Eur Biophys J (2009) 39:51–59
DOI 10.1007/s00249-009-0455-8

REVIEW

Ubiquitous SPRY domains and their role in the skeletal type ryanodine receptor

HanShen Tae · Marco G. Casarotto · Angela Fay Dulhunty

Received: 23 January 2009 / Revised: 28 March 2009 / Accepted: 7 April 2009 / Published online: 28 April 2009

Abstract We recently identified the second of three SPRY domains in the skeletal muscle ryanodine receptor type 1 (RyR1) as a potential binding partner in the RyR1 ion channel for the recombinant II–III loop of the skeletal muscle dihydropyridine receptor, for a scorpion toxin, Imperatoxin A and for an interdomain interaction within RyR1. SPRY domains are structural domains that were first described in the fungal *Dictyostelium discoideum* tyrosine kinase spore lysis A and all three isoforms of the mammalian ryanodine receptor (RyR). Our studies are the first to assign a function to any of the three SPRY domains in the RyR. However, in other systems SPRY domains provide binding sites for regulatory proteins or intramolecular binding sites that maintain the structural integrity of a protein. In this article, we review the general characteristics of a range of SPRY domains and discuss evidence that the SPRY2 domain in RyR1 supports interactions with binding partners that contain a structural surface of aligned basic residues.

Keywords Ryanodine receptor · SPRY domains · Protein–protein interactions · Calcium signalling · Excitation–contraction coupling · Skeletal muscle

"Proteins, membranes and cells: the structure–function nexus". Contributions from the annual scientific meeting (including a special symposium in honour of Professor Alex Hope of Flinders University, South Australia) of the Australian Society for Biophysics held in Canberra, ACT, Australia, September 28–October 1, 2008.

H. Tae · M. G. Casarotto · A. F. Dulhunty (✉)
Muscle Research Group, John Curtin School of Medical Research, Australian National University, PO Box 334, Canberra, ACT 2601, Australia
e-mail: angela.dulhunty@anu.edu.au

Introduction

The ryanodine receptor (RyR) is the major Ca^{2+} release channel in the membranes of sarcoplasmic reticulum (SR). Ca^{2+} is stored in the SR in striated muscle and is found in membranes of endoplasmic reticulum (ER) in a variety of cell types. There are three RyR genes in mammals named after the three tissues types in which each was first identified, the skeletal RyR (RyR1), the cardiac RyR (RyR2) and the brain RyR (RyR3). The homology between the three isoforms is high. The RyR protein is a large (>2 MDa) homoteramer. Each monomeric subunit has a molecular mass of ~560 kDa and contains a number of structural domains, which regulate the gating of the ion channel in an integrated manner, and ultimately determine the amount of Ca^{2+} release from the SR and the strength of contraction. These structural domains provide catalytic sites (Baker et al. 2002), binding domains for regulatory proteins (Samso et al. 2006) and intermolecular binding sites between adjacent RyR proteins (Yin et al. 2005). These structural entities include three β sandwich SPRY domains (a β sandwich is formed by two pleated beta sheets, each consisting of beta strands connected by hydrogen bonds), which are conserved in each of the three mammalian RyR isoforms as well as in amphibian and insect RyR isoforms.

RyR1 in skeletal muscle releases Ca^{2+} from the SR following an action potential which travels along the surface membrane from the neuromuscular junction and penetrates the cross-section of the fibre along transverse (T-) tubule membranes. The T-tubule membrane is closely aligned with the SR membrane at triad junctions, where RyR1 channels in the junctional face membrane of the SR are in close contact with dihydropyridine receptor (DHPR) L-type Ca^{2+} channels located in the T-tubule membrane. The membrane spanning $\alpha 1_S$ subunit of the DHPR is a voltage

sensor, and it is thought to communicate the action potential to the RyR via a conformational coupling process between the intracellular II–III loop of the DHPR and RyR1. This communication has been shown to specifically involve the central "C" region of the II–III loop (Dulhunty et al. 2002). The II–III loop was divided into three regions (A–C) for simplicity by El-Hayek et al. (1995) and those definitions are used in here. It is important to note that despite extensive searches the specific binding residues on the RyR for the essential C region of the II–III loop have not been located.

The SPRY domains that are highly conserved across RyR isoforms had not been assigned any specific function until we found that the second of the three SPRY domains (SPRY2) is an in vitro binding partner for the II–III loop from $\alpha 1_S$ subunit of the skeletal muscle dihydropryidine receptor (DHPR). We found that the recombinant SPRY2 domain binds to the DHPR II–III loop through its N-terminal A region. The SPRY2 domain also binds to a peptide corresponding only to the A sequence. In addition, the SPRY2 domain interacts with the scorpion toxin Imperatoxin A, which contains a stretch of basic residues similar to those in the A region of the II–III loop (Cui et al. 2009). The A peptide and Imperatoxin A are commonly used peptide probes in a wide variety of studies exploring RyR function, and thus their binding site in the RyR is of particular interest. In this article, we review the structure and function of SPRY domains in general and consider their function in RyR channels.

SPRY and B30.2 domains

The SPRY domain structure was first described in the *Dictyostelium discoideum* tyrosine kinase spore lysis A (SplA) and the mammalian RyRs, hence the name SPRY (Ponting et al. 1997). SPRY-containing protein families have since been found in many proteins and shown to be involved in a myriad of cellular processes including RNA metabolism (DDDX1 and hnRNP proteins), developmental pathways (HERC1 proteins), regulation of cytokine signalling (SOCS box, SS-B proteins) and in Ca^{2+} signalling (RyR proteins) (Rhodes et al. 2005). The definition of a SPRY domain depends on the β sandwich structure rather than amino acid sequence. For example the three SPRY domains in RyR1 have similar predicted structures but have $\leq$29% sequence identity (29% between SPRY1 and SPRY2, 11% between SPRY1 and SPRY3 and 18% between SPRY2 and SPRY3). Interestingly, the sequence conservation of each domain between the RyR isoforms is considerably greater and in the three mammalian isoforms is: 78–80% for SPRY1, 52–64% for SPRY2 and 62–64% for SPRY3.

Another similar domain, the B30.2 domain, has been described (Henry et al. 1998; Tazi-Ahnini et al. 1997). The B30.2 domain contains a SPRY subdomain structure. Additional residues in the N-termini of the B30.2 domain form a distinct PRY domain structure. In other words, the B30.2 domain consists of PRY and SPRY subdomains. Generally, B30.2 domains are ~170 residues long while SPRY2 domains contain ~100 residues. A B30.2 domain is encoded by the B30-2 exon in the human class 1 major histocompatibility complex (MHCI) region of the human chromosome, 6p21.3 (Henry et al. 1998; Tazi-Ahnini et al. 1997). The B30.2 domain is present in two major protein groups, the tripartite motif (TRIM) family and the butyrophilin (BTN) family. The TRIM5α protein is a cellular retroviral restriction factor which blocks infection soon after the viral entry into the cell (Kar et al. 2008; Li et al. 2006). Meanwhile, BTN proteins are suggested to have regulatory role in the immune system (Guggenmos et al. 2004; Mana et al. 2004) in which they inhibit T cell activation (Compte et al. 2004).

