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Immuno-proteomic approach to excitation–contraction coupling in skeletal and cardiac muscle: Molecular insights revealed by the mitsugumins

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Abstract

A comprehensive understanding of excitation–contraction (E–C) coupling in skeletal and cardiac muscle requires that all the major components of the Ca^{2+} release machinery be resolved. We utilized a unique immuno-proteomic approach to generate a monoclonal antibody library that targets proteins localized to the skeletal muscle triad junction, which provides a structural context to allow efficient E–C coupling. Screening of this library has identified several mitsugumins (MG); proteins that can be localized to the triad junction in mammalian skeletal muscle. Many of these proteins, including MG29 and junctophilin, are important components in maintaining the structural integrity of the triad junction. Other triad proteins, such as calumen, play a more direct role in regulation of muscle Ca^{2+} homeostasis. We have recently identified a family of trimeric intracellular cation-selective (TRIC) channels that allow for K^{+} movement into the endoplasmic or sarcoplasmic reticulum to counter a portion of the transient negative charge produced by Ca^{2+} release into the cytosol. Further study of TRIC channel function and other novel mitsugumins will increase our understanding of E–C coupling and Ca^{2+} homeostasis in muscle physiology and pathophysiology.

Keywords

E–C coupling; Triad junction; Dyad junction; Skeletal muscle; Cardiac muscle; Counter ion

1. Introduction

Ca^{2+} ions are important second messengers in many cellular signal transduction pathways. Two principle sources provide Ca^{2+} to the cell: channels in the plasma membrane (PM) that open to allow external Ca^{2+} to enter the cell, and internal stores sequestered in the endoplasmic reticulum (ER) or sarcoplasmic reticulum (SR) that release Ca^{2+} . Junctional membrane complexes between PM and ER/SR are present in all excitable cells, providing effective mechanisms for crosstalk between Ca^{2+} -dependent channels/transporters on the plasma membrane and Ca^{2+} release channels on intracellular membranes [1].

Coupling of electrical excitation across the PM to intracellular Ca^{2+} release from the SR and subsequent muscle contraction (E–C coupling) occurs in the junction between the transverse-tubular (TT) invagination of PM and the terminal cisternae of SR (“triad junction”), where two major protein components are located: the dihydropyridine receptors (DHPR) function as voltage sensor of the TT membrane, and the ryanodine receptors (RyR) function as the Ca^{2+} release channel in the SR [2–6]. In skeletal muscle, the triad junction provides the structural framework for interaction between DHPR and RyR and mediates direct signal transduction of voltage-induced Ca^{2+} release (VICR) [7–9]. A process called Ca^{2+} -induced Ca^{2+} release (CICR) is essential for SR Ca^{2+} release in cardiac muscle, and also plays an important role by amplifying Ca^{2+} release in mammalian skeletal muscle, particularly under stress conditions in muscle aging, fatigue and dystrophy. Extensive research has demonstrated that maintenance of an intact triad junction in skeletal muscle is not only essential for the efficient operation of VICR and CICR, but also important for maintaining a tight control of Ca^{2+} homeostasis inside the SR.

It is well known that during the acute phase of VICR or CICR, the rapid flux of Ca^{2+} across the SR membrane generates a transient negative potential within the SR lumen. Movement of ions across the SR membrane to counter this negative potential has been implicated in the efficient operation of VICR in skeletal muscle and CICR in cardiac muscle [10–15]. Although channels selective for monovalent cations have been reported in SR membrane [10,14,16–18], until recently no gene has been identified that encodes an ER/SR localized cation-selective channel.

2. Immuno-proteomic approach to resolve important E–C coupling components

In an attempt to identify novel components of the E–C coupling machinery, we have used a combination of monoclonal antibody (mAb) immunohistochemistry, cDNA library screening and gene knockout techniques (see Fig. 1), to identify a group of novel proteins termed mitsugumins (MG), Japanese for triad proteins, that in skeletal and cardiac muscle are generally localized to the triad and dyad junctions, respectively.

