Mapping domains and mutations on the skeletal muscle ryanodine receptor channel

Jean H. Hwang[1], Francesco Zorzato[2,3], Nigel F. Clarke[1,4]*, Susan Treves[2,3]*

1. Institute for Neuroscience and Muscle Research, Children's Hospital at Westmead, Sydney, 2145, Australia
2. Departments of Anesthesia and Biomedizin, Basel University Hospital, 4031 Basel, Switzerland
3. Department of Experimental and Diagnostic Medicine General Pathology section, University of Ferrara, Ferrara, 44100 Italy
4. Discipline of Paediatrics and Child Health, University of Sydney, Sydney, NSW 2006 Australia

* These authors have contributed equally to the work.

Corresponding author: Treves, S. (susan.treves@unibas.ch)

Key Words: ryanodine receptor type 1, excitation-contraction coupling, calcium signalling, skeletal muscle, protein interactions, domains, disease pathogenesis

ABSTRACT

The skeletal muscle ryanodine receptor isoform 1 (RyR1) is a calcium release channel involved in excitation-contraction coupling, the process whereby an action potential is translated to a cytoplasmic Ca^{2+} signal that activates muscle contraction. Dominant and recessive mutations in *RYR1* cause a range of muscle disorders, including malignant hyperthermia and several forms of congenital myopathies. Many aspects of disease pathogenesis in ryanodinopathies remain uncertain, particularly for those myopathies due to recessive mutations. A thorough understanding of the ryanodine receptor macromolecular complex and its interactions with proteins and small molecular modulators is an essential starting point from which to investigate disease mechanisms.

Ryanodine Receptor Function in Normal and Diseased Muscle

Rapid changes in the intracellular calcium concentration ($[Ca^{2+}]_i$) are important signalling events in most eukaryotic cells, necessitating tight regulation of cytoplasmic Ca^{2+} levels. Global increases in $[Ca^{2+}]_i$ are triggered by the release of Ca^{2+} from the endoplasmic reticulum (ER)/sarcoplasmic reticulum (SR) intracellular stores and/or by calcium influx from the extracellular environment via opening of specific plasma membrane channels. The primary organelle involved in storing rapidly releasable Ca^{2+} is the SR in striated muscles and the ER in most other mammalian cell types. Two families of channels are responsible for mediating rapid calcium release from these intracellular stores: ryanodine receptors (RyRs) which are predominantly expressed in excitable cells and inositol 1,4,5-triphosphate receptors (IP_3Rs) which are expressed in most cells.

The skeletal muscle SR calcium release channel protein was first identified at the biochemical level in the 1980s and was named for its ability to bind the plant alkaloid *ryanodine*. Three RyR isoforms, encoded by separate genes sharing a high level of homology, have been identified in mammalian tissues. Ryanodine receptor type 1 (RyR1) is the primary isoform expressed in skeletal muscle. RyR1 is also expressed in some areas of the central nervous system and in some haemopoietic cells. RyR2 is the predominant isoform in cardiac muscle and is expressed in various regions of the brain whereas RyR3 has widespread expression, particularly during development. In skeletal muscle, RyR1 is a key protein involved in excitation-contraction (EC) coupling. In a simplified overview of this process, an electrical signal generated by an action potential travelling along the transverse (t)-tubules is detected by the dihydropyridine receptor (DHPR), a voltage-sensitive Ca^{2+} channel. In response to membrane depolarization, DHPR channels undergo a conformational change and induce the opening of RyR1 channels that are closely apposed and located on the SR terminal cisternae. This leads to the release of Ca^{2+} from SR stores into the cytoplasm. The cytoplasmic Ca^{2+} binds to troponin and initiates muscle contraction in sarcomeres. Muscle contraction is terminated upon closure of RyR1 and reuptake of Ca^{2+} from the cytoplasm into the SR by sarcoplasmic/endoplasmic Ca^{2+} ATPase (SERCA) pumps.

Ablation of RyR1 in mice (dyspedic mice) results in a lethal phenotype most likely due to respiratory failure. Mutant neonates also display skeletal abnormalities, including spined curvature, arched vertebral column, thin limbs and a thick neck, and resembled dysgenic mice that lack DHPR [1]. Primary dyspedic muscle cell cultures exhibit severely impaired Ca^{2+} release after depolarization, demonstrating the pivotal role RyR1 plays in skeletal muscle EC coupling. Over the

past decades it has emerged that, mutations in *RYR1* are a major cause of muscle disease. Both dominant and recessive mutations have been identified throughout the *RYR1* coding sequence and are responsible for a wide range of muscle disorders including malignant hyperthermia (MH), central core disease (CCD), multi-minicore disease, centronuclear myopathy, core-rod myopathy and congenital fiber type disproportion (Box 1) [2-4]. Dantrolene, used in the emergency treatment of malignant hyperthermia, is currently the only clinically relevant drug for RyR1 disorders and drugs that address the chronic, often life-threatening muscle weakness associated with many RyR1 disorders are much needed.

The aim of this review is to summarize the current understanding of RyR1 structure and function and, in particular, the domains that mediate RyR1-protein and RyR1-ion interactions, which finely regulate channel function. The interactions of RyR1 with a host of modulators, such as the dihydropyridine receptor (DHPR), FKBP12, homer, calmodulin, S100A1, and calcium have been well studied and are summarized in Tables 1 and 2 and Figure 1. By comparing this information with recently reported recessive mutations, it can be seen that many of these mutations fall within RyR1 protein binding domains , leading to the hypothesis that abnormal RyR1-protein interactions may be an underlying cause of ryanodine receptor malfunction and disease (Table 3).

Structure of the ryanodine receptor

The ryanodine receptor is the largest known ion channel and forms a homotetrameric structure of approximately 2.3 MDa. Each 565-kDa monomeric subunit consists of 5038 amino acids. Approximately four-fifths of the channel is made up of the large N-terminal cytoplasmic domain, which contains binding sites for a number of modulators. The C-terminal region contains the transmembrane domains and ion-conducting pore. RyR1 is characterized by a very large-conductance with moderate selectivity for divalent cations over monovalent cations [5]. The selectivity filter contains a widely conserved sequence (Gly-X-Arg-X-Gly_3-X-Gly-Asp) found in several different cation channels, including potassium channels and IP_3Rs. Aspartate 4900 and glycine within the RyR1 motif 4895Gly_3-Ile-Gly-Asp are particularly important for ion selectivity (unless otherwise indicated all amino acid numbers refer to the human sequence)[6, 7]. Figure 2 illustrates the overall structural information of the RyR1, including the distinct cytoplasmic and transmembrane assemblies. For detailed reviews, see [8, 9].

Interdomain Interactions

Cryo-electron microscopy (cryo-EM) studies indicate that the RyR undergoes widespread long-range conformational changes during the transition from closed to open states and that binding of various modulators increases the probability of the 'on' or 'off' state [10-12]. A domain-switch hypothesis has been proposed to explain the transition between 'on' and 'off' states [13], whereby several subdomains throughout the MH/CCD hotspots N-terminal domain 1 (amino acids 35-614) and the central domain 2 (amino acids 2163-2458) interact to stabilize the basal closed conformational state (Figure 3). Upon activation, the interdomain interactions are disrupted, leading to a conformational change in which the channel 'opens'. Perturbation of the interdomain interactions with a monoclonal antibody against the N-terminal domain 1 results in RyR1 channel activation [14]. Interestingly, many pathogenic dominant RyR1 mutations associated with MH fall within MH/CCD hotspots of domains 1 and 2. It is thought that some of the mutations associated with MH weaken interdomain interactions and decrease the energy barrier required for channel opening, resulting in hypersensitive channels [15].

Alternative Splicing

Analysis of rabbit RyR1 cDNA reveals two alternatively spliced regions that likely modulate RyR1 channel properties by altering interdomain interactions or interactions with other proteins. Exclusion of exon 70 results in the ASI(-) RyR1 isoform, lacking five residues (3481-3485), and the exclusion of exon 83 results in the ASII(-) isoform, which also lacks five residues (3865-3870) [16]. There is conflicting data on whether variant expression is tissue-specific and developmentally regulated. Zorzato and colleagues reported that both AS variants are found in fast- and slow-twitch rabbit skeletal muscles irrespective of their developmental stage [17], whereas others have shown that the ASI(-)/ASII(-) forms predominate during early development and the ASI(+)/ASII(+) forms predominate in adulthood [16, 18]. Abnormal overexpression of the ASI(-) isoform of RyR1 occurs in myotonic dystrophy type 1 and may contribute to muscle dysfunction [18].

Data describing the functional consequences of expressing alternatively spliced variants is inconclusive. Peptide studies suggest that the ASI region together with an adjacent basic amino acid sequence (the combined region Thr3471-Gly3500) are involved in interdomain interactions that stabilize the closed channel conformation, and that the ASI(-) variant stabilizes the closed channel more effectively [19]. Less is known about the function of the ASII region. Both alternatively spliced sequences sit within a broader region encompassing: (i) a calmodulin binding

region and (ii) the Arg-Gly-Asp (RGD) adhesive sequence, a well-established protein-binding motif; [16].

Interactions with Cytoplasmic Protein Modulators

RyR1 interacts with a large number of proteins and ionic modulators. Some protein partners have been investigated in detail and their corresponding binding sites on RyR1 determined with confidence whereas details for others are sparse and uncertain.

Dihydropyridine Receptor (L-type Ca^{2+} channel)

In skeletal muscle, there is a regular geometric arrangement of RyR1 and DHPR channels in which alternate RyR1 channels associate with four DHPR channels [5]. This close association permits direct signalling between the DHPR$\alpha_{1.1}$ and RyR1 both in the forward direction (orthograde signalling in which DHPR activation causes the RyR1 to open) and retrograde signalling (whereby RyR1 interactions enhance current through the DHPR channel) [20, 21]. DHPRs are composed of 5 subunits The $\alpha_{1.1}$ subunit contains the voltage sensor, forms the pore region of the channel, and is believed to be one of the main sites of interaction with RyR1, though recent research supports the importance of other sites [21, 22]. Specifically, there is good evidence that the $\alpha_{1.1}$ II-III loop (between the second and third transmembrane domains of the $\alpha_{1.1}$ subunit) and the β_{1a} subunit bind to different regions of RyR1.