As mentioned, the B30.2 domain is found only in the BTN and TRIM protein families, and is usually located at the C-terminal end of the proteins. The SPRY domain on the other hand is distributed over a range of protein families, and more than one copy can occur in some proteins including the RyR (Ponting et al. 1997), FLJ14800 protein and in the fungal tyrosine kinase SplA (Rhodes et al. 2005). In contrast to the preferred C-terminal location of the B30.2 domain, SPRY domains have no positional preference and are found at both the N- and C-termini of the proteins as well as in central locations (Godbout et al. 2002; Ponting et al. 1997; Shtifman et al. 2002; Wang et al. 2005). The similarities in the primary sequence of SPRY and B30.2 domains led to the proposal that they share a common evolutionary relationship. SPRY domains are highly conserved across all taxa whereas the B30.2 domains have so far only been discovered in vertebrate species with an adaptive immune system. Therefore, SPRY domains are more ancient than B30.2 domains which evolved recently and incorporated the PRY domain into the N-terminal end of the SPRY domain. B30.2 domains are thought to have been selectively maintained as a component of immune defence. In support of this hypothesis, the TRIM5α B30.2 domain plays an important role in innate immune retroviral recognition (Perron et al. 2006; Richardson et al. 2008; Song et al. 2005; Stremlau et al. 2004; Yap et al. 2005). The plasticity in the gene organisation of B30.2 domain in mammalian genome is often found with immune receptors, and is thought to be an ongoing progression as a result of selective-adaptation (Emes et al. 2003; James et al. 2007). Therefore, it is likely that the evolutionary adaptation from SPRY to B30.2 has been selectively maintained for immune defence.

Disease-causing point mutations in the SPRY domain

The functional importance of SPRY domains is indicated by diseases caused by point mutations in their sequences. Familial Mediterranean fever (FMF), a recessively inherited disorder, is characterised by recurrent episodes of fever and serosal inflammation (Bakkaloglu 2003; Centola et al. 1998; Chae et al. 2003; Papin et al. 2007) without apparent high-titer antibodies or antigen specific T-cells (Chae et al. 2000; Stojanov and Kastner 2005). More than 50 missense mutations in the MEFV (Mediterranean fever) gene have been identified (Chae et al. 2006). The product of this gene is a 781-residue pyrin protein, which contains the canonical SPRY domain at its C-terminus (Henry et al. 1998) and an adjacent PRY domain. Although the exact role of pyrin is unknown, it has been postulated to have a modulatory effect on the inflammatory response through its SPRY domain (Chae et al. 2006; Papin et al. 2007). Pyrin is commonly found in neutrophils, cytokine-regulated monocytes and serosal and synovial fibroblasts (Centola et al. 2000). Most of the mutations are located in the SPRY domain and are thought to alter pyrin protein function.

The Opitz syndrome (OS) is the result of mutations in the MID1 gene, which encodes a 72-kDa MID1 protein with several conserved functional domains. MID1 protein regulates the microtubule complex, which is associated with the ventral midline development. The pathology of OS includes abnormalities in ventral midline structures. These include defects of the upper airways and cleft lip and palate, mental retardation and malformations of the gastrointestinal tract (Hsieh et al. 2008). Mutations causing OS are located in the C-terminal SPRY domain of the MID1 protein (Cox et al. 2000; De Falco et al. 2003; Mnayer et al. 2006). It is proposed that the OS mutations dissociate the MID1 protein from the microtubule complex and remove its regulatory role on ventral midline development (Aranda-Orgilles et al. 2008a; b; Cainarca et al. 1999; Schweiger et al. 1999). So far, no mutations in the SPRY domains of RyRs have been linked to any disease.

The structure of the B30.2/SPRY domain

One common feature of all B30.2/SPRY domains is a core double β-sheet layer (Fig. 1). Each sheet is made up of 4–8 β strands arranged in an anti-parallel array interconnected by varying length α-helices (shown as red helices in Fig. 4 below). Each of the 4–8 β strands are connected by either unstructured loops or helical turns. The differences between the unstructured regions endow unique properties on SPRY domains in different SPRY proteins which allow interactions with specific protein binding partners. There are subtle structural differences between common parts of the

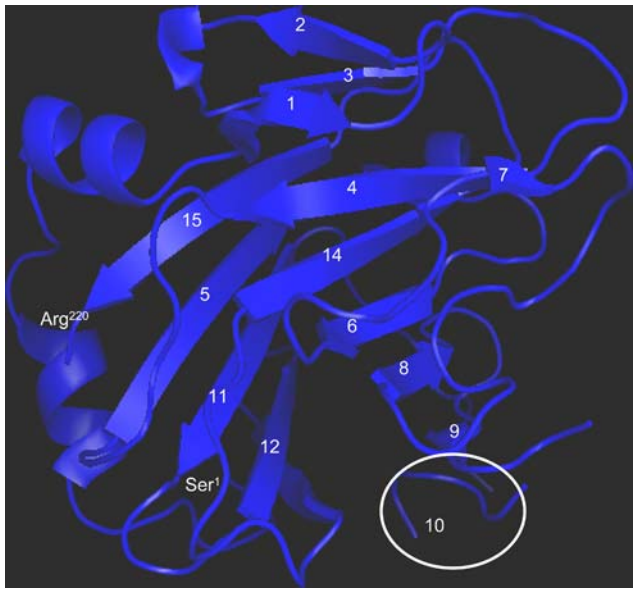

Fig. 1 Ribbon diagram of the crystal structure of SSB-2 SPRY domain. The N- and C-terminal residues are labelled. The *arrows* indicate the C-terminal ends of each β-strand and the *numbers* on each strand indicate its position in the sequence of the β-strands through the domain (N- to C-terminal). Note that there are no atomic coordinates for residues flanking β10 (*white circle*) as they are disordered (Kuang et al. 2009)

SPRY and B30.2 domains. A helix-turn-helix and a β-hairpin are found immediately before the SPRY domain, as in the SSB-2 (Kuang et al. 2009), whereas the B30.2 domain normally has ordered β-strands at the N-terminal of its SPRY component. The structures of the B30.2/SPRY domain from two members of the SSB protein family and TRIM21 are described below.

The suppressor of cytokine signalling (SOCS) box protein 2 (SSB-2) is thought to have a vital role in maintaining platelet count and the level of blood urea nitrogen in the mouse (Masters et al. 2005). The SSB-2 SPRY domain has been suggested to interact directly with c-MET, which has diverse biological roles. Some studies associate its deregulation with metastatic and invasive tumour generation (Trusolino and Comoglio 2002). It has also been shown to bind to an upregulated apoptotic cancer protein (Masters et al. 2006), the prostate apoptosis response protein (PAR-4) that sensitises the cells to apoptotic stimuli (Gurumurthy and Rangnekar 2004).