Our approach involved developing an immuno-proteomic monoclonal antibody library from mice immunized with triad- or T-tubule-enriched membranes from rabbit skeletal muscle. These mice were used to prepare hybridoma cells that produce a monoclonal antibody library against antigens present in the triad junction of skeletal muscle. Antibodies of interest were selected based on the A–I junction staining patterns in striated muscle sections under an immunofluorescence microscope. The target-proteins were purified through antibody-affinity column, and partial amino acid sequences of the purified proteins were obtained. Based on the partial amino acid sequence, the complete cDNA coding for the target gene was isolated from a skeletal muscle cDNA library. Homologous gene screening was then used to search for the presence of different isoforms of the identified genes in other excitable tissues. Finally, transgenic or knockout mouse models were generated to study the *in vivo* physiological function of genes of interest. This approach has led to the discovery of several novel genes with important roles in Ca^{2+} signaling and the physiological function of striated muscle (Fig. 2).

As this approach has proved to be effective in the study of proteins found in the triad junction of skeletal muscle there is potential that a similar immuno-proteomic library could be generated for other cell types. The only requirement is that sufficient quantities of highly purified protein can be isolated to produce an immune response in a mouse. However, screening of such a library requires a dedicated effort as one can expect to detect a number of known proteins in the process of screening for novel proteins.

3. MG29, a synaptophysin-like protein with roles in E–C coupling and aging

One protein isolated from this immuno-proteomic library is MG29, a synaptophysin-related membrane protein that is essential for the development and maintenance of triad structure in skeletal muscle [19–26]. Abnormalities of membrane ultrastructure around the triad junction were detected in skeletal muscle from the *mg29*($-/-$) mice: the TTs were swollen and sometimes missing from the A–I junction, and the SR networks were poorly formed with vacuolated and fragmented structures, leading to misalignment of triad junctions. Treadmill exercise and muscle contractility measurements revealed a distinct phenotype of the *mg29*($-/-$) mice, e.g. the mutant mice fails to complete an endurance test and their skeletal muscles exhibit increased susceptibility to fatigue [27]. In further studies, we discovered a dysfunctional property of store-operated Ca^{2+} entry (SOCE) in the *mg29*($-/-$) muscle and established a functional role of SOCE in muscle fatigue [22,27,28]. We also identified a functional interaction between MG29 and RyR, through co-expression of MG29 and RyR in heterologous cells and reconstitutions studies of RyR in lipid bilayer membranes [29].

Recently, our studies suggest that MG29 act as a sentinel during muscle aging, as MG29 expression in skeletal muscle decreases during aging, and that young *mg29*($-/-$) muscle shows dysfunction in skeletal muscle Ca^{2+} handling that is similar to that seen in aged wild type muscle [30]. Aged wild type skeletal muscle appears to have a fragmented SR Ca^{2+} store, which results in segregation of a portion of stored Ca^{2+} away from the normal E–C coupling process. Additionally, the plastic Ca^{2+} spark response observed following stress in young, healthy muscle is severely abrogated in aged skeletal muscle [31]. Both of these phenotypes are also observed in young *mg29*($-/-$) muscle, however they are not present in young wild type muscle. Additional structural defects, including disruption of the triad junction complex, can be observed in aged wild type and young *mg29*($-/-$) mouse muscle. The complementary phenotype observed in muscle tissue with decreased MG29 expression supports the conclusion that MG29 expression is essential to maintain skeletal muscle triad junction structural integrity and normal Ca^{2+} handling in skeletal muscle physiology and aging.

4. Junctophilins span the cytoplasmic space in junctions to link the PM to ER/SR

Another important class of genes isolated from our immuno-proteomic library are related to MG72, also known as the junctophilins (JP), which possesses a unique primary structure with a large cytoplasmic region and a C-terminal transmembrane segment spanning the SR/ER [32]. The cytoplasmic region of JP contains repeated motifs of 14 amino acid residues (termed “MORN motifs”) and exhibit selective binding affinity to the plasma membrane. A proposed α -helical rich domain lies between the amino-terminal MORN motif and carboxyl-terminal transmembrane segment in JP. Therefore, JP possesses the property of physically linking the TT and SR membrane, allowing the formation of triad junctions in skeletal muscle [32–35] and other junctional complexes in different cell types. There are four junctophilin genes, which all display some degree of tissue specificity in their expression pattern (Fig. 3). Junctophilin-1 is primarily expressed in skeletal muscle, while junctophilin-2 is expressed throughout muscle cell types. Junctophilin-3 and -4 are expressed in neuronal tissues and appear to be involved in coupling of ER to PM to allow for excitability of neurons [36–38].