Although experiments using RyR1/RyR2 chimeras and RyR1 peptide fragments have sometimes yielded contrasting results [21, 23-27], current evidence implicates the SPRY2 domain, located in the N-terminal cytoplasmic region of RyR1. SPRY domains are protein-protein interaction motifs defined by a characteristic pattern of β-sheets. The RyR1 isoform has three highly conserved SPRY domains (Table 2). Interestingly, cryo-EM mapping of the SPRY2 domain reveals that its location on the RyR1 overlaps with the previously proposed positioning of DHPR [28]. *In vitro* studies indicate that the SPRY2 region binds the DHPR $\alpha_{1.1}$ II-III loop [27, 29] and may also participate in an interdomain interaction within RyR1 [19], binding to the broader ASI region via the positive amino acid cluster Lys3495-Arg3502, which is homologous to a similar sequence on the II-III loop of the DHPR $\alpha_{1.1}$ subunit. The physiologic importance of this SPRY2/ASI interaction is currently uncertain, as are the roles of the SPRY1 and SPRY3 domains.

Aside from the $\alpha_{1.1}$ subunit, there is good evidence that RyR1 also directly interacts with the β_{1a} subunit (Table 2) [22, 30] and that the strength of this interaction is governed by the

positively charged motif Lys(3495)-Lys-Lys-Arg-Arg-Gly-Asp-Arg(3502) [30]. The β_{1a} subunit may play a dual role in augmenting EC coupling, both by activating RyR1 during EC coupling, and by targeting the DHPR to precisely aligned tetrad arrays on the t-tubules that oppose the RyR1 complex (reviewed in [31]).

Early studies implicated other RyR1 regions in DHPR interactions but the domains are large and their importance is uncertain. The second of three divergent regions (D1-D3), so named because the sequences represent areas of variance between RyR isoforms, has been proposed as a DHPR binding site because the presence of residues 1272-1455 (region D2) rescues skeletal-type EC coupling [21, 32, 33], whereas deletion of this region abolished this coupling [34]; however, other studies dispute this finding [35]. In addition, D2 has been shown to be critical in the formation of DHPR tetrads [21]. Experiments using chimeric RyR containing segments of different isoforms (ie: RyR1/RyR2 or RyR1/RyR3 chimera) suggest that RyR1 residues 1837–2168 (rabbit cDNA), contribute to both EC coupling and calcium release and residues 2659-3720 may be important in retrograde signalling [25, 35].

Calmodulin (CaM)

Calmodulin is a ubiquitously expressed, highly-conserved, 17-kDa protein that binds the cytoplasmic surface of the ryanodine receptor and modulates its activity in a complex manner. Four calmodulin molecules bind per RyR subunit. Calmodulin differentially regulates RyR1 activity depending on whether or not it has Ca^{2+} bound. In its Ca^{2+}-free state (apoCaM), it is a weak partial agonist whereas the Ca^{2+}-bound form (CaCaM) is a stronger antagonist of RyR calcium release [36]. Thus, calmodulin likely acts as a cytoplasmic calcium sensor for RyR. It may also affect channel activity by influencing binding to DHPR (which binds RyR1 in close spatial proximity) or by affecting RyR1 interdomain interactions [37, 38].

Calmodulin binding sites on RyR1 have been extensively researched and although a number of different sequences have been proposed based on the experimental protocols used, there is some consensus regarding the importance of particular sequences (Figures 1 and 2, Table 2). Although apoCaM and CaCaM bind to distinct domains of RyR1, some studies show that residues 3614-3643 and 2937-3225, among others, bind both forms of calmodulin [39-42]. There is evidence that CaCaM binds residues 3553-3662 and 4303-4431 [40, 41]. More recent data also suggests that the N-terminus of calmodulin interacts with sequence 1975-1999 [43]. Interestingly, residues 1975-1999 and 3614-3643 are likely to be in close proximity in the three-dimensional

RyR1 structure which would make it feasible for one CaM molecule to bind both sites simultaneously [43].

S100A1

Like calmodulin, the S100 proteins are cytoplasmic calcium-binding proteins containing two EF-hand motifs. S100A1 is one of at least 21 different S100 isoforms expressed in humans, and is most highly expressed in cardiac and skeletal muscle where it regulates SR calcium release and mitochondrial function. There is reasonable consensus regarding the S100A1 binding domains on RyR1 (Figure 1 and Table 2). Competition assays using CaCaM and binding experiments with RyR1 peptides defined residues 3616-3627 as the Ca^{2+}-S100A1 binding site and show that Ca^{2+}-S100A1 likely competes with CaCaM for this binding domain [44]. This finding is not surprising, as S100A1 proteins and calmodulin bind similar structural motifs in other proteins. The physiological consequence of the competitive binding of S100A1 and calmodulin to RyR is not well understood.

Physiologically relevant concentrations of S100A1 can activate RyR1 *in vitro*. When applied to purified RyR1 channels reconstituted in lipid bilayers, S100A1 enhances the normalized open probability of RyR1 at nM calcium concentrations [45]. S100A1 -/- knockout mice and mice containing a mutation in the S100A1 binding domain on RyR1 exhibit reduced SR calcium release, reduced global cytoplasmic calcium transients, and reduced force generation [46-48]. Further experiments suggested that the S100A1 protein is important in activating RyR1 in response to a single action potential [47, 48]. With repetitive action potentials, S100/CaM binding may have an inhibitory effect because mouse muscles carrying a mutation that eliminates S100/CaM binding showed increased SR calcium release when stimulated at 100 Hz [48].

In addition to residues 3616-3627, initial studies that measured the ability of digoxigenin-labelled S100A1 to bind RyR1 found evidence for three other binding regions (amino acids 1861-2155, 3773-3873, and 4426-4622) [45].

FKBP12 (also known as FK506BP, calstabin)

The FK506-binding proteins (FKBPs) are immunophilins, a family of highly conserved proteins that bind immunosuppressive drugs, such as FK506 (tacrolimus). FKBPs are expressed in most tissues and are involved in a variety of cellular processes including protein folding, receptor signalling, and transcription. Two isoforms, FKBP12 and FKBP12.6 (also known as calstabin 1 and 2, respectively) can interact with all three RyR isoforms, but likely have distinct roles owing to

different binding affinities and patterns of tissue expression. Specifically, FKBP12 is the predominant isoform expressed in skeletal muscle [49], whereas FKBP12.6 co-purifies with RyR2 in the heart.

In normal skeletal muscle, FKBP12 binds to the cytoplasmic side of RyR with high affinity and stabilizes the closed conformation. Experiments using immunosuppressive drugs that disrupt FKBP12 binding to RyR and FKBP-deficient mice have shown that loss of FKBP binding increases the open probability of the ryanodine receptor and induces the appearance of sub-conductance states of the channel [50, 51]. In addition to inhibiting RyR, FKBP12 likely influences EC coupling by modifying orthograde signalling and possibly retrograde signalling between RyR1 and DHPR [52-54]. FKBP12 has also been implicated in coordinating gating between adjacent RyR molecules, which results in neighbouring channels opening simultaneously in response to a voltage change [55].

Four FKBP12 molecules can bind to one ryanodine receptor tetramer and three-dimensional cryo-EM difference mapping and FRET studies have revealed that the FKBP12 binding site is close to the clamp domain [56, 57]. A study that mapped four particular mutations (human residues Glu160, Arg163, Arg401, and Ile403) known to cause malignant hyperthermia and central core disease onto the cryo-EM RyR1 structure suggest that the mutations may reside within the FKBP12 binding region [58], but this has not been experimentally confirmed. Several lines of evidence implicate residues 2458-2468 as an important FKBP12 binding site on RyR1 with amino acid 2461 being particularly important [53].

Interactions with SR Luminal Proteins

The ryanodine receptor is also modulated by proteins that reside in the SR lumen, primarily triadin, junctin and calsequestrin (CSQ), which likely function in a coordinated manner.

Triadin and Junctin

Triadin and junctin are two SR luminal proteins that interact with RyR to regulate its function. Both are integral SR membrane proteins with similar features, such as a short cytoplasmic N-terminal tail, a single transmembrane domain, and a longer C-terminal tail that extends into the SR lumen. The C-terminal tails of both proteins contain multiple clusters of alternating lysine and glutamic acid residues (KEKE motifs) that likely bind CSQ and possibly RyR as

well [59, 60]. Recent research emphasizes the importance of RyR2-triadin interactions for channel function in the heart. In skeletal muscle, triadin knockdown affects voltage-dependent calcium release and reduces muscle function [61, 62], whereas disruption of triadin binding reduces both voltage- and ligand-gated SR calcium release, suggesting that triadin may play a role in skeletal muscle EC coupling [63]. The primary triadin binding site on RyR1 has determined by *in vitro* binding and site-directed mutagenesis is the second intraluminal loop of RyR1 (residues 4861-4918). In particular, three acidic residues (Asp4879, Asp4908, and Glu4909) are particularly important [60]. The second intraluminal loop may also contain the pore and selectivity filter of the calcium channel. A number of dominant *RYR1* mutations found in patients with central core disease fall within the triadin binding domain, but their effects on protein-protein interaction have not been investigated.

It has been suggested that junctin may be the main protein mediator between CSQ and RyR [64]. Knockdown of junctin in myotubes reduces both depolarization-induced SR Ca^{2+} release and reduced SR Ca^{2+} stores [61], possibly by disrupting the signalling complex between CSQ and RyR. The role of junctin in maintaining SR Ca^{2+} stores as well as its binding site on RyR1 remain uncertain.

Calsequestrin (CSQ)

CSQ is the primary calcium-binding protein in the SR lumen and its high-capacity/low-affinity binding properties for calcium ions make it a highly effective calcium storage protein, with around 40-50 calcium ions bound per CSQ molecule in skeletal muscle [65]. Though *in vitro* studies demonstrate the ability of CSQ to bind directly to RyR1, it is uncertain whether this occurs *in vivo* or if CSQ interacts with RyR through triadin/junctin interactions [66]. Purified RyR channels freed from triadin and junctin were not inhibited in response to exogenous CSQ, suggesting that CSQ does not bind directly to RyR1 [67].

Other Potential Protein Modulators

A number of other potential protein modulators of the RyR1 channel have been discovered but the specific RyR1 binding sites are currently undefined for these proteins.