Suppressor of cytokine signalling (SOCS) box protein 2 (SSB-2)

Four members of the SOCS box (SSB) protein family (SSB-1 to -4) contain a central conserved SPRY domain and a C-terminal SOCS box motif. SSB-2 maintains platelet count and blood urea nitrogen in the mouse (Masters et al. 2005). The SSB-2 SPRY domain is thought to interact

directly with c-MET, which has diverse biological roles. Some studies associate its deregulation with metastatic and invasive tumour generation (Trusolino and Comoglio 2002). It has also been shown to bind to an upregulated prostate apoptosis response protein (PAR-4) (Masters et al. 2006) that sensitises the cells to apoptotic stimuli (Gurumurthy and Rangnekar 2004). The crystal structure of the murine SSB-2 SPRY domain is composed of a 2–7 stranded β-sandwich-core and several flexible loop regions that interact with target proteins (Fig. 1) (Kuang et al. 2009). A conserved motif has been identified within these loop regions that binds to Par-4 and c-Met.

GUSTAVUS

GUSTAVUS also belongs to the family of SPRY domain-containing SOCS box proteins (Hilton et al. 1998; Kile et al. 2002; Krebs and Hilton 2000), and is the only SSB protein found in *D. melanogaster* (Styhler et al. 2002). The protein is required for the specification of germ cells in sexual reproduction. GUSTAVUS has a short stretch of 40 N-terminal residues, followed by a central SPRY domain and a SOCS box at the C-terminal end (Fig. 2). A functional BC box at the far C-terminal end binds to the Elongin B and C heterodimer (Fig. 2) (Kamura et al. 1998) and prevents the degradation SOCS proteins. A BC box (elongin BC complex binding-site) is a conserved region with the consensus sequence (T/S)(L/M)*XXX*(C/S)-*XXX*(V/L/I). GUSTAVUS without the Elongin B and C complex interacts with an RNA helicase VASA, an important protein in the development of the *D. melanogaster* oocyte (Carrera et al. 2000). The localisation of VASA is dependent on its interaction with GUSTAVUS (Styhler et al. 2002). The SPRY domain of GUSTAVUS is unique. It adopts a highly distorted compact sandwich of 7–8 antiparallel strand β-sheets with two short α-helices at its N-terminal. The N- and C-termini of the domain are close to each other. A third α-helix forms the BC box, which binds to the Elongin B and C heterodimer complex via Elongin C (Fig. 2).

Tripartite motif (TRIM) 21

The tripartite motif (TRIM) protein family contains at least 70 members, and is involved in myriads of cellular pathways from differentiation and development to immune responses (Meroni and Diez-Roux 2005; Nisole et al. 2005). TRIM proteins share a conserved multidomain architecture consisting of a RING domain (a type of Zn finger that binds two Zn^{2+}), a B box and a coiled-coil domain (Reddy et al. 1992). The B30.2 (PRYSPRY) domain is found at the far C-terminal end of the protein, adjacent to the coiled coil domain and determines the function of TRIM proteins by acting as a targeting module (Li et al. 2006). TRIM21 is a major autoantigen in diseases such as rheumatoid arthritis, systemic lupus erythematosus and Sjögren's syndrome (Ben-Chetrit et al. 1990). In addition to being a target for autoantibodies, TRIM21 interacts with normal serum IgG through its B30.2 domain (James et al. 2007). The TRIM21 B30.2 domain in a complex with the C_H2 and C_H3 domains of one Fc heavy chain forms a twisted two β-sheet core with the direct contact sites on the Fc on loops arranged around a convex β-sheet (Fig. 3).

Several studies suggest that SPRY domains function as protein-interaction modules. The VASA peptide interaction with the truncated GUSTAVUS protein (GUS) is critical in the development of fruit fly oocyte (Styhler et al. 2002). The cytoplasmic domain of butyrophilin, which contains a SPRY domain that interacts with xanthine dehydrogenase (Ishii et al. 1995) and the SPRY domain of TRIM7 protein interacts with glycogenin (Zhai et al. 2004). In addition the B30.2/SPRY domain of all SSB proteins interacts with c-Met, a hepatocyte growth factor receptor (Wang et al. 2005).

The SPRY domain of the skeletal RyR1

As mentioned above, SPRY domains are present as three structural repeats in all three isoforms of the mammalian

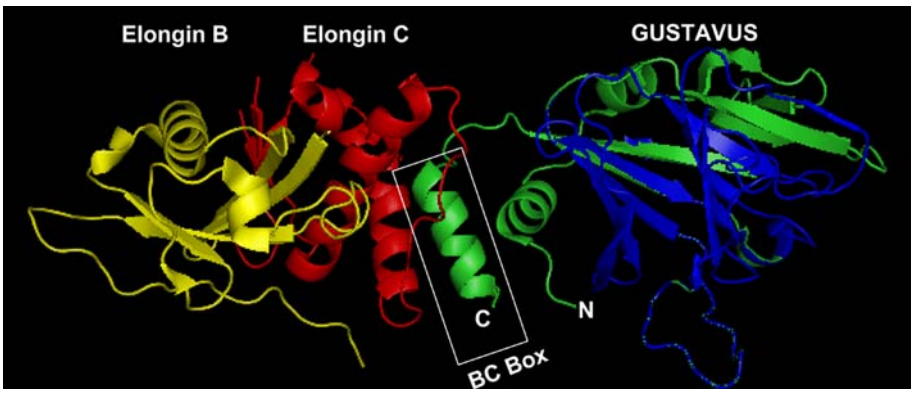

Fig. 2 GUSTAVUS protein in complex with Elongin B and C (Woo et al. 2006). Elongin B is shown in *yellow*, Elongin C is shown in *red*, the SPRY domain is shown in *blue* and other (non-SPRY) regions of the GUSTAVUS are in *green*. The C-terminal BC box helix is shown in the *white box*

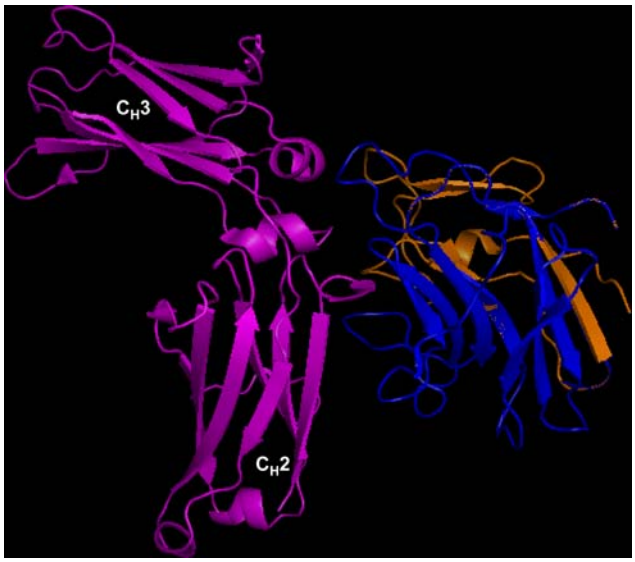

Fig. 3 Solution structure of TRIM21 B30.2 (PRYSPRY) domain in a complex with one Fc heavy chain (Keeble et al. 2008). The PRY domain is shown in *orange*, the SPRY domain is shown in *blue* and the C_H2 and C_H3 domains are shown in *magenta*