Genetic ablation or siRNA-mediated suppression of JP-1 and JP-2 leads to uncoupling of the triad and dyad junction and abnormal intracellular Ca^{2+} homeostasis in both skeletal and cardiac muscle cells. Our collaborative studies have also demonstrated that preservation of an intact triad junction is essential for the maintenance of quiescent Ca^{2+} release machinery

during the resting state of skeletal muscle contraction, as well as for the efficient control of VICR during the activation process of muscle contraction and for the coordinated activation of SOCE to maintain the long-term Ca^{2+} homeostasis in muscle cells [39].

An increasing body of evidence links junctophilin genes with various human diseases as several junctophilin mutations have been linked with both muscle and neurological disorders. Recent studies have identified mutations in the human JP-2 gene that lead to hypertrophic cardiomyopathy (HCM) and consequences in cellular Ca^{2+} handling [40]. Other JP-2 mutations in a separate cohort suggest that polymorphisms in the JP-2 gene that link to HCM may be common in various populations [41]. Additional studies have also established that JP-2 levels are down-regulated in mouse models of hypertrophic and dilated cardiomyopathies [42]. Decreases expression of JP-2 has also been observed in aortic stenosis rat models of compensated and decompensated hypertrophy, a modification that results in desynchronized CICR at early stages of cardiac compensation before the progression into heart failure [43]. These studies present a strong case that JP-2 is an important component to maintain the morphological integrity of the dyad for establishing efficient E-C coupling in cardiac muscle, and that disruption of junctophilin function can result in severe pathology. This is the case beyond the cardiovascular system, as several mutations in JP-3 have been linked to the development of Huntington's Disease type 2 [44–46].

5. Calumin, a structural homolog of SOCE regulator STIM1

Our efforts recently identified calumin, a novel high capacity Ca^{2+} binding protein with moderate affinity for Ca^{2+} that is highly conserved in multi-cellular eukaryotes [47]. Calumin contains a single transmembrane domain that spans the ER membrane in many different cell types, with a highly acidic, amino-terminal Ca^{2+} binding domain within the lumen of the ER and a long carboxy-terminal domain on the cytoplasmic side that contains a putative coiled-coil region (Fig. 4). This configuration of domains lead to the hypothesis that this protein may act as a sensor of Ca^{2+} concentration within the ER lumen. Knockout mice for calumin die *in utero* or shortly after birth, implying calumin is an important component of that machinery controlling Ca^{2+} homeostasis in a variety of tissues. Embryonic fibroblasts from these calumin-null mice showed increased cell death in response to stress, reduced Ca^{2+} storage within the ER and compromised SOCE.

The observation that calumin ablation decreases SOCE function has interesting implications in the context of calumin's putative role in sensing ER lumen Ca^{2+} concentration. Calumin shares a similar domain configuration to STIM1 (Fig. 4), an ER resident protein of great interest in the SOCE field that senses ER Ca^{2+} store depletion [48]. Following store depletion, STIM1 translocates [49,50] to activate the channel protein Orai-1/CRACM [49,51] and allow entry of Ca^{2+} into the cell to replenish the depleted SR Ca^{2+} store [52]. STIM1 has also been shown to activate TRPC channels that may also mediate SOCE in some cell types [53–55]. Considering the similarities in domain configuration between STIM1 and calumin, and the reduced SOCE in *calumin*($-/-$) cells, it is possible that under certain conditions calumin may play a role similar to that of STIM1, perhaps interacting with other Orai isoforms that display less robust activation by STIM1, such as Orai-2 and Orai-3 [56]. Further investigation should resolve how calumin affects SOCE in various cell types, and any partner proteins that function with calumin to regulate cellular Ca^{2+} homeostasis.

6. TRIC channel, a trimeric intracellular cation-selective channel in ER/SR membrane

Continuing our immuno-proteomic approach, we have recently identified a new protein, MG33, and named the gene trimeric intracellular cation-selective channel type A (TRIC-A) (Fig. 2). TRIC-A mRNA is preferentially expressed in excitable tissues, particular within striated muscle and brain. Biochemical analysis and electron microscopy reveals that TRIC-A is specifically distributed throughout the SR and nuclear membrane in skeletal muscle, without the protein appearing on the PM.