Calcium Homeostasis Endoplasmic Reticulum Protein (CHERP)

CHERP is a recently identified integral SR/ER membrane protein shown to interact with RyR1 through pull-down and co-localization studies [68]. CHERP participates in cytosolic Ca^{2+} cycling within cells and affects diverse cellular processes, such as DNA synthesis, growth and proliferation [69]. siRNA-mediated suppression of CHERP expression in HEK-293 cells transfected with the full-length RyR1 resulted in decreased calcium flux through the ryanodine receptor [68] but further work is required to confirm that CHERP is a direct binding partner of RyR1 *in vivo* and to clarify its physiological role.

Homer/Ves1

Homer proteins are high-affinity ligands that modify RyR channel function. Within neurons and other tissues, homer proteins are important anchoring or adaptor proteins that facilitate the formation of multimeric protein complexes [70]. Homer may play a similar role in the SR by stabilizing interactions between RyR1 and possibly DHPR. Different homer isoforms (e.g. 1b, 1c, and 2b) activate RyR1 in single-channel experiments, induce calcium release from SR vesicles, and increase the frequency of calcium sparks from permeabilized skeletal muscle fibers (reviewed in [71]).

Homer proteins contain EVH1 domains that facilitate binding to proline-rich consensus sequences on target proteins, defined by Pro-Pro-X-X-ϕ (where X=any amino acid and ϕ=aromatic residue), flanked by charged residues [71]. There are many homer consensus sequences in RyR1 (Figure 1 and Table 2).

Selenoprotein

Selenoproteins contain a selenocysteine residue (a modified cysteine residue where selenium replaces the sulphur moity), and are implicated in diverse processes that capitalize on the high redox potential of the selenium ion. One study demonstrated that selenium compounds induce calcium release from SR vesicles, affect ryanodine binding at varying concentrations, and stimulate or inhibit RyR by binding free thiols within the protein [72]. Selenoprotein N (SelN) is a SR transmembrane protein found to be associated with RyR1 *in vivo* and required for normal muscle development and differentiation in zebrafish muscle [73]. When SelN is absent, RyR loses its normal sensitivity to redox modulation [73]. Mutations in *SEPN1*, which encodes SelN, cause a skeletal myopathy with many histological and phenotypic similarities to *RYR1*-related multi-minicore disease and it is hypothesized that the disease mechanism may involve abnormal redox

status and function of RyR1 [74]. It has not yet been clarified whether SelN and RyR1 interact directly and, if they do, the binding sites that are involved.

Interactions with Ions and Small Molecular Modulators

Aside from protein ligands, the ryanodine receptor is also regulated by several ions and small molecules, including calcium, magnesium, and ATP. Calcium and magnesium, in particular, likely interact at numerous sites to influence channel function.

Calcium (Ca^{2+})

Ca^{2+} modulates RyR1 function both directly and indirectly through interactions with both the cytoplasmic and SR luminal surfaces of RyR1. The indirect effect is mediated primarily by calmodulin and CaMKII, but also via S100A1, triadin, junctin, and calsequestrin (discussed above). The direct effects of calcium on channel function are discussed in this section.

The Ca^{2+} concentration in the SR lumen may directly modulate calcium release from RyR1. In lipid bilayer single channel experiments as the SR lumen Ca^{2+} levels are increased there is an enhanced sensitivity of RyR1 to cytosolic agonists, such as caffeine and ATP [75-77]. However, it remains uncertain whether there are specific Ca^{2+}-binding sites on the SR luminal face of RyR1 or whether Ca^{2+} can modulate activity by passing through the channel pore and binding to sites on the cytoplasmic surface. Recent evidence suggests the presence of luminal sites in RyR2 [78, 79].

RyR1 activity exhibits a bell-shaped dependence on cytoplasmic Ca^{2+} arising from the binding of Ca^{2+} to two distinct classes of binding sites: low [Ca^{2+}] bind to high-affinity activation sites and high [Ca^{2+}] bind to low-affinity inactivation sites. Evidence suggests that the C-terminal third of RyR1 contains several important high-affinity Ca^{2+}-binding sites [80]. Polyclonal antibodies raised against residues 4380-4625 inhibit Ca^{2+}-induced calcium release from isolated SR [81]. Overlay studies and site-directed polyclonal antibodies indicate Ca^{2+}-binding residues at 4245-4378 and 4479-4515 [82]. A negatively charged Pro-Glu repeat sequence (PEPEPEPEPEPE) at residues 4490-4502 is likely the critical Ca^{2+}-binding domain in this region because antibodies raised against this sequence inhibit Ca^{2+}-dependent RyR1 activation [83]. Site-directed mutagenesis provides evidence for physiologically-important calcium-binding at human RyR1 residue Glu4031 (equivalent to rabbit RyR3 residue Asp3885) [84]. The RyR1 likely has other high-affinity Ca^{2+}-binding sites that have yet to be identified.

Less is known about the low-affinity Ca^{2+}-inactivation sites and the available data is contradictory. A truncated RyR channel lacking residues 1-3660 failed to close at high [Ca^{2+}], which may indicate that Ca^{2+} inactivation sites are located in the N-terminal foot of the protein [85]; however, the absence of such a large portion of the protein likely affects the overall structure of the RyR1. Other studies suggest the importance of the C-terminus for inactivating Ca^{2+}-binding sites. Indeed, RyR1/RyR2 chimeras containing an RyR2 C-terminal domain show reduced inhibition of the channel at elevated Ca^{2+} levels, suggesting that the C-terminal region (residues 3726-5038) contains important Ca^{2+}-inactivation sites [86]. Specifically, mutation of a putative EF hand-homology region (4080-4091) greatly enhances channel activity, supporting the idea that this region is an important calcium-inactivation site [87]. Other possible Ca^{2+}-inactivation sites have been identified throughout the RyR1 protein sequence such as between residues 4063-4209 [88].

Magnesium

The effect of magnesium on channel function is intimately linked to Ca^{2+} levels. Based on single channel experiments and studies of calcium release from SR vesicles, there is good evidence that Mg^{2+} competes with Ca^{2+} for the high-affinity Ca^{2+}-activation binding sites and prevents channel activation. A second likely mechanism involves the low-affinity Ca^{2+}-inactivation binding sites, which are less-selective to divalent ions and permit Mg^{2+} to inhibit channel function [89]. It was recently shown that the relative concentrations of SR luminal Ca^{2+} and Mg^{2+} play an important role in determining the unitary calcium current amplitude through the RyR pore. As the SR Mg^{2+} load increases the open probability decreases, leading to lower intracellular calcium levels [90]. The current model predicts that the Mg^{2+} binding sites on RyR1 overlap with those for Ca^{2+}.

ATP

Overall, ATP is an activator of RyR1 but the strength of its effects depends on the concentration of Ca^{2+}, Mg^{2+} and other exogenous modulators that may modify the number of accessible ATP binding sites [91, 92]. In [^{3}H]ryanodine binding and single channel measurements, ATP only minimally activates the channel at low Ca^{2+} concentrations and µM Ca^{2+} is required for maximal activation of RyR by ATP [91]. Other adenine nucleotides, such as AMP and adenosine, are even weaker RyR1 agonists.

Based on a proposed nucleotide binding motif, there are several possible ATP binding sites on RyR1. Photoaffinity labelling has confirmed possible ATP-binding regions [93]; however, further resolution of these segments is required.

NADH and NADH Oxidase

Cyclic adenosine diphosphate (cADP) ribose and nicotinamide-adenine dinucleotides (NAD^+/NADH) modulate RyR function in an isoform specific way. cADP ribose increases Ca^{2+} transients through RyR1, either by acting directly on the receptor or by affecting ancillary protein binding partners [94]. NAD species (NAD^+/NADH, $NADP^+$/NADPH) are redox-based signalling molecules affecting a wide range of biological pathways. Direct redox modulation of reactive thiol groups on the RyR has been described (see "Redox Modulation" section), but the mechanism by which NAD species affect RyR channel function may be isoform-specific. Some studies demonstrate that both NAD+ and NADH activate RyR1 [95, 96], while others found that NADH has minimal effects [97]. NAD species may influence RyR1 activity through several mechanisms. NAD^+/NADH may interact directly with RyR1 at ATP-binding sites due to structural similarities between these two molecules [95]. An NADH-dependent oxidase (NOX) present in the SR membrane interacts with RyR1 and reduces reduced oxygen to superoxide, which in turn activates RyR1 [96]. In addition, a potential oxidoreductase-like domain is present in the N-terminus of RyR1 (residues 40-419) that may bind NAD^+ and act as a redox sensor discussed in [58].

Posttranslational Modification of the Ryanodine Receptor

The ryanodine receptor undergoes posttranslational modification through a range of physiologic processes such as phosphorylation, oxidation, and nitrosylation, all of which may be elicited by stress-dependent signalling pathways [98]. The effects of these modifications on the ryanodine receptor are complex and controversial, but recent studies are beginning to clarify this field.

Kinases and Phosphatases

The ryanodine receptor can be phosphorylated and dephosphorylated by a range of kinases and phosphatases such as protein kinase A (PKA), Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), cGMP-dependent kinase, and protein phosphatase (PP) [99-102]. The largest

body of data relates to RyR2 in cardiac muscle. Most studies indicate that phosphorylation by PKA, which is activated by β-adrenergic signals, activates RyR2 and Ca^{2+} release, thereby inducing a positive inotropic effect on the heart [103]. Initial studies in skeletal muscle found variable effects of phosphorylation on channel activity, but there is an emerging consensus that phosphorylation by PKA or CAMKII activates RyR1 [100, 104, 105].

There is good evidence that interactions between RyR and various kinases and phosphatases require binding of specific targeting proteins to highly conserved leucine/isoleucine zipper (LIZ) motifs located on RyR channels [106]. These LIZ motifs interact with targeting proteins, such as muscle A-kinase anchoring protein (mAKAP) and spinophilin, which localize specific kinases and phosphatases, such as PKA and protein phosphatase I (PPI) respectively, to the RyR. Although these results were first demonstrated for the RyR2 macromolecular complex, the RyR2 LIZ motifs that bind PKA and PPI are highly conserved in RyR1. RyR1 residues 553-602 co-precipitated with PPI and residues 3039-3075 co-precipitated with PKA [106]. A consensus sequence (Arg-His-Arg-(Val)-Ser-Leu) within the calmodulin binding domain also binds PKA (residues 3051-3056) and Ser3055 is the likely phosphorylation target [107]. Ser3055 phosphorylation is inhibited when calmodulin binds RyR1 and the binding of calmodulin is inhibited by Ser3055 phosphorylation.