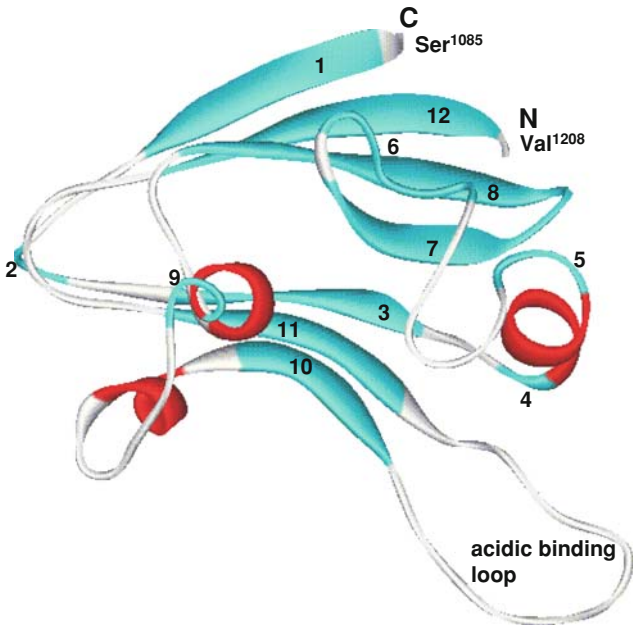

Fig. 4 RyR1-SPRY2 homology model with a β-sheet core consisting of two sheets of 3–4 antiparallel β-strands (Tae et al. 2009). The N- and C-terminal residues are shown with the residue numbers in the RyR1 sequence. The *numbers* on each strand indicate the position in the sequence of the β-strands through the domain (N- to C-terminal). The acidic loop that binds to the DHPR II–III loop, the A peptide, Imperatoxin A and the ASI peptide is indicated. β-strands are shown in *cyan*, unstructured loops in *grey* and α-helices in *red*

RyR. The three SPRY domains are located in the N-terminal part of the cytoplasmic region of the RyRs (SPRY1, Ser^{659}-Gly^{797}; SPRY2, Arg^{1085}-Asp^{1208}; SPRY3, Thr^{1430}-Asn^{1570}). The second of the three SPRY domains (SPRY2) in RyR1 is of particular interest. Although the domain was not specifically identified, it is contained within residues 1,021–1,631 which support protein–protein interactions with peptide A and the structurally similar scorpion toxin, maurocalcine (Altafaj et al. 2005) and overlaps with residues 1,076–1,112 which bind to the recombinant II–III loop (Leong and MacLennan 1998). Since these residues form protein–protein interaction modules in RyR1, it was possible that SPRY2 is the interaction module. This was confirmed with the recent observations (described in detail in "The SPRY2 domain binds to the DHPR II–III loop and the ASI region of RyR1") that the recombinant SPRY2 domain is (1) a binding site for the A region of the DHPR II–III loop and for another structurally similar scorpion toxin Imperatoxin A (Cui et al. 2009), which contains a structural surface similar to that of the A region (Green et al. 2003) and (2) a binding partner for the variably spliced AS1 region (Tae et al., unpublished observations), which is implicated in myotonic dystrophy and participates in an interdomain interaction within RyR1 that strongly influences EC coupling (Kimura et al. 2005, 2007, 2009).

A homology model of the RyR1-SPRY2 domain

We have generated a homology model for the SPRY2 domain (Fig. 4) in order to predict specific binding sites and provide better understanding of this RyR1 domain. The structure was modelled on the SPRY domain of GUSTAVUS (Woo et al. 2006) and the NMR structure of SSB-2 (Masters et al. 2006; Yao et al. 2006). Multiple sequence alignment analysis revealed a conserved β sheets sandwich core flanked by several flexible loop regions. A secondary structural assessment of the recombinant SPRY2 domain using circular dichroism spectroscopy also revealed an overall β-sheet structure. The molar ellipticity spectra of SPRY2 domain displayed a negative peak at around 215 nm (Fig. 5; Tae et al. 2009), which is a canonical feature of a β-sheet structured protein (Kelly and Price 2000).

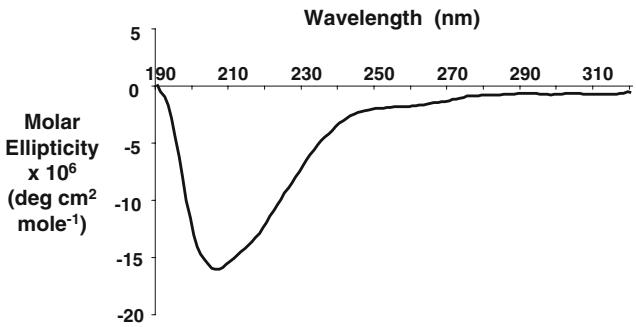

Fig. 5 CD spectrum of purified recombinant RyR1-SPRY2 domain with the molar ellipticity (deg cm^2 $mole^{-1}$) as a function of wavelength (nm). A single negative peak at 215 nm is the signature of a β-sheet structure (Cui et al. 2009)

The SPRY2 domain binds to the DHPR II–III loop and the ASI region of RyR1

Skeletal muscle excitation–contraction (EC) coupling is tightly regulated by the physical coupling between two Ca^{2+} channels in two different membranes, the DHPR in the surface/transverse tubule membrane and the skeletal muscle RyR1 in the SR membrane. EC coupling is an essential signal transduction pathway that allows a surface action potential to release of Ca^{2+} from the SR in striated muscle. A cytoplasmic loop (II–III loop) of the DHPR, spanning the second and third transmembrane domains of the $\alpha 1_S$ subunit ($\alpha 1_S$ residues 671–790), is a critical determinant of the skeletal muscle EC coupling process (Tanabe et al. 1990), which is independent of external Ca^{2+} ion entry, but thought to be dependent on a physical coupling between the II–III loop and RyR1 (Dulhunty et al. 2002). In vitro experiments provide evidence for a direct interaction between the II–III loop and the RyR1 (Dulhunty et al. 2005a; El-Hayek et al. 1995; Leong and MacLennan 1998; Lu et al. 1994; Stange et al. 2001).

There is whole cell evidence that the macro region of the RyR required for skeletal EC coupling is contained in residues 1–1,680 which contains all the three SPRY domains (Perez et al. 2003a). However, many other regions also appear to be involved in supporting skeletal EC coupling (Perez et al. 2003b; Protasi et al. 2002). It is worth noting that in vivo experiments have thus far failed to demonstrate a direct interaction between the II–III loop and the RyR, although this remains the preferred explanation for the EC coupling process.

