.

Collectively, the work to date shows that the STIM function is not only dictated by its structural composition, but also by the influences of local environmental factors. Oxidative stress, localization and activity of modifying enzymes ultimately play important roles in regulating STIM structure and function. Thus, future structural targets should include both post-translationally modified and unmodified versions of the protein to fully appreciate the underlying mechanisms.

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