Phosphorylation of Ser2843 occurs *in vivo* and there are several further potential phosphorylation consensus sites in RyR1 that require confirmation (Figure 1). Phosphorylation at Ser2843 may increase the sensitivity of RyR1 to cytoplasmic Ca^{2+} and promote release of FKBP from RyR1 thereby destabilizing the closed conformation [103] but other studies challenge this finding [108].

Redox Modulation

It is well-established that RyR channel function is altered by reversible redox processes, such as cysteine (disulphide) oxidation, *S*-nitrosylation, and *S*-glutathionylation. Abnormal oxidation of RyR1 has been implicated in several disease states, such as muscular dystrophy and heart failure [98, 109] but a detailed understanding is lacking.

Reactive oxygen species and reactive nitrogen species are continuously generated within active skeletal muscle and can modulate muscle function. RyR1 is a major cellular redox sensor in skeletal muscle, having ~100 cysteine thiol groups on its surface. Three general classes of thiols are proposed: (i) most are likely inert and resist oxidation during normal activity; (ii) 6-8 thiol groups are hyper-reactive and sensitive to the local oxidative environment, modulating RyR1 channel

function in conditions of moderate oxidative stress such as during vigorous exercise; and (iii) thiols that can be oxidized by extreme oxidative stress, impairing RyR channel function [110]. Some redox-active cysteines in RyR1 are located in calmodulin- and FKBP12-binding regions [111], and changes in binding affinity for these effectors is one way that the oxidative environment modulates RyR1 function [109].

As a general rule, oxidizing reagents activate and reducing reagents inhibit RyR channel activity [112]. *In vitro* RyR1 activity is increased by many oxidizing species, including superoxide anion, hydrogen peroxide, hydroxyl radicals and nitric oxide although channel function is inhibited in extreme oxidative environments. The relationships between redox state, other factors such as membrane potential or the concentrations of RyR1 protein modulators, and channel function are extremely complex and beyond the scope of this article.

There is little consensus on the location of the specific cysteine residues that are redox-active. Cysteine residues at positions 35, 314, 810, 905, 1039, 1302, 1590, 2326, 2363, 2436, 2565, 2606, 2611, 3193 and 3635 have been implicated in at least one study [113, 114]. Cys3635 has been confirmed as redox-sensitive in two studies and has been implicated in voltage-gated EC coupling and regulation of calmodulin binding [43, 115].

Using information about RyR1 functional domains to understand disease pathogenesis

As an example of how information on RyR1 functional domains can be used to investigate the possible disease mechanisms, we have analyzed a range of myopathy cases caused by recessive *RYR1* mutations (Table 3). Some patients are compound heterozygous for two different missense mutations, but interestingly, over half of patients are compound heterozygous for a missense mutation and a 'null' mutation (including protein-truncation mutations or frameshift/splice-site mutations that disrupt the reading frame). Such 'null' mutations likely abolish protein expression from that allele and the phenotype is probably determined primarily by the sequence variant on the other *RYR1* allele (carrying the missense mutation). Analysis of these missense mutations can give insights into disease pathogenesis. For example, the Arg2435Leu mutation changes a conserved arginine residue that likely alters the redox-sensing properties of Cys2436. Three mutations, His2035Leu, Arg3772Gln and Arg3772Trp sit within or immediately adjacent to S100A1 binding domains and the changes in surface charge may alter S100A1-RyR1

interactions. In this way, *in-silico* analysis of different mutations can generate hypotheses that can inform research into disease pathogenesis.

VIII. Concluding Remarks

After reviewing the existing knowledge about the structure of RyR1 and the relationship to its function, it is clear there is much left to discover. For most parts of the protein, it remains uncertain how the amino acid sequences fold to form the tertiary protein structure and the role of specific residues in specific channel functions is based on partial information. Achieving a crystal structure for the full tetrameric complex will be a major milestone for the field and for understanding the molecular basis of various channel properties. It is also clear that our current knowledge of how channel function is modulated by various interacting proteins and small molecules is rudimentary. Many other interacting proteins likely remain undiscovered and the RyR1 binding sites for many recognized interacting proteins are uncertain. Advancing this field is not only important for understanding normal skeletal muscle function, but will influence the treatment of many muscle disorders. Drugs can now be designed to modulate specific molecular interactions and the potential to normalize RyR1 function using small molecules in primary RYR1-myopathies and other skeletal muscle disorders is great. At present this a distant goal but we hope that consolidating our knowledge of the functional domains of RyR1 is a useful early step on this journey. We also hope that it helps the investigation of the ways that different *RYR1* mutations cause muscle dysfunction and directs us to the most fruitful areas for therapy development.

Acknowledgements

This work was supported by the National Health & Medical Research Council (NC; grants 1035828 & 1022707), the Swiss National Science Foundation (SNF 310030-129785), the Association Française contre les Myopathies (AFM), and the Albert Einstein College of Medicine Senior Research Fellowship.

References

1 Takeshima, H.*, et al.* (1994) Excitation-contraction uncoupling and muscular degeneration in mice lacking functional skeletal muscle ryanodine-receptor gene. *Nature* 369, 556-559

2 Treves, S.*, et al.* (2008) Congenital muscle disorders with cores: the ryanodine receptor calcium channel paradigm. *Curr. Opin. Pharmacol.* 8, 319-326

1 **3 Clarke, N.F.*, et al.* (2010) Recessive mutations in RYR1 are a common cause of congenital fiber type disproportion. *Hum. Mutat.* 31, E1544-E1550**
2 **4 Wilmshurst, J.*, et al.* (2010) RYR1 mutations are a common cause of congenital myopathies with central nuclei. *Ann. Neurol.* 68, 717-726**
3 **5 Fill, M. and Copello, J.A. (2002) Ryanodine receptor calcium release channels. *Physiol. Rev.* 82, 893-922**
4 **6 Zhao, M.*, et al.* (1999) Molecular identification of the ryanodine receptor pore-forming segment. *J. Biol. Chem.* 274, 25971-25974**
5 **7 Gillespie, D.*, et al.* (2005) (De) constructing the ryanodine receptor: Modeling ion permeation and selectivity of the calcium release channel. *J. Phys. Chem. B* 109, 15598-15610**
6 **8 Ramachandran, S.*, et al.* (2009) A structural model of the pore-forming region of the skeletal muscle ryanodine receptor (RyR1). *PLoS Comput. Biol.* 5, e1000367**
7 **9 Van Petegem, F. (2012) Ryanodine Receptors: Structure and Function. *J. Biol. Chem.***
8 **10 Serysheva, I.*, et al.* (1999) Structure of the skeletal muscle calcium release channel activated with Ca2+ and AMP-PCP. *Biophys. J.* 77, 1936-1944**
9 **11 Ikemoto, N. and Yamamoto, T. (2000) Postulated role of inter-domain interaction within the ryanodine receptor in Ca2+ channel regulation. *Trends Cardiovasc. Med.* 10, 310-316**
10 **12 Sharma, M.R.*, et al.* (2000) Three-dimensional structure of ryanodine receptor isoform three in two conformational states as visualized by cryo-electron microscopy. *J. Biol. Chem.* 275, 9485-9491**
11 **13 Ikemoto, N. and Yamamoto, T. (2002) Regulation of calcium release by interdomain interaction within ryanodine receptors. *Front. Biosci.* 7, d671-d683**
12 **14 Zorzato, F.*, et al.* (1996) Role of malignant hyperthermia domain in the regulation of Ca2+ release channel (ryanodine receptor) of skeletal muscle sarcoplasmic reticulum. *J. Biol. Chem.* 271, 22759-22763**
13 **15 Bannister, M.L.*, et al.* (2007) Malignant hyperthermia mutation sites in the Leu2442–Pro2477 (DP4) region of RyR1 (ryanodine receptor 1) are clustered in a structurally and functionally definable area. *Biochem. J.* 401, 333-339**
14 **16 Futatsugi, A.*, et al.* (1995) Tissue-specific and developmentally regulated alternative splicing in mouse skeletal muscle ryanodine receptor mRNA. *Biochem. J.* 305, 373-378**
15 **17 Zorzato, F.*, et al.* (1994) Identification of two ryanodine receptor transcripts in neonatal, slow-, and fast-twitch rabbit skeletal muscles. *Biochem. Biophys. Res. Commun.* 203, 1725-1730**
16 **18 Kimura, T.*, et al.* (2005) Altered mRNA splicing of the skeletal muscle ryanodine receptor and sarcoplasmic/endoplasmic reticulum Ca2+-ATPase in myotonic dystrophy type 1. *Hum. Mol. Genet.* 14, 2189-2200**
17 **19 Kimura, T.*, et al.* (2007) A variably spliced region in the type 1 ryanodine receptor may participate in an inter-domain interaction. *Biochem. J.* 401, 317-324**
18 **20 Nakai, J.*, et al.* (1996) Enhanced dihydropyridine receptor channel activity in the presence of ryanodine receptor. *Nature* 380, 72-75**
19 **21 Sheridan, D.C.*, et al.* (2006) Bidirectional signaling between calcium channels of skeletal muscle requires multiple direct and indirect interactions. *Proc. Natl. Acad. Sci. U.S.A.* 103, 19760-19765**
20 **22 Rebbeck, R.T.*, et al.* (2011) The beta(1a) subunit of the skeletal DHPR binds to skeletal RyR1 and activates the channel via its 35-residue C-terminal tail. *Biophys. J.* 100, 922-930**
21 **23 Leong, P. and MacLennan, D.H. (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**
22 **24 Protasi, F.*, et al.* (2002) Multiple Regions of RyR1 Mediate Functional and Structural Interactions with [alpha] 1S-Dihydropyridine Receptors in Skeletal Muscle. *Biophys. J.* 83, 3230-3244**
23 **25 Proenza, C.*, et al.* (2002) Identification of a region of RyR1 that participates in allosteric coupling with the 1S (CaV1. 1) II–III loop. *J. Biol. Chem.* 277, 6530-6535**
24 **26 Altafaj, X.*, et al.* (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**
25 **27 Casarotto, M.G.*, et al.* (2006) Structural and functional characterization of interactions between the dihydropyridine receptor II–III loop and the ryanodine receptor. *Clin. Exp. Pharmacol. Physiol.* 33, 1114-1117**
26 **28 Peralvarez-Marin, A.*, et al.* (2011) 3D Mapping of the SPRY2 domain of ryanodine receptor 1 by single-particle cryo-EM. *PloS ONE* 6, e25813**
27 **29 Cui, Y.*, et al.* (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**
28 **30 Cheng, W.*, et al.* (2005) Interaction between the dihydropyridine receptor Ca2+ channel -subunit and ryanodine receptor type 1 strengthens excitation-contraction coupling. *Proc. Natl. Acad. Sci. U.S.A.* 102, 19225-19230**
29 **31 Beam, K.G. and Bannister, R.A. (2010) Looking for answers to EC coupling's persistent questions. *J. Gen. Physiol.* 136, 7-12**
30 **32 Yamazawa, T.*, et al.* (1997) A region of the ryanodine receptor critical for excitation-contraction coupling in skeletal muscle. *J. Biol. Chem.* 272, 8161-8164**
31 **33 Perez, C.F.*, et al.* (2003) Amino acids 1–1,680 of ryanodine receptor type 1 hold critical determinants of skeletal type for excitation-contraction coupling. *J. Biol. Chem.* 278, 39644-39652**
32 **34 Yamazawa, T.*, et al.* (1996) Subtype specificity of the ryanodine receptor for Ca2+ signal amplification in excitation-contraction coupling. *EMBO J.* 15, 6172-6177**
33 **35 Nakai, J.*, et al.* (1998) Two Regions of the Ryanodine Receptor Involved in Coupling withl-Type Ca2+ Channels. *J. Biol. Chem.* 273, 13403-13406**
34 **36 Prosser, B.L.*, et al.* (2011) S100A1 and calmodulin regulation of ryanodine receptor in striated muscle. *Cell Calcium* 50, 323-331**