The recombinant II–III loop binds to a 37-residue fragment of RyR1 (Arg^{1076}-Asp^{1112}) (Leong and MacLennan 1998). In addition, the N-terminus of the II–III loop interacts with a similar fragment, Val^{1021}-Glu^{1631} of the RyR1 (Altafaj et al. 2005). Both RyR1 fragments contain the second repeat of the RyR1 SPRY domain (SPRY2) in residues Ser^{1085}-Val^{1208}; thus it is possible that the II–III loop interacts with RyR1 via this domain. Cui et al. (2009) demonstrated an interaction between the recombinant DHPR II–III loop and the recombinant SPRY2 domain, thus providing the first evidence for an interactive role of the SPRY domain in the RyR. The interaction was demonstrated using NMR spectroscopy and spectrofluorimetric analysis, which revealed a weak (2–3 μM) binding affinity (Cui et al. 2009). The main sites on the II–III loop that interacted with SPRY2 were in the A region (residues Glu^{666}-Leu^{690}, which is highly populated with positively charged residues. The adjacent B region (residues Pro^{691}-Leu^{720}) also interacted with SPRY2 (Cui et al. 2009). The interaction with the full loop had affinity of ~2.3 μM. A 20 residue synthetic peptide, peptide A, which corresponds to the A region of the loop had a lower affinity of ~8.3 μM (Tae et al. 2009). In addition Imperatoxin A, which competes with peptide A in activating RyR1 (Dulhunty et al. 2004) and has structural surfaces similar to peptide A (Green et al. 2003), binds to SPRY2 with an affinity of ~10.3 μM (Cui et al. 2009).

A noteworthy feature of the SPRY2 homology model is a loop containing residues Pro^{1107}-Ala^{1121} (Fig. 4) which is not present in the other two SPRY domains in RyR1. The high density of negatively charged residues in the loop (Fig. 6) led us to postulate that it may be the interaction site for the basic A region of the DHPR II–III loop. The stretch of positively charged residues the A region, residues Arg^{681}-Lys^{685}, is responsible for the activation of RyR1 (Dulhunty et al. 1999; El-Hayek and Ikemoto 1998; Zhu et al. 1999). The removal of the positively charged residues (Arg^{681}-Lys^{685}) from the II–III loop results in a tenfold reduction in the dissociation constant for the SPRY2 interaction with the loop (Cui et al. 2009). Similarly, removal of these residues from peptide A resulted in a threefold reduction in the affinity of the peptide for SPRY2 (Tae et al. 2009). These results suggest an involvement of the II–III loop basic residues through electrostatic interactions with the SPRY2 domain. Several of the acidic residues in the Pro^{1107}-Ala^{1121} loop of SPRY2 were mutated, and the ability of the mutated SPRY2 domain to interact with to the II–III loop and peptide A were examined spectrofluorimetrically (Tae et al. 2009). The Glu^{1108} residue in SPRY2 was shown to be a key residue in the binding of the SPRY2 domain to the II–III loop. Multiple point mutations in other negatively charged residues along the loop did not produce any further reduction in the binding affinity.

The variably spliced ASI region of RyR1 also interacts with the SPRY2 domain. The ASI region is structurally homologous with the A region of the II–III loop and competes with the A peptide to activate RyR1 (Kimura et al. 2009). The ASI region is also implicated in determining the gain of EC coupling (Kimura et al. 2009). Our preliminary data suggests that the ASI region and the A region of the II–III loop bind to the same acidic residues in the SPRY2 domain (Tae et al., unpublished observations). However, other preliminary data show that mutation of the residues in SPRY2 that bind to peptide A and the ASI region does not alter EC coupling in RyR1-null myotubes expressing the RyR1 with mutations in key acidic residues the SPRY2

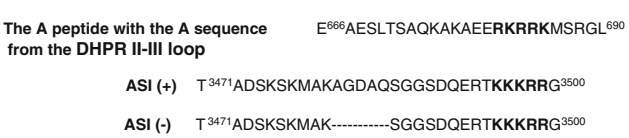

Fig. 6 The sequence of the basic A region of the II–III loop (and sequence of the A peptide) and the sequence of the ASI+ and ASI− regions of RyR1 (and sequence of the ASI+ and ASI− peptides). Note the sequence similarity between the RyR1 ASI region and the A region of the DHPR II–III loop

domain (Wei et al., unpublished observations). The lack of an effect of mutation of these residues on EC coupling suggests that if II–III loop and the ASI region do bind to the SPRY2 domain in vivo, this binding is not involved in the role of the two domains in EC coupling. An alternative hypothesis is that additional binding partners are required and have yet to be identified. Thus, although the acidic SPRY2 residues support the in vitro interaction with the II–III loop, they do not support EC coupling. It is perhaps not surprising that mutation of the A-region binding site on RyR1 did not alter EC coupling, since in vivo EC coupling is independent of the A region, but requires the more central C region of the loop (residues 720–765) (Grabner et al. 1999; Proenza et al. 2000; Wilkens et al. 2001). Curiously, however, mutations in this C region that influence EC coupling (Kugler et al. 2004) also influence isolated II–III loop interactions with RyR1 (Dulhunty et al. 2005b).

What is clear from our recent studies is that the SPRY2 domain is likely to be involved in an inter-domain interaction within RyR1, and that it can also bind to α-helical peptides like peptide A and the ASI peptides, which have positively charged residues aligned along one surface. This indicates that the in vivo binding partner for SPRY2 may have such a structure. There are six other regions within RyR1 with stretches of four or more basic residues, five of these within the N-terminal regulatory domain (residues 1,381–3,614) and the possibility that these are binding partners for SPRY2 remains to be investigated. The conservation of the three repeats of the SPRY domain in RyRs and the evidence that SPRY domains can support protein–protein interactions that underlie a range of cellular functions suggest that the three RyR SPRY domains may contribute to Ca^{2+}-signalling pathways in many if not all RyR containing cell types.

Conclusion

SPRY structural domains have been demonstrated to be a vital modulatory element in a number of cellular protein–protein interactions. Members of this family share a canonical β-sheet core. The critical interaction interface of the SPRY domain is usually located on an unstructured peripheral loop. Our homology model of the SPRY2 domain in RyR1 predicts a high β-sheet content and the presence of a distinct acidic loop that is likely to be involved in its interaction with basic residues in other protein domains. We have shown that the acidic loop interacts with Imperatoxin A and with basic α-helices in the skeletal DHPR II–III loop as well as with the ASI region of RyR1. This indicates that interactions involving the RyR1 SPRY2 domain are dependent on the charge and the alignment of positive residues in its binding partners. These findings provide evidence that SPRY2 domain functions as a protein-interaction module in the RyR and suggest a general role for SPRY domains in maintaining RyR dependent Ca^{2+} signalling.