Homology searching of protein databases indicated a structural homologue, namely TRIC-B, was present in several species. These two TRIC subtypes display only a minimal homology at the amino acid residue level, however the same protein domains are present in the same order in these TRIC subtypes. Hydrophobicity analysis and biochemical assays establish that the amino- and carboxyl-terminus of TRIC appear on the luminal and cytoplasmic sides, respectively, of the ER/SR membrane. Such analysis further suggests that TRIC subtypes contain three membrane-spanning domains. A putative cytoplasmic loop between the first and second transmembrane segments is enriched in hydrophobic residues that would allow this loop to incorporate into the SR/ER membrane to form an ion-conducting pore, resembling the overall structure of glutamate receptor channels [57].

Using highly purified TRIC-A preparations, we found that chemical cross-linking reagents can produce dimeric and trimeric complexes. Using gold-labeled Fab fragment recognizing the carboxyl-terminus of TRIC-A, we observed that the antigen-antibody complexes frequently display tripartite patterns that converge at near equal angles in the complex. Reconstruction of the three dimensional volume of purified TRIC-A particles from electron microscopy images reveal a clear threefold symmetric structure bearing a putative central pore, characteristics reminiscent of other recently reported trimeric ionic channels [58,59].

Considering the putative pore containing structure of TRIC-A, we tested if this protein could form functional ion channels using the lipid bilayer reconstitution system. Indeed, functional monovalent cation-selective channel could be observed with the native TRIC-A protein purified from rabbit skeletal muscle. While the lipid bilayer method is highly effective at resolving channel moieties, it does select for functional channels within a protein preparation even if these result from minor impurities within the preparation. Considering that minor impurities in our TRIC-A preparations from skeletal muscle could complicate our single channel measurements and subsequent data, we developed a protocol for expression and purification of TRIC-A in *E. coli*. TRIC-A protein isolated from *E. coli* can recapitulate channels with characteristics identical to those produced by native TRIC-A preparations from rabbit muscle. Additionally, both recombinant and native channels could be blocked by TRIC-A monoclonal antibody against TRIC-A. Thus, these channel activities are unlikely to result from minor impurities in TRIC-A protein preparations.

7. Role for TRIC in skeletal and cardiac E-C coupling

As a first step toward understanding the physiological function of TRIC subtypes, we have produced knockout mice carrying deletion of either *TRIC-A* or *TRIC-B*. Perhaps not unexpected due to its ubiquitous expression pattern, homozygous ablation of *TRIC-B* is lethal; the *TRIC-B*(-/-) mice died at the neonatal stage, likely due to respiratory defects. Interestingly, mutant mice lacking the TRIC-A subtype, *TRIC-A*(-/-), survive into adulthood. Further studies suggest a complementary function for the two TRIC subtypes in cardiac muscle, as aggravated embryonic lethality is apparent in the *TRIC-A*(-/-)*TRIC-B*(-/-) that die *in utero* at E9-E10, due to cardiac arrest. Ultrastructural analysis detected that

SR/ER elements in *TRIC-A(-/-)TRIC-B(-/-)* cardiomyocytes become swollen toward the terminal asystole, suggesting Ca^{2+} -overloading of intracellular stores.

Overloading of the SR Ca^{2+} store is also evident in measurements of caffeine stimulated Ca^{2+} release. Following exposure to caffeine, isolated *TRIC-A(-/-)TRIC-B(-/-)* embryonic cardiomyocytes display greater Ca^{2+} release than wild type myocytes. While spontaneous cytoplasmic Ca^{2+} oscillations in *TRIC-A(-/-)TRIC-B(-/-)* cardiomyocytes have a significantly depressed amplitude compared to wild type cells. This phenotype is suggestive that CICR is significantly compromised in *TRIC-A(-/-)TRIC-B(-/-)* embryonic cardiomyocytes due to reduced activity of RyR2 [60]. Considering that TRIC subtypes are localized to membrane systems associated with intracellular Ca^{2+} stores and that the purified TRIC protein can form a cation-selective channel in lipid bilayer membrane, we hypothesized that TRIC could provide certain aspects of the counter-ion movement mechanism during the acute phase of Ca^{2+} release in muscle and potentially in other cell types.