37 Samsó, M. and Wagenknecht, T. (2002) Apocalmodulin and Ca2+-calmodulin bind to neighboring locations on the ryanodine receptor. *J. Biol. Chem.* 277, 1349-1353

38 Rodney, G.G.*, et al.* (2005) A calmodulin binding domain of RyR increases activation of spontaneous Ca2+ sparks in frog skeletal muscle. *J. Biol. Chem.* 280, 11713-11722

39 Menegazzi, P.*, et al.* (1994) Identification and characterization of three calmodulin binding sites of the skeletal muscle ryanodine receptor. *Biochemistry* 33, 9078-9084

40 Chen, S. and MacLennan, D.H. (1994) Identification of calmodulin-, Ca (2+)-, and ruthenium red-binding domains in the Ca2+ release channel (ryanodine receptor) of rabbit skeletal muscle sarcoplasmic reticulum. *J. Biol. Chem.* 269, 22698-22704

41 Yamaguchi, N.*, et al.* (2001) Identification of apocalmodulin and Ca2+-calmodulin regulatory domain in skeletal muscle Ca2+ release channel, ryanodine receptor. *J. Biol. Chem.* 276, 22579-22585

42 Rodney, G.G.*, et al.* (2001) Calcium binding to calmodulin leads to an N-terminal shift in its binding site on the ryanodine receptor. *J. Biol. Chem.* 276, 2069-2074

43 Zhang, H.*, et al.* (2003) A noncontiguous, intersubunit binding site for calmodulin on the skeletal muscle Ca2+ release channel. *J. Biol. Chem.* 278, 8348-8355

44 Wright, N.T.*, et al.* (2008) S100A1 and calmodulin compete for the same binding site on ryanodine receptor. *J. Biol. Chem.*283, 26676-26683

45 Treves, S.*, et al.* (1997) Interaction of S100A1 with the Ca2+ release channel (ryanodine receptor) of skeletal muscle. *Biochemistry* 36, 11496-11503

46 Prosser, B.L.*, et al.* (2008) S100A1 binds to the calmodulin-binding site of ryanodine receptor and modulates skeletal muscle excitation-contraction coupling. *J. Biol. Chem.* 283, 5046-5057

47 Prosser, B.L.*, et al.* (2010) S100A1 promotes action potential-initiated calcium release flux and force production in skeletal muscle. *Am. J. Physiol. Cell Physiol.* 299, C891-C902

48 Yamaguchi, N.*, et al.* (2011) Modulation of sarcoplasmic reticulum Ca2+ release in skeletal muscle expressing ryanodine receptor impaired in regulation by calmodulin and S100A1. *Am. J. Physiol. Cell Physiol.* 300, C998-C1012

49 Qi, Y.*, et al.* (1998) FK-binding protein is associated with the ryanodine receptor of skeletal muscle in vertebrate animals. *J. Biol. Chem.*273, 34813-34819

50 Ahern, G.P.*, et al.* (1997) Subconductance states in single-channel activity of skeletal muscle ryanodine receptors after removal of FKBP12. *Biophys. J.* 72, 146-162

51 Shou, W.*, et al.* (1998) Cardiac defects and altered ryanodine receptor function in mice lacking FKBP12. *Nature* 391, 489-492

52 Avila, G.*, et al.* (2003) FKBP12 binding to RyR1 modulates excitation-contraction coupling in mouse skeletal myotubes. *J. Biol. Chem.* 278, 22600-22608

53 Gaburjakova, M.*, et al.* (2001) FKBP12 binding modulates ryanodine receptor channel gating. *J. Biol. Chem.* 276, 16931-16935

54 Tang, W.*, et al.* (2004) Altered excitation-contraction coupling with skeletal muscle specific FKBP12 deficiency. *FASEB J.* 18, 1597-1599

55 Marx, S.O.*, et al.* (1998) Coupled gating between individual skeletal muscle Ca2+ release channels (ryanodine receptors). *Science* 281, 818-821

56 Wagenknecht, T.*, et al.* (1997) Locations of calmodulin and FK506-binding protein on the three-dimensional architecture of the skeletal muscle ryanodine receptor. *J. Biol. Chem.* 272, 32463-32471

57 Cornea, R.L.*, et al.* (2010) Mapping the ryanodine receptor FK506-binding protein subunit using fluorescence resonance energy transfer. *J. Biol. Chem.* 285, 19219-19226

58 Serysheva, I.I.*, et al.* (2008) Subnanometer-resolution electron cryomicroscopy-based domain models for the cytoplasmic region of skeletal muscle RyR channel. *Proc. Natl. Acad. Sci. U.S.A.* 105, 9610-9615

59 Kobayashi, Y.M.*, et al.* (2000) Localization and characterization of the calsequestrin-binding domain of triadin 1. *J. Biol. Chem.* 275, 17639-17646

60 Lee, J.M.*, et al.* (2004) Negatively charged amino acids within the intraluminal loop of ryanodine receptor are involved in the interaction with triadin. *J. Biol. Chem.* 279, 6994-7000

61 Wang, Y.*, et al.* (2009) Altered stored calcium release in skeletal myotubes deficient of triadin and junctin. *Cell Calcium* 45, 29-37

62 Oddoux, S.*, et al.* (2009) Triadin deletion induces impaired skeletal muscle function. *J. Biol. Chem.*284, 34918-34929

63 Goonasekera, S.A.*, et al.* (2007) Triadin binding to the C-Terminal luminal loop of the ryanodine receptor is important for skeletal muscle excitation–contraction coupling. *J. Gen. Physiol.* 130, 365-378

64 Wei, L.*, et al.* (2009) Junctin and triadin each activate skeletal ryanodine receptors but junctin alone mediates functional interactions with calsequestrin. *Int. J. Biochem. Cell. Biol.* 41, 2214-2224

65 MacLennan, D.H. and Wong, P. (1971) Isolation of a calcium-sequestering protein from sarcoplasmic reticulum. *Proc. Natl. Acad. Sci. U.S.A.* 68, 1231-1235

66 Perez, C.F. (2011) On the footsteps of Triadin and its role in skeletal muscle. *World J. Biol. Chem.* 2, 177-183

67 Beard, N.A.*, et al.* (2002) Calsequestrin is an inhibitor of skeletal muscle ryanodine receptor calcium release channels. *Biophys. J.* 82, 310-320

68 Ryan, T.*, et al.* (2011) Identification of novel ryanodine receptor 1 (RyR1) protein interaction with calcium homeostasis endoplasmic reticulum protein (CHERP). *J. Biol. Chem.* 286, 17060-17068

69 Laplante, J.M.*, et al.* (2000) Cloning of human Ca2+ homoeostasis endoplasmic reticulum protein (CHERP): regulated expression of antisense cDNA depletes CHERP, inhibits intracellular Ca2+ mobilization and decreases cell proliferation. *Biochem. J.* 348, 189-199

70 Duncan, R.S.*, et al.* (2005) Effects of Vesl/Homer proteins on intracellular signaling. *Exp. Biol. Med.* 230, 527-535