References

Altafaj X, Cheng W, Esteve E, Urbani J, Grunwald D, Sabatier JM, Coronado R, De Waard M, Ronjat M (2005) Maurocalcine and domain A of the II-III loop of the dihydropyridine receptor Cav 1.1 subunit share common binding sites on the skeletal ryanodine receptor. J Biol Chem 280:4013–4016. doi:10.1074/jbc.C400433200

Aranda-Orgilles B, Aigner J, Kunath M, Lurz R, Schneider R, Schweiger S (2008a) Active transport of the ubiquitin ligase MID1 along the microtubules is regulated by protein phosphatase 2A. PLoS ONE 3:e3507. doi:10.1371/journal.pone.0003507

Aranda-Orgilles B, Trockenbacher A, Winter J, Aigner J, Kohler A, Jastrzebska E, Stahl J, Muller EC, Otto A, Wanker EE, Schneider R, Schweiger S (2008b) The Opitz syndrome gene product MID1 assembles a microtubule-associated ribonucleoprotein complex. Hum Genet 123:163–176. doi:10.1007/s00439-007-0456-6

Baker ML, Serysheva II, Sencer S, Wu Y, Ludtke SJ, Jiang W, Hamilton SL, Chiu W (2002) The skeletal muscle Ca^{2+} release channel has an oxidoreductase-like domain. Proc Natl Acad Sci USA 99:12155–12160. doi:10.1073/pnas.182058899

Bakkaloglu A (2003) Familial Mediterranean fever. Pediatr Nephrol 18:853–859. doi:10.1007/s00467-003-1185-2

Ben-Chetrit E, Fox RI, Tan EM (1990) Dissociation of immune responses to the SS-A (Ro) 52-kd and 60-kd polypeptides in systemic lupus erythematosus and Sjogren's syndrome. Arthritis Rheum 33:349–355. doi:10.1002/art.1780330307

Cainarca S, Messali S, Ballabio A, Meroni G (1999) Functional characterization of the Opitz syndrome gene product (midin): evidence for homodimerization and association with microtubules throughout the cell cycle. Hum Mol Genet 8:1387–1396. doi:10.1093/hmg/8.8.1387

Carrera P, Johnstone O, Nakamura A, Casanova J, Jackle H, Lasko P (2000) VASA mediates translation through interaction with a Drosophila yIF2 homolog. Mol Cell 5:181–187. doi:10.1016/S1097-2765(00)80414-1

Centola M, Chen X, Sood R, Deng Z, Aksentijevich I, Blake T, Ricke DO, Chen X, Wood G, Zaks N, Richards N, Krizman D, Mansfield E, Apostolou S, Liu J, Shafran N, Vedula A, Hamon M, Cercek A, Kahan T, Gumucio D, Callen DF, Richards RI, Moyzis RK, Doggett NA, Collins FS, Liu PP, Fischel-Ghodsian N, Kastner DL (1998) Construction of an approximately 700-kb transcript map around the familial Mediterranean fever locus on human chromosome 16p13.3. Genome Res 8:1172–1191

Centola M, Wood G, Frucht DM, Galon J, Aringer M, Farrell C, Kingma DW, Horwitz ME, Mansfield E, Holland SM, O'Shea JJ, Rosenberg HF, Malech HL, Kastner DL (2000) The gene for familial Mediterranean fever, MEFV, is expressed in early leukocyte development and is regulated in response to inflammatory mediators. Blood 95:3223–3231

Chae JJ, Centola M, Aksentijevich I, Dutra A, Tran M, Wood G, Nagaraju K, Kingma DW, Liu PP, Kastner DL (2000) Isolation, genomic organization, and expression analysis of the mouse and rat homologs of MEFV, the gene for familial mediterranean fever. Mamm Genome 11:428–435. doi:10.1007/s003350010082

Chae JJ, Komarow HD, Cheng J, Wood G, Raben N, Liu PP, Kastner DL (2003) Targeted disruption of pyrin, the FMF protein, causes heightened sensitivity to endotoxin and a defect in macrophage apoptosis. Mol Cell 11:591–604. doi:10.1016/S1097-2765(03)00056-X

Chae JJ, Wood G, Masters SL, Richard K, Park G, Smith BJ, Kastner DL (2006) The B30.2 domain of pyrin, the familial Mediterranean fever protein, interacts directly with caspase-1 to modulate IL-1beta production. Proc Natl Acad Sci USA 103:9982–9987. doi:10.1073/pnas.0602081103

Compte E, Pontarotti P, Collette Y, Lopez M, Olive D (2004) Frontline: characterization of BT3 molecules belonging to the B7 family expressed on immune cells. Eur J Immunol 34:2089–2099. doi:10.1002/eji.200425227

Cox TC, Allen LR, Cox LL, Hopwood B, Goodwin B, Haan E, Suthers GK (2000) New mutations in MID1 provide support for loss of function as the cause of X-linked Opitz syndrome. Hum Mol Genet 9:2553–2562. doi:10.1093/hmg/9.17.2553

Cui Y, Tae HS, Norris NC, Karunasekara Y, Pouliquin P, Board PG, Dulhunty AF, Casarotto MG (2009) A dihydropyridine receptor alpha(1s) loop region critical for skeletal muscle contraction is intrinsically unstructured and binds to a SPRY domain of the type 1 ryanodine receptor. Int J Biochem Cell Biol 41:677–686

De Falco F, Cainarca S, Andolfi G, Ferrentino R, Berti C, Rodriguez Criado G, Rittinger O, Dennis N, Odent S, Rastogi A, Liebelt J, Chitayat D, Winter R, Jawanda H, Ballabio A, Franco B, Meroni G (2003) X-linked Opitz syndrome: novel mutations in the MID1 gene and redefinition of the clinical spectrum. Am J Med Genet A 120A:222–228

Dulhunty AF, Laver DR, Gallant EM, Casarotto MG, Pace SM, Curtis S (1999) Activation and inhibition of skeletal RyR channels by a part of the skeletal DHPR II-III loop: effects of DHPR Ser687 and FKBP12. Biophys J 77:189–203. doi:10.1016/S0006-3495(99)76881-5

Dulhunty AF, Haarmann CS, Green D, Laver DR, Board PG, Casarotto MG (2002) Interactions between dihydropyridine receptors and ryanodine receptors in striated muscle. Prog Biophys Mol Biol 79:45–75. doi:10.1016/S0079-6107(02)00013-5

Dulhunty AF, Curtis SM, Watson S, Cengia L, Casarotto MG (2004) Multiple actions of imperatoxin A on ryanodine receptors: interactions with the II-III loop "A" fragment. J Biol Chem 279:11853–11862. doi:10.1074/jbc.M310466200

Dulhunty AF, Karunasekara Y, Curtis SM, Harvey PJ, Board PG, Casarotto MG (2005a) The recombinant dihydropyridine receptor II-III loop and partly structured 'C' region peptides modify cardiac ryanodine receptor activity. Biochem J 385:803–813. doi:10.1042/BJ20041152

Dulhunty AF, Karunasekara Y, Curtis SM, Harvey PJ, Board PG, Casarotto MG (2005b) Role of some unconserved residues in the "C" region of the skeletal DHPR II-III loop. Front Biosci 10:1368–1381. doi:10.2741/1626

El-Hayek R, Ikemoto N (1998) Identification of the minimum essential region in the II-III loop of the dihydropyridine receptor alpha 1 subunit required for activation of skeletal muscle-type excitation-contraction coupling. Biochemistry 37:7015–7020

El-Hayek R, Antoniu B, Wang J, Hamilton SL, Ikemoto N (1995) Identification of calcium release-triggering and blocking regions of the II-III loop of the skeletal muscle dihydropyridine receptor. J Biol Chem 270:22116–22118