To further test TRIC function in counter ion movement across the SR, we examined Ca^{2+} signaling in adult skeletal muscle from viable *TRIC-A(-/-)TRIC-B(-/+)* mice. Ca^{2+} sparks are elementary events of RyR-mediated Ca^{2+} release from SR in striated muscle cells. Ever since the discovery by Cheng et al. of Ca^{2+} sparks in heart muscle [61], there has been a general belief that these localized Ca^{2+} release events rarely appear in intact adult mammalian skeletal muscle. Our laboratory recently discovered a measurement of Ca^{2+} sparks in intact adult mammalian skeletal muscle [62,63]. We found that subtle membrane-deformations induced by an osmotic shock of the isolated muscle fiber leads to fluttered Ca^{2+} sparks and Ca^{2+} bursts, that are confined to the peripheral region of the muscle fiber. To test the contribution of TRIC-A to the elemental Ca^{2+} spark signal in adult skeletal muscle, we tested the Ca^{2+} spark response in *TRIC-A(-/-)TRIC-B(+/-)* muscle fibers. We observed decreased amplitude and increased duration of Ca^{2+} spark in the mutant muscle. These observations suggest that the TRIC deficiency results in impaired Ca^{2+} release through RyR1, leading to reduced Ca^{2+} -dependent inactivation mechanism of RyR1 and prolongation of its channel opening at the level of the individual Ca^{2+} release unit [3,64].

While our findings in TRIC mutant skeletal and cardiac muscle support a role for TRIC channels in SR ionic permeability, we directly tested this hypothesis by monitoring changes in membrane potential of isolated SR vesicles from *TRIC-A(-/-)* mice using di-8-ANEPPQ as a fluorescent voltage indicator [65]. Ca^{2+} -loaded *TRIC-A(-/-)* vesicles in an ATP-containing environment displayed a slightly negative potential in their lumen compared to wild type vesicles. This negative potential was increased by Ca^{2+} leakage triggered by thapsigargin, suggesting that counter ion flow to counteract the negative changes that accumulate with Ca^{2+} release is reduced in *TRIC-A(-/-)* vesicles. Application of valinomycin, a K^{+} ionophore, eliminates the difference in membrane potential between *TRIC-A(-/-)* and wild type vesicles, thus indicating the difference in membrane potential at the resting state and following Ca^{2+} results from altered K^{+} permeability in *TRIC-A(-/-)* vesicles. This observation is complemented by the ability of A23187, a Ca^{2+} ionophore, to return the elevated membrane potential in *TRIC-A(-/-)* vesicles to similar levels observed in wild type vesicles (unpublished observation). As a whole, our findings with SR vesicles support our lipid bilayer results to demonstrate that TRIC-A deficiency reduces K^{+} permeability without affecting the basal Ca^{2+} permeability across the SR membrane.

8. Importance of TRIC in physiology and pathophysiology

If TRIC provides some aspects of the counter current across the SR to compensate for Ca^{2+} release from the SR, it is possible that TRIC could represent a current that has been

previously identified. The conductance characteristics for TRIC-A in the lipid bilayer reconstitution assay are similar to those of the SR K⁺ channel reported previously [10,66]. Under bi-ionic conditions of 200 mM KCl (*cis*)/200 mM NaCl (*trans*), outward current was measured at a holding potential of 0mV that reverses at a potential of -10 mV. This negative reversal potential corresponds to a moderate selectivity for K⁺ over Na ($P_K/P_{Na} = 1.5$), which corresponds with the moderate selectivity of the SR K⁺ channel. However, the SR K⁺ channel is highly sensitive to decamethonium [10], while the TRIC-A channel is insensitive to decamethonium even at high concentrations. Since it is possible that accessory proteins not present in the TRIC-A protein preparation contribute to the decamethonium sensitivity of the SR K⁺ channel, we cannot rule out that TRIC-A represents the molecular identity of the SR K⁺ channel. Further characterization of TRIC-A channel functions and interacting partners should resolve the extent that TRIC contributes to counter ion flow across the SR membrane and allow a better understanding of TRIC-A function in physiology and pathophysiology.

The discovery of TRIC isoforms has great potential importance for our understanding of Ca²⁺ signaling and homeostasis in several cell types. While monovalent cation-selective currents are known to exist in ER/SR membrane [10,14,16–18], the identity of gene(s) encoding these channels was completely unknown. TRIC constitutes a candidate gene product for one such long-sought, counter ion channel in the ER/SR membrane. One question that requires further investigation is the extent that TRIC contributes to counter ion movement. It is likely that additional molecules function in balancing the ion flux across the SR/ER membrane (Fig. 2). High H⁺ permeability is detected in the SR/ER membrane, which comes in part from SERCA as it provides counter-transport for H⁺ and Ca²⁺. Along with the SR K⁺ channel, several other K⁺ and Cl⁻-selective currents are known to present in the ER/SR membrane. As TRIC characteristics most closely match those of the SR K⁺ channel, future studies on the molecular identity of these additional ER/SR channels should be targeted to improve our understanding of muscle E–C coupling as well as Ca²⁺ homeostasis in other cell types.