71 Pouliquin, P. and Dulhunty, A.F. (2009) Homer and the ryanodine receptor. *Eur. Biophys. J.* 39, 91-102

1 **72 Xia, R.*, et al.* (2004) Selenium compounds modulate the calcium release channel/ryanodine receptor of rabbit skeletal muscle**
2 **by oxidizing functional thiols. *Biochem. Pharmacol.* 67, 2071-2079**
3 **73 Jurynec, M.J.*, et al.* (2008) Selenoprotein N is required for ryanodine receptor calcium release channel activity in human and**
4 **zebrafish muscle. *Proc. Natl. Acad. Sci. U.S.A.* 105, 12485-12490**
5 **74 Zorzato, F.*, et al.* (2007) Functional effects of mutations identified in patients with multiminicore disease. *IUBMB Life* 59, 14-**
6 **20**
7 **75 Smith, J.S.*, et al.* (1986) Single channel measurements of the calcium release channel from skeletal muscle sarcoplasmic**
8 **reticulum. Activation by Ca2+ and ATP and modulation by Mg2+. *J. Gen. Physiol.* 88, 573-588**
9 **76 Sitsapesan, R. and Williams, A. (1997) Regulation of current flow through ryanodine receptors by luminal Ca 2+. *J. Membr.***
10 ***Biol.* 159, 179-185**
11 **77 Laver, D.R.*, et al.* (2004) Luminal Ca2+–regulated Mg2+ Inhibition of Skeletal RyRs Reconstituted as Isolated Channels or**
12 **Coupled Clusters. *J. Gen. Physiol.* 124, 741-758**
13 **78 Laver, D.R. (2007) Ca2+ stores regulate ryanodine receptor Ca2+ release channels via luminal and cytosolic Ca2+ sites.**
14 ***Biophys. J.* 92, 3541-3555**
15 **79 Diaz-Sylvester, P.L.*, et al.* (2011) Modulation of cardiac ryanodine receptor channels by alkaline earth cations. *PLoS ONE* 6,**
16 **e26693**
17 **80 Meissner, G. (2002) Regulation of mammalian ryanodine receptors. *Front. Biosci.* 7, d2072-d2080**
18 **81 Treves, S.*, et al.* (1993) Identification of the domain recognized by anti-(ryanodine receptor) antibodies which affect Ca(2+)-**
19 **induced Ca2+ release. *Biochem. J.* 291 (Pt 3), 757-763**
20 **82 Chen, S.*, et al.* (1992) Characterization of a Ca2+ binding and regulatory site in the Ca2+ release channel (ryanodine receptor)**
21 **of rabbit skeletal muscle sarcoplasmic reticulum. *J. Biol. Chem.* 267, 23318-23326**
22 **83 Chen, S.*, et al.* (1993) Antibodies as probes for Ca2+ activation sites in the Ca2+ release channel (ryanodine receptor) of rabbit**
23 **skeletal muscle sarcoplasmic reticulum. *J. Biol. Chem.* 268, 13414-13421**
24 **84 Chen, S.*, et al.* (1998) Molecular identification of the ryanodine receptor Ca2+ sensor. *J. Biol. Chem.* 273, 14675-14678**
25 **85 Bhat, M.B.*, et al.* (1997) Functional calcium release channel formed by the carboxyl-terminal portion of ryanodine receptor.**
26 ***Biophys. J.* 73, 1329-1336**
27 **86 Du, G.G. and MacLennan, D.H. (1999) Ca2+ inactivation sites are located in the COOH-terminal quarter of recombinant rabbit**
28 **skeletal muscle Ca2+ release channels (ryanodine receptors). *J. Biol. Chem.* 274, 26120-26126**
29 **87 Fessenden, J.D.*, et al.* (2004) Mutational analysis of putative calcium binding motifs within the skeletal ryanodine receptor**
30 **isoform, RyR1. *J. Biol. Chem.* 279, 53028-53035**
31 **88 Xiong, L.*, et al.* (2006) A Ca2+-binding domain in RyR1 that interacts with the calmodulin binding site and modulates channel**
32 **activity. *Biophys. J.* 90, 173-182**
33 **89 Laver, D.*, et al.* (1997) Magnesium inhibition of ryanodine-receptor calcium channels: evidence for two independent**
34 **mechanisms. *J. Membr. Biol.* 156, 213-229**
35 **90 Gillespie, D.*, et al.* (2012) Is ryanodine receptor a calcium or magnesium channel? Roles of K(+) and Mg(2+) during Ca(2+)**
36 **release. *Cell Calcium* doi.org 10.1016/j.ceca.2012.02.001**
37 **91 Meissner, G.*, et al.* (1986) Kinetics of rapid Ca2+ release by sarcoplasmic reticulum. Effects of Ca2+, Mg2+, and adenine**
38 **nucleotides. *Biochemistry* 25, 236-244**
39 **92 Dias, J.M.*, et al.* (2006) Insights into the regulation of the ryanodine receptor: differential effects of Mg2+ and Ca2+ on ATP**
40 **binding. *Biochemistry* 45, 9408-9415**
41 **93 Laver, D.*, et al.* (1997) Reduced inhibitory effect of Mg2+ on ryanodine receptor-Ca2+ release channels in malignant**
42 **hyperthermia. *Biophys. J.* 73, 1913-1924**
43 **94 Ogunbayo, O.A.*, et al.* (2011) Cyclic adenosine diphosphate ribose activates ryanodine receptors, whereas NAADP activates**
44 **two-pore domain channels. *J. Biol. Chem.*286, 9136-9140**
45 **95 Zima, A.V.*, et al.* (2003) Differential modulation of cardiac and skeletal muscle ryanodine receptors by NADH. *FEBS Lett.* 547,**
46 **32-36**
47 **96 Xia, R.*, et al.* (2003) Skeletal muscle sarcoplasmic reticulum contains a NADH-dependent oxidase that generates superoxide.**
48 ***Am. J. Physiol. Cell Physiol.* 285, C215-221**
49 **97 Baker, M.L.*, et al.* (2002) The skeletal muscle Ca2+ release channel has an oxidoreductase-like domain. *Proc. Natl. Acad. Sci.**
50 ***U.S.A.* 99, 12155-12160**
51 **98 Bellinger, A.M.*, et al.* (2008) Stressed out: the skeletal muscle ryanodine receptor as a target of stress. *J. Clin. Invest.* 118, 445-**
52 **453.**
53 **99 Witcher, D.*, et al.* (1991) Unique phosphorylation site on the cardiac ryanodine receptor regulates calcium channel activity. *J.**
54 ***Biol. Chem.* 266, 11144-11152**
55 **100 Dulhunty, A.F.*, et al.* (2001) Characteristics of irreversible ATP activation suggest that native skeletal ryanodine receptors**
56 **can be phosphorylated via an endogenous CaMKII. *Biophys. J.* 81, 3240-3252**
57 **101 Rodriguez, P.*, et al.* (2003) Stoichiometric phosphorylation of cardiac ryanodine receptor on serine 2809 by calmodulin-**
58 **dependent kinase II and protein kinase A. *J. Biol. Chem.* 278, 38593-38600**
59 **102 Wehrens, X.H.T.*, et al.* (2004) Ca2+/calmodulin-dependent protein kinase II phosphorylation regulates the cardiac ryanodine**
60 **receptor. *Circ. Res.* 94, e61-e70**
61 **103 Reiken, S.*, et al.* (2003) PKA phosphorylation activates the calcium release channel (ryanodine receptor) in skeletal muscle. *J.**
62 ***Cell Biol.* 160, 919-928**
63 **104 Hain, J.*, et al.* (1994) Phosphorylation modulates the function of the calcium release channel of sarcoplasmic reticulum from**
64 **skeletal muscle. *Biophys. J.* 67, 1823-1833**

105 Sonnleitner, A.*, et al.* (1997) Gating of the skeletal calcium release channel by ATP is inhibited by protein phosphatase 1 but not by Mg2+. *Cell Calcium* 21, 283-290
106 Marks, A.R.*, et al.* (2002) Regulation of ryanodine receptors via macromolecular complexes: a novel role for leucine/isoleucine zippers. *Trends Cardiovasc. Med.* 12, 166-170
107 Guerrini, R.*, et al.* (1995) Calmodulin binding sites of the skeletal, cardiac, and brain ryanodine receptor Ca2+ channels: modulation by the catalytic subunit of cAMP-dependent protein kinase? *Biochemistry* 34, 5120-5129
108 Stange, M.*, et al.* (2003) Characterization of recombinant skeletal muscle (Ser-2843) and cardiac muscle (Ser-2809) ryanodine receptor phosphorylation mutants. *J. Biol. Chem.* 278, 51693-51702
109 Bellinger, A.M.*, et al.* (2009) Hypernitrosylated ryanodine receptor/calcium release channels are leaky in dystrophic muscle. *Nat. Med.* 15, 325-330
110 Sun, J.*, et al.* (2001) Classes of thiols that influence the activity of the skeletal muscle calcium release channel. *J. Biol. Chem.* 276, 15625-15630
111 Zissimopoulos, S.*, et al.* (2007) Redox sensitivity of the ryanodine receptor interaction with FK506-binding protein. *J. Biol. Chem.* 282, 6976-6983
112 Zissimopoulos, S. and Lai, F. (2006) Redox regulation of the ryanodine receptor/calcium release channel. *Biochem. Soc. Trans.* 34, 919-921
113 Aracena-Parks, P.*, et al.* (2006) Identification of cysteines involved in S-nitrosylation, S-glutathionylation, and oxidation to disulfides in ryanodine receptor type 1. *J. Biol. Chem.* 281, 40354-40368
114 Voss, A.A.*, et al.* (2004) Identification of hyperreactive cysteines within ryanodine receptor type 1 by mass spectrometry. *J. Biol. Chem.* 279, 34514-34520
115 Moore, C.P.*, et al.* (1999) A role for cysteine 3635 of RYR1 in redox modulation and calmodulin binding. *J. Biol. Chem.* 274, 36831-36834
116 Samsó, M.*, et al.* (2005) Internal structure and visualization of transmembrane domains of the RyR1 calcium release channel by cryo EM. *Nat. Struct. Mol. Biol.* 12, 539-544
117 Yano, M.*, et al.* (2006) Mechanisms of disease: ryanodine receptor defects in heart failure and fatal arrhythmia. *Nat. Clin. Pract. Cardiovasc. Med.* 3, 43-52
118 Yamamoto, T.*, et al.* (2000) Postulated role of interdomain interaction within the ryanodine receptor in Ca2+ channel regulation. *J. Biol. Chem.* 275, 11618-11625
119 Hayek, S.M.*, et al.* (2000) Characterization of a calcium-regulation domain of the skeletal-muscle ryanodine receptor. *Biochem. J.* 351, 57-65
120 Dulhunty, A.*, et al.* (2006) Novel regulators of RyR Ca 2+ release channels: insight into molecular changes in genetically-linked myopathies. *J. Muscle Res. Cell Motil.* 27, 351-365
121 Hamada, T.*, et al.* (2007) Peptide probe study of the role of interaction between the cytoplasmic and transmembrane domains of the ryanodine receptor in the channel regulation mechanism. *Biochemistry* 46, 4272-4279
122 Monnier, N.*, et al.* (2008) Null mutations causing depletion of the type 1 ryanodine receptor (RYR1) are commonly associated with recessive structural congenital myopathies with cores. *Hum. Mutat.* 29, 670-678
123 Duarte, S.T.*, et al.* (2011) Dominant and recessive RYR1 mutations in adults with core lesions and mild muscle symptoms. *Muscle Nerve* 44, 102-108
124 Zhou, H.*, et al.* (2010) Multi-minicore disease and atypical periodic paralysis associated with novel mutations in the skeletal muscle ryanodine receptor (RYR1) gene. *Neuromusc. Disord.* 20, 166-173
125 Bevilacqua, J.A.*, et al.* (2011) Recessive RYR1 mutations cause unusual congenital myopathy with prominent nuclear internalization and large areas of myofibrillar disorganization. *Neuropathol. Appl. Neurobiol.* 37, 271-284

TEXT BOXES

Box 1. RyR1-related muscle disorders.