Emes RD, Goodstadt L, Winter EE, Ponting CP (2003) Comparison of the genomes of human and mouse lays the foundation of genome zoology. Hum Mol Genet 12:701–709

Godbout R, Packer M, Katyal S, Bleoo S (2002) Cloning and expression analysis of the chicken DEAD box gene DDX1. Biochim Biophys Acta 1574:63–71

Grabner M, Dirksen RT, Suda N, Beam KG (1999) The II-III loop of the skeletal muscle dihydropyridine receptor is responsible for the Bi-directional coupling with the ryanodine receptor. J Biol Chem 274:21913–21919

Green D, Pace S, Curtis SM, Sakowska M, Lamb GD, Dulhunty AF, Casarotto MG (2003) The three-dimensional structural surface of two beta-sheet scorpion toxins mimics that of an alpha-helical dihydropyridine receptor segment. Biochem J 370:517–527

Guggenmos J, Schubart AS, Ogg S, Andersson M, Olsson T, Mather IH, Linington C (2004) Antibody cross-reactivity between myelin oligodendrocyte glycoprotein and the milk protein butyrophilin in multiple sclerosis. J Immunol 172:661–668

Gurumurthy S, Rangnekar VM (2004) Par-4 inducible apoptosis in prostate cancer cells. J Cell Biochem 91:504–512

Henry J, Mather IH, McDermott MF, Pontarotti P (1998) B30.2-like domain proteins: update and new insights into a rapidly expanding family of proteins. Mol Biol Evol 15:1696–1705

Hilton DJ, Richardson RT, Alexander WS, Viney EM, Willson TA, Sprigg NS, Starr R, Nicholson SE, Metcalf D, Nicola NA (1998) Twenty proteins containing a C-terminal SOCS box form five structural classes. Proc Natl Acad Sci USA 95:114–119

Hsieh EW, Vargervik K, Slavotinek AM (2008) Clinical and molecular studies of patients with characteristics of Opitz G/BBB syndrome shows a novel MID1 mutation. Am J Med Genet A 146A:2337–2345

Ishii T, Aoki N, Noda A, Adachi T, Nakamura R, Matsuda T (1995) Carboxy-terminal cytoplasmic domain of mouse butyrophilin specifically associates with a 150-kDa protein of mammary epithelial cells and milk fat globule membrane. Biochim Biophys Acta 1245:285–292

James LC, Keeble AH, Khan Z, Rhodes DA, Trowsdale J (2007) Structural basis for PRYSPRY-mediated tripartite motif (TRIM) protein function. Proc Natl Acad Sci USA 104:6200–6205

Kamura T, Sato S, Haque D, Liu L, Kaelin WG Jr, Conaway RC, Conaway JW (1998) The Elongin BC complex interacts with the conserved SOCS-box motif present in members of the SOCS, ras, WD-40 repeat, and ankyrin repeat families. Genes Dev 12:3872–3881

Kar AK, Diaz-Griffero F, Li Y, Li X, Sodroski J (2008) Biochemical and biophysical characterization of a chimeric TRIM21-TRIM5alpha protein. J Virol 82:11669–11681

Keeble AH, Khan Z, Forster A, James LC (2008) TRIM21 is an IgG receptor that is structurally, thermodynamically, and kinetically conserved. Proc Natl Acad Sci USA 105:6045–6050

Kelly SM, Price NC (2000) The use of circular dichroism in the investigation of protein structure and function. Curr Protein Pept Sci 1:349–384

Kile BT, Schulman BA, Alexander WS, Nicola NA, Martin HM, Hilton DJ (2002) The SOCS box: a tale of destruction and degradation. Trends Biochem Sci 27:235–241

Kimura T, Nakamori M, Lueck JD, Pouliquin P, Aoike F, Fujimura H, Dirksen RT, Takahashi MP, Dulhunty AF, Sakoda S (2005) Altered mRNA splicing of the skeletal muscle ryanodine receptor and sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase in myotonic dystrophy type 1. Hum Mol Genet 14:2189–2200

Kimura T, Pace SM, Wei L, Beard NA, Dirksen RT, Dulhunty AF (2007) A variably spliced region in the type 1 ryanodine receptor may participate in an inter-domain interaction. Biochem J 401:317–324

Kimura T, Lueck JD, Harvey PJ, Pace SM, Ikemoto N, Casarotto MG, Dirksen RT, Dulhunty AF (2009) Alternative splicing of RyR1 alters the efficacy of skeletal EC coupling. Cell Calcium 45:264–274

Krebs DL, Hilton DJ (2000) SOCS: physiological suppressors of cytokine signaling. J Cell Sci 113(Pt 16):2813–2819

Kuang Z, Yao S, Xu Y, Lewis RS, Low A, Masters SL, Willson TA, Kolesnik TB, Nicholson SE, Garrett TJ, Norton RS (2009) SPRY Domain-containing SOCS box protein 2: crystal structure and residues critical for protein binding. J Mol Biol 386:662–674

Kugler G, Weiss RG, Flucher BE, Grabner M (2004) Structural requirements of the dihydropyridine receptor alpha1S II-III loop for skeletal-type excitation-contraction coupling. J Biol Chem 279:4721–4728

Leong P, MacLennan DH (1998) A 37-amino acid sequence in the skeletal muscle ryanodine receptor interacts with the cytoplasmic loop between domains II and III in the skeletal muscle dihydropyridine receptor. J Biol Chem 273:7791–7794

Li X, Li Y, Stremlau M, Yuan W, Song B, Perron M, Sodroski J (2006) Functional replacement of the RING, B-box 2, and coiled-coil domains of tripartite motif 5alpha (TRIM5alpha) by heterologous TRIM domains. J Virol 80:6198–6206

Lu X, Xu L, Meissner G (1994) Activation of the skeletal muscle calcium release channel by a cytoplasmic loop of the dihydropyridine receptor. J Biol Chem 269:6511–6516

Mana P, Goodyear M, Bernard C, Tomioka R, Freire-Garabal M, Linares D (2004) Tolerance induction by molecular mimicry: prevention and suppression of experimental autoimmune encephalomyelitis with the milk protein butyrophilin. Int Immunol 16:489–499

Masters SL, Palmer KR, Stevenson WS, Metcalf D, Viney EM, Sprigg NS, Alexander WS, Nicola NA, Nicholson SE (2005) Genetic deletion of murine SPRY domain-containing SOCS box protein 2 (SSB-2) results in very mild thrombocytopenia. Mol Cell Biol 25:5639–5647

Masters SL, Yao S, Willson TA, Zhang JG, Palmer KR, Smith BJ, Babon JJ, Nicola NA, Norton RS, Nicholson SE (2006) The SPRY domain of SSB-2 adopts a novel fold that presents conserved Par-4-binding residues. Nat Struct Mol Biol 13:77–84

Meroni G, Diez-Roux G (2005) TRIM/RBCC, a novel class of 'single protein RING finger' E3 ubiquitin ligases. Bioessays 27:1147–1157