As RyR or IP3 receptor mediated Ca²⁺ release from SR and ER is observed in all cell types, including excitable and non-excitable tissues, it is likely that ionic channels conducting counter ions during Ca²⁺ release are ubiquitously expressed. Indeed, TRIC-B is expressed in all tissues tested and, considering the lethal phenotype produced by TRIC-B ablation, one can envision that TRIC-B plays an essential role in maintaining normal cellular function in a wide variety of cell types. TRIC-A expression is predominantly targeted to tissues containing excitable cell types, such as the brain and muscle, suggesting that this subtype may function to facilitate the particular kinetic demands of Ca²⁺ release within excitable cells. A reduction in TRIC protein level would likely lead to instability in ER/SR Ca²⁺ release process, which would have wide reaching effects in cellular physiology. For example, in viable TRIC-A knockout animals it is possible that altered SR Ca²⁺ release may result in altered contractility in striated muscle types. While progress made to this point has illustrated the importance of TRIC channels in muscle Ca²⁺ homeostasis, major challenges remain towards developing an understanding of the cellular and molecular function of TRIC in the physiology and pathophysiology of different cell types.

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Fig. 1.

Using an immuno-proteomic monoclonal antibody library to discover novel proteins involved in E-C coupling and muscle physiology. This scheme illustrates the steps used to generate and screen a monoclonal antibody library against proteins that localize to the triad junction in skeletal muscle. Antibodies that show localization at the triad junction are used to isolated proteins for sequencing, which provides the sequence information to clone the genes of interest. Knockout mice can then be generated to study the physiological role of the gene of interest.

Fig. 2.

Molecular components of E–C coupling in striated muscle. While dihydropyridine receptor (DHPR) and ryanodine receptor (RyR) Ca²⁺ channels are major components of the E–C coupling machinery at the triad (in skeletal muscle) or dyad (in cardiac muscle) junctions, other accessory proteins are essential to maintain structural integrity and efficient RyR function. Mitsugumin-29 (MG29) acts in transverse (T)-tubule maintenance and can directly interact with RyR to modulate channel activity. Junctophilin (JP) maintains proper spacing between the T-tubule and sarcoplasmic reticulum (SR) by spanning the gap between the two membranes to physically link them together. Calu may interact with Orai to regulate SOCE. Calsequestrin (CSQ) and sarcalumenin (SAR) are Ca²⁺ binding proteins that buffer SR Ca²⁺ stores. TRIC channels in the SR gate K⁺ movement to counteract transient negative potential produced by Ca²⁺ release from the SR. Other SR resident proteins, such as SERCA and K⁺ or Cl⁻ channels of unknown molecular identity, can also provide some aspects of this counter ion movement.

Fig. 3.

Various junctophilin mutations have been linked to human disease. Members of the JP family all contain a well-conserved series of MORN motifs (red) that interact with the PM and a transmembrane domain (TM, green) that inserts into the ER membrane. A divergent region that contains some alpha-helical sequences links these conserved domains. Mutations have been identified in JP-2 (arrows) that are linked to hypertrophic cardiomyopathy in different studies (Ref. [40] in black; Ref. [41] in blue). Numerous studies have identified triplet repeats in JP-3 exon 2a that result in Huntington's disease-like symptoms. Sequences are based on human protein sequences for JP-1 (NP_065698), JP-2 (NP_065166), JP-3 (NP_065706) and JP-4 (NP_115828). Black numbers indicate the number of amino acid residues in each isoform. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Fig. 4.

Caluim and STIM1 share a similar domain structure. Caluim contains a SP (signal peptide), an ER/SR lumen domain that can bind Ca^{2+} (lum), a single transmembrane domain (TM) with a long cytoplasmic (cyt) domain that contains a coiled coil region. Similarly, STIM1 contains a SP and Ca^{2+} -binding EF-hand domain (EF) on a section of the protein localized within the lumen of the ER/SR, which also contains a sterile α -helix motif (SAM) domain. STIM1 also contains a single TM and a cytosolic coiled-coil domain. Black numbers indicate the number of amino acid residues in each isoform.