Malignant hyperthermia (MH) is a potentially fatal pharmacogenetic disorder in which a hypermetabolic state is evoked by volatile anaesthetics or depolarizing muscle agents. Symptoms include hypercapnia, metabolic acidosis, tachycardia, generalized muscle rigidity, rhabdomyolysis, and hyperthermia. Most MH-susceptible individuals have normal muscle function so long as triggering agents are avoided but some individuals with congenital myopathies because of *RYR1* mutations may be at risk of developing an MH reaction. Dantrolene, which acts on RyR1, is currently the only available treatment. Dominant *RYR1* mutations are the leading cause of MH.

Congenital myopathies are uncommon muscle disorders that usually present at birth or during infancy with generalized muscle weakness and low muscle tone. There are many forms of congenital myopathy, each defined by a specific pattern of histologic abnormalities. The clinical severity varies. Most individuals with congenital myopathies follow a stable clinical course and current medical treatment is based on supportive care. Patients may develop respiratory involvement, joint contractures, distinctive weakness of eye movements (ophthalmoplegia), scoliosis, or difficulty swallowing (dysphagia). Mutations in *RYR1* are an important cause of the following congenital myopathies.

- The **core myopathies** are characterized by regions devoid of mitochondria in muscle fibers that appear as 'cores' on oxidative stains. In **central core disease (CCD)**, cores are large and extend longitudinally. In **multi-minicore disease (MmD)** the cores are short and vary in size and location.

- **Centronuclear myopathy (CNM)** is characterized by the presence of numerous internally located nuclei within muscle fibers.

- **Congenital fiber type disproportion (CFTD)** is characterized by a marked consistent difference in size between type 1 fibers (which are small) and type 2 fibers (which are large), when there are no other histological abnormalities.

FIGURE LEGENDS

Figure 1. Protein/ion binding domains distributed across the RyR1 protein sequence.

Figure 2. Proposed architecture of RyR1 based on cryo-EM at 10 Å resolution. Adapted from [116].

Figure 3. Interdomain interaction within RyR1. The N-terminal and central domains interact with one another to stabilize the closed configuration of the channel. When this interaction is disrupted and "unzipped," either by means of a competing synthetic domain peptide, pathogenic mutation, or physiologic activation, the channel becomes destabilized and allows the passage of calcium ions through the pore. aa = amino acids. Adapted from [117].

Figure 1:

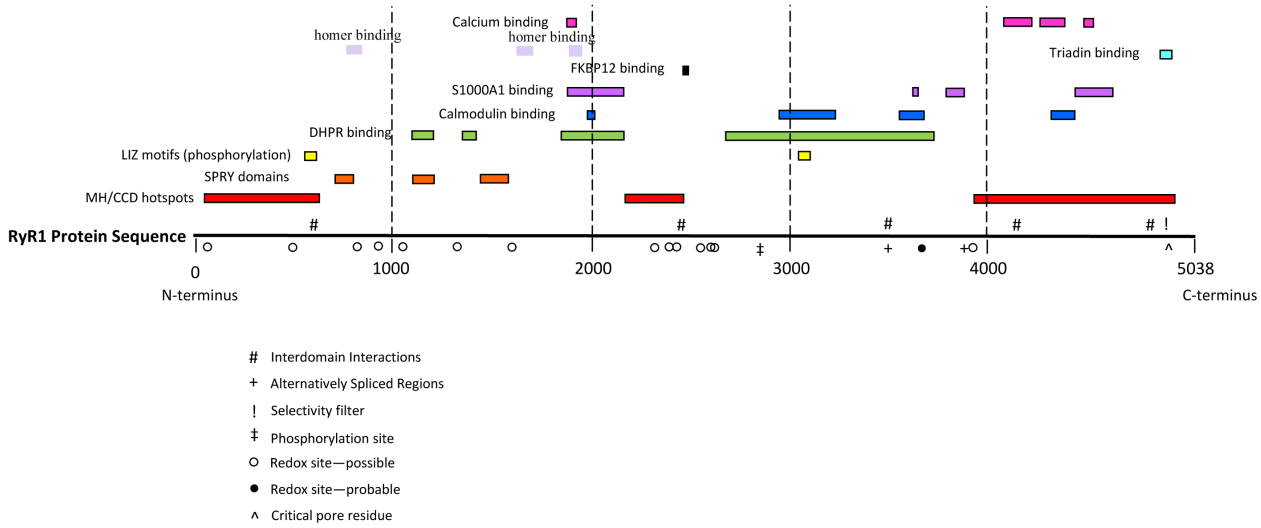
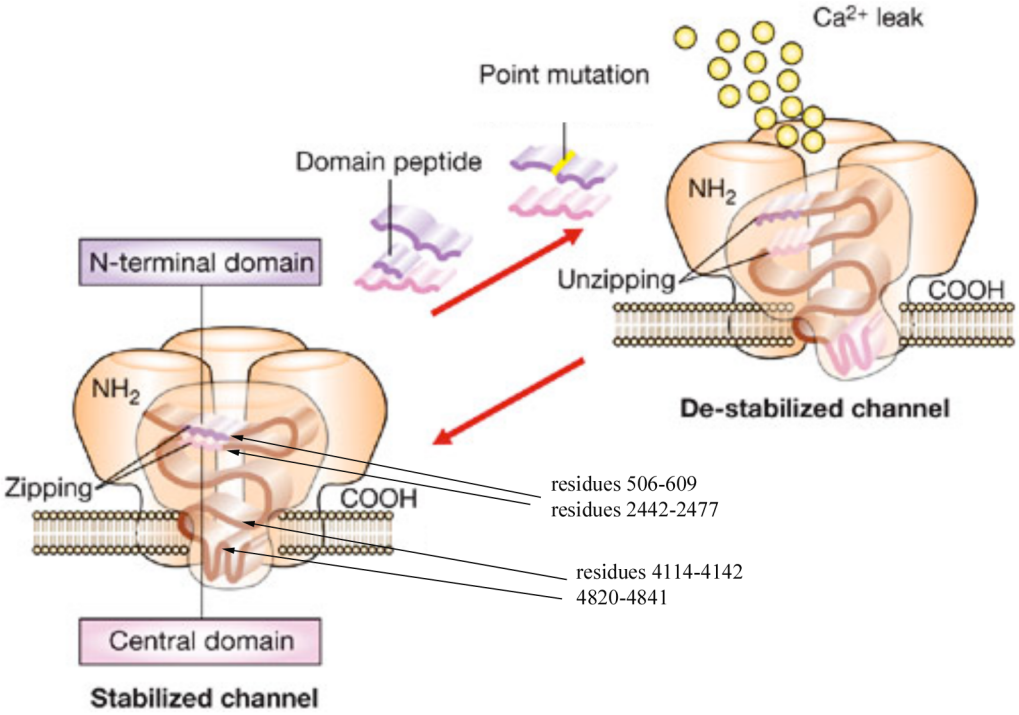


Figure 2

Figure 3


Ca2+ leak
Point mutation
Domain peptide
NH2
N-terminal domain
Unzipping
COOH
De-stabilized channel
NH2
Zipping
COOH
residues 506-609
residues 2442-2477
residues 4114-4142
4820-4841
Central domain
Stabilized channel

1 **Table 1.** Human RyR1 domains and effects on channel function arranged by amino acid range.

Amino Acid Range	Domain	Function/Effect on RyR1	Supporting Evidence	Refs
35 – 614	MH/CCD hotspot region 1	--	Human genetic studies	[2]
553 – 602	Leucine/Isoleucine zipper (LIZ) motifs	Binds to and localizes kinases/phosphatases on RyR1, phosphorylation activates RyR1	Experimental studies	[106]
589 – 608	Interdomain interactions	Stabilizes closed conformation, inactivates RyR1	Experimental studies	[118]
659 – 797	SPRY1 domain	Protein-protein interaction motif, exact effect on RyR1 unknown	*In silico* analysis	--
801 FLPPP 805	Binding to homer	Likely mediates protein-protein interactions, may activate RyR1	*In silico* analysis	[71]
1084 – 1207	SPRY2 domain	Binding to DHPR α_{1s} II-III loop, activates RyR1	*In silico* analysis, experimental studies	[27, 29]
1272-1455	Divergent region 2 (D2)	Binding to DHPR and responsible for formation of DHPR tetrads	Experimental studies	[21, 32, 33]
1430 – 1570	SPRY3 domain	Protein-protein interaction motif, exact effect on RyR1 unknown	*In silico* analysis	--
1773 PPHHF 1777	Binding to homer	Likely mediates protein-protein interactions, may activate RyR1	*In silico* analysis	[71]
1837 – 2168	Binding to DHPR	Activates RyR1	Experimental studies	[25, 35]
1861 – 2155	Binding to S100A1	Activates RyR1	Experimental studies	[45]
1872 – 1922	Low affinity Ca^{2+} inactivation site	Inactivates RyR1	Experimental studies	[119]
1975 – 1999	Binding to calmodulin	Effect depends on which form is bound (apoCaM is a partial agonist vs. CaCaM is	Experimental studies	[43]