Mnayer L, Khuri S, Merheby HA, Meroni G, Elsas LJ (2006) A structure-function study of MID1 mutations associated with a mild Opitz phenotype. Mol Genet Metab 87:198–203

Nisole S, Stoye JP, Saib A (2005) TRIM family proteins: retroviral restriction and antiviral defence. Nat Rev Microbiol 3:799–808

Papin S, Cuenin S, Agostini L, Martinon F, Werner S, Beer HD, Grutter C, Grutter M, Tschopp J (2007) The SPRY domain of Pyrin, mutated in familial Mediterranean fever patients, interacts with inflammasome components and inhibits proIL-1beta processing. Cell Death Differ 14:1457–1466

Perez CF, Mukherjee S, Allen PD (2003a) Amino acids 1–1,680 of ryanodine receptor type 1 hold critical determinants of skeletal type for excitation-contraction coupling. Role of divergence domain D2. J Biol Chem 278:39644–39652

Perez CF, Voss A, Pessah IN, Allen PD (2003b) RyR1/RyR3 chimeras reveal that multiple domains of RyR1 are involved in skeletal-type E-C coupling. Biophys J 84:2655–2663

Perron MJ, Stremlau M, Sodroski J (2006) Two surface-exposed elements of the B30.2/SPRY domain as potency determinants of N-tropic murine leukemia virus restriction by human TRIM5alpha. J Virol 80:5631–5636

Ponting C, Schultz J, Bork P (1997) SPRY domains in ryanodine receptors (Ca(2 +)-release channels). Trends Biochem Sci 22:193–194

Proenza C, Wilkens CM, Beam KG (2000) Excitation-contraction coupling is not affected by scrambled sequence in residues 681–690 of the dihydropyridine receptor II-III loop. J Biol Chem 275:29935–29937

Protasi F, Paolini C, Nakai J, Beam KG, Franzini-Armstrong C, Allen PD (2002) Multiple regions of RyR1 mediate functional and structural interactions with alpha(1S)-dihydropyridine receptors in skeletal muscle. Biophys J 83:3230–3244

Reddy BA, Etkin LD, Freemont PS (1992) A novel zinc finger coiled-coil domain in a family of nuclear proteins. Trends Biochem Sci 17:344–345

Rhodes DA, de Bono B, Trowsdale J (2005) Relationship between SPRY and B30.2 protein domains. Evolution of a component of immune defence? Immunology 116:411–417

Richardson MW, Carroll RG, Stremlau M, Korokhov N, Humeau LM, Silvestri G, Sodroski J, Riley JL (2008) Mode of transmission affects the sensitivity of human immunodeficiency virus type 1 to restriction by rhesus TRIM5alpha. J Virol 82:11117–11128

Samso M, Shen X, Allen PD (2006) Structural characterization of the RyR1-FKBP12 interaction. J Mol Biol 356:917–927

Schweiger S, Foerster J, Lehmann T, Suckow V, Muller YA, Walter G, Davies T, Porter H, van Bokhoven H, Lunt PW, Traub P, Ropers HH (1999) The Opitz syndrome gene product, MID1, associates with microtubules. Proc Natl Acad Sci USA 96:2794–2799

Shtifman A, Ward CW, Yamamoto T, Wang J, Olbinski B, Valdivia HH, Ikemoto N, Schneider MF (2002) Interdomain interactions within ryanodine receptors regulate Ca^{2+} spark frequency in skeletal muscle. J Gen Physiol 119:15–32

Song B, Javanbakht H, Perron M, Park DH, Stremlau M, Sodroski J (2005) Retrovirus restriction by TRIM5alpha variants from Old World and New World primates. J Virol 79:3930–3937

Stange M, Tripathy A, Meissner G (2001) Two domains in dihydropyridine receptor activate the skeletal muscle Ca(2+) release channel. Biophys J 81:1419–1429

Stojanov S, Kastner DL (2005) Familial autoinflammatory diseases: genetics, pathogenesis and treatment. Curr Opin Rheumatol 17:586–599

Stremlau M, Owens CM, Perron MJ, Kiessling M, Autissier P, Sodroski J (2004) The cytoplasmic body component TRIM5alpha restricts HIV-1 infection in Old World monkeys. Nature 427:848–853

Styhler S, Nakamura A, Lasko P (2002) VASA localization requires the SPRY-domain and SOCS-box containing protein, GUSTAVUS. Dev Cell 3:865–876

Tae HS, Norris NC, Cui Y, Karunasekara Y, Board PG, Dulhunty AF, Casarotto MG (2009) Molecular recognition of the disordered dihydropyridine receptor II-III loop by a conserved SPRY domain of the type 1 ryanodine receptor. Clin Exp Pharmacol Physiol 36:346–349

Tanabe T, Beam KG, Adams BA, Niidome T, Numa S (1990) Regions of the skeletal muscle dihydropyridine receptor critical for excitation-contraction coupling. Nature 346:567–569

Tazi-Ahnini R, Henry J, Offer C, Bouissou-Bouchouata C, Mather IH, Pontarotti P (1997) Cloning, localization, and structure of new members of the butyrophilin gene family in the juxta-telomeric region of the major histocompatibility complex. Immunogenetics 47:55–63

Trusolino L, Comoglio PM (2002) Scatter-factor and semaphorin receptors: cell signalling for invasive growth. Nat Rev Cancer 2:289–300

Wang D, Li Z, Messing EM, Wu G (2005) The SPRY domain-containing SOCS box protein 1 (SSB-1) interacts with MET and enhances the hepatocyte growth factor-induced Erk-Elk-1-serum response element pathway. J Biol Chem 280:16393–16401

Wilkens CM, Kasielke N, Flucher BE, Beam KG, Grabner M (2001) Excitation-contraction coupling is unaffected by drastic alteration of the sequence surrounding residues L720–L764 of the alpha 1S II-III loop. Proc Natl Acad Sci USA 98:5892–5897

Woo JS, Suh HY, Park SY, Oh BH (2006) Structural basis for protein recognition by B30.2/SPRY domains. Mol Cell 24:967–976

Yao S, Liu MS, Masters SL, Zhang JG, Babon JJ, Nicola NA, Nicholson SE, Norton RS (2006) Dynamics of the SPRY domain-containing SOCS box protein 2: flexibility of key functional loops. Protein Sci 15:2761–2772

Yap MW, Nisole S, Stoye JP (2005) A single amino acid change in the SPRY domain of human Trim5alpha leads to HIV-1 restriction. Curr Biol 15:73–78

Yin CC, Blayney LM, Lai FA (2005) Physical coupling between ryanodine receptor-calcium release channels. J Mol Biol 349:538–546

Zhai L, Dietrich A, Skurat AV, Roach PJ (2004) Structure-function analysis of GNIP, the glycogenin-interacting protein. Arch Biochem Biophys 421:236–242

Zhu X, Gurrola G, Jiang MT, Walker JW, Valdivia HH (1999) Conversion of an inactive cardiac dihydropyridine receptor II-III loop segment into forms that activate skeletal ryanodine receptors. FEBS Lett 450:221–226