		an antagonist of RyR1)		
1998 FRSPP 2002	Binding to homer	Likely mediates protein-protein interactions, may activate RyR1	*In silico* analysis	[71]
2163 – 2458	MH/CCD hotspot region 2	--	Human genetic studies	--
2442 – 2477	Interdomain interactions	Stabilizes closed conformation, inactivates RyR1	Experimental studies	[118]
2458 – 2468	Binding to FKBP12	Stabilizes closed conformation, inactivates RyR1	Experimental studies	[52, 53, 58]
2659 – 3720	Binding to DHPR	Activates RyR1	Experimental studies	[25, 35]
2843S	Phosphorylation site	Likely activates RyR1	Experimental studies	[103]
2937 – 3225	Binding to apoCaM	Weak partial agonist, activates RyR1	Experimental studies	[39]
3039 – 3075	Leucine/Isoleucine zipper (LIZ) motifs	Binds to and localizes kinases/phosphatases on RyR1, phosphorylation activates RyR1	Experimental studies	[106]
3471 – 3500	ASI region + basic sequence	Possible interdomain interaction which stabilizes closed conformation	Experimental studies	[16, 120]
3481 – 3485	ASI region	See above	Experimental studies	[16, 30, 120]
3495 – 3502	Basic sequence upstream from ASI region	Possible binding to DHPR β_{1a} subunit, may stabilize interaction between DHPR and RyR1	Experimental studies	[30]
3546 – 3655	Binding to apoCaM	Weak partial agonist, activates RyR1	Experimental studies	[39-41]
3553 – 3662	Binding to CaCaM	Antagonist, inactivates RyR1	Experimental studies	[39-41]
3614 – 3643	Binding to calmodulin (apoCaM and CaCaM)	Effect depends on which form is bound (apoCaM is a partial agonist vs. CaCaM is an antagonist of RyR1)	Experimental studies	[39, 40]
3616 – 3627	Binding to S100A1	Activates RyR1	Experimental studies	[44]
3635C	Redox modulation	Likely activates RyR1	Experimental	[113, 114]

	site		studies	
3773 – 3873	Binding to S100A1	Activates RyR1	Experimental studies	[45]
3865 – 3870	ASII region	Unknown	Experimental studies	[16]
3916 – 4943	MH/CCD hotspot region 3	--	Human genetic studies	--
4063 – 4209	Broad region containing Ca^{2+} binding sites	Unknown(?)	Experimental studies	[88]
4080 – 4091	Low affinity Ca^{2+} inactivation site	Inactivates RyR1	Experimental studies	[87]
4113 – 4141	Interdomain interactions	Stabilizes closed conformation, inactivates RyR1	Experimental studies	[121]
4245 – 4378	High affinity Ca^{2+} activation site	Activates RyR1	Experimental studies	[82]
4303 - 4431	Binding to apoCaM	Weak partial agonist, activates RyR1	Experimental studies	[40, 41]
4426 – 4622	Binding to S100A1	Activates RyR1	Experimental studies	[45]
4490 – 4502	High affinity Ca^{2+} activation site	Activates RyR1	Experimental studies	[83]
4821 – 4842	Interdomain interactions	Stabilizes closed conformation, inactivates RyR1	Experimental studies	[121]
4861 – 4918	Binding to triadin	Activates RyR1	Experimental studies	[60]
4895 GGGIGD 4900	Selectivity filter	Channel conductance and cation selectivity	Experimental and molecular dynamics simulations studies	[6, 7]

Abbreviations: MH, malignant hyperthermia; CCD, central core disease; DHPR, dihydropyridine receptor; apoCaM, calmodulin with unbound calcium; CaCaM, calmodulin with bound calcium; AS, alternatively spliced.

Table 2. RyR1 domains and effects on channel function arranged by binding protein/ion.

Binding proteins/ intrinsic regions/ions	Amino acid range H (human)/R (rabbit) cDNA sequence	Domain	Functional effect on RyR1	Refs
Interdomain interaction	H 589-608 H 2442-2477 H 4113-4141; 4821-4842	MH/CCD domain 1 MH/CCD domain 2 MH/CCD domain 3	Stabilize closed conformation	[121]
Alternative splice variants	H 3481-3485 ASI(-) H 3864-3869 ASII(-)	- -	Possible interdomain interaction stabilizing closed conformation	[16, 120]
SPRY	H 659-797 SPRY1 H 1084-1207 SPRY2 H 1430-1570 SPRY3	- - -	Protein-protein interacting domain; SPRY2 identified as binding DHPR α_{1s} II-III loop	
DHPR α_{1s} II-III loop	H 1076-1112 R 1837-2168 R 2642-3770 R 3351-3507	SPRY2 domain MH/CCD domain 2	Orthograde signaling Contribute to skeletal EC coupling (orthograde and retrograde)	[21, 23, 25, 26, 33]
DHPR ß1	H 3495-3502 R 3201-3661	ASI (-)	Strengthens effect of interaction via +ve charges	[30]
Tetrad formation	H 1272-1455		Tetrad formation enhanced but sequence not sufficient	[21]
FKBP12	H 2458-2468	MH/CCD domain 2	Stabilizes closed state	[52, 53, 58]
Calmodulin	H 2937-3225 ApoCaM H 3553-3662 CaCaM H 3614-3663 Apo and CaCaM H 4303-4431 CaCaM	 MH/CCD domain 3		[41]
S100A1	H 1861-2155 H 3616-3627 H 3773-3873 H 4426-4622	Overlaps with CaM binding	Activates RyR1 Activates RyR1 Activates RyR1 Activates RyR1	[45, 46]
Triadin	H 4861-4918	MH/CCD domain 3	Rapid Ca^{2+} release	[60]
Kinases/ phosphotases	H 552-602 PPI H 3039-3075 PKA	Leu/Ile zipper (LIZ) motifs Leu/Ile zipper (LIZ) motifs	Phosphorylation activates RyR1	[106]
Homer	H 801-805 H 1773-1777 H 1998-2002		Enhances RyR1 function	[71]
Redox sensitive Cys	H Cys3635		Activates RyR1	[111, 113, 114]
Phosphorylation site	H Ser2843	-	Channel opening	[103]

Ca^{2+} binding domain	H 1872-1922 H 4080-4091 H 4245-4378 H 4490-4502	 MH/CCD domain 3 MH/CCD domain 3 MH/CCD domain 3	Low affinity, Ca^{2+} inactivation Low affinity Ca^{2+} inactivation Ca^{2+} binding High affinity Ca^{2+} activation	[82, 83, 87, 119]

Abbreviations: H, human; R, rabbit; MH, malignant hyperthermia; CCD, central core disease; AS, alternatively sliced; DHPR, dihydropyridine receptor.

Table 3. Recessive RYR1 mutations and neuromuscular diseases. Genetic *RYR1* mutations and the corresponding possible protein/ion binding domains affected are listed, along with a brief description of clinical phenotypes.

RYR1 Mutation 1	RYR1 Mutation 2	RyR1 alleles expressed in muscle	Predicted maximum level of RyR1 expressed *	Observed level of RyR1 expressed	Histological diagnosis	RyR1 domain implicated in disease pathogenesis	Refs
c.212C>A p.S71Y	c.6847A>C p.N2283H	S71Y & N2283H	100%	Normal	Cores		[2]
c.325C>T p.R109W	Monoallelic	R109W	50%	Reduced	Cores		[2]
c.1205T>C p.M402L	c.5333C>A p.S1778X	M402L	50%	Reduced	CFTD		[3]
c.1205T>C p.M402T	Monoallelic	M402T	50%	Reduced	Some cores		[2]
c.4024A>G p.S1342G	c.8360C>G p.T2787S	S1342G & T2787S	100%	-	CNM	**Tetrad formation** (1341-1402) + **DHPR $Cav_{1.1}$** (2642-3770)	[4]
c.5110G>A p.G1704S	c.12801_12802ins p.A4268RfsX7	G1704S	50%	Slightly reduced	Cores		[122]
c.6104A>T p.H2035L	c.738T>G p.Y246X	H2035L	50%	-	CFTD	**S100A1** (1861-2155)	[3]
c.6612C>G p.H2204G	c.14228G>A p.G4743D	H2204G & G4743D	100%	-	MmD/MHS, fiber type 1 predominance	**High affinity Ca^{2+} binding** (4490-4502)	[123]
c.7268T>A p.M2423K	Monoallelic	M2423K	50%	-	Cores		[2]
c.7268T>A p.M2423K	c.34delT; c.37C>G Stop +p.L13V	M2423K	50%	Slightly reduced	Cores		[122]
c.7304G>T p.R2435L	c.7304G>T p.R2435L	R2435L	50%	Normal	Cores		[2]
c.6721C>T p.R2241X+ c.2122G>A p.D708N	c.8816G>A p.R2939K	R2939K	50%	Reduced	MmD	**CaM** (2937-3225)	[124]
c.9247T>C p.M3081T	c.5726_27ΔAG p.E1909GfsX39	M3081T	50%	-	CNM	**DHPR $Cav_{1.1}$** (Rabbit 2642-3770)	[4]
c.9413C>T p.P3138L	c.11314C>T p.R3772W	P3138L & R3772W	100%	-		**CaM** (2937-3225) + **S100A1**? (3773-3873)	[125]
c.9605C>T p.P3202L	c.10561G>T p.G3521C	P3202L & G3521C	100%	-		**CaM** (2937-3225) + **DHPR retrograde** (2659-3720)	[125]

c.9605C>T p.P3202L	c.12536G>A p.R4179H	P3202L & R4179H	100%	-		**CaM** (2937-3225) + **DHPR retrograde** (2659-3720)	[125]
c.9978C>A p.N3326K	c.9000+1G>T exon 95 skipped	N3326K	50%	Reduced	CFTD	**DHPR $Cav_{1.1}$** (2642-3770)	[3]
c.10097G>A p.R3366H	c.11798G p.Y3933C	R3366H & Y3933C	100%	-	MmD/rhabdo, fiber type 1 predominance	**DHPR $Cav_{1.1}$** (2642-3770)	[123]
c.10204T>G p.C3402G	c.13480G>T p.Glu4494X	C3402G	50%	-	CFTD	**DHPRβ1**(3201- 3661)	[3]
c.10343C>T p.S3448F	c.14365-2AT+	S3448F	50%	-	MmD	**DHPR $Cav_{1.1}$** (Rabbit 3351- 3507)	[2]
c.10579G>A p.P3527S	c.10579G>A p.P3527S	P3527S	50%	-	CCD/MmD	**CaM**? (2937-3225)	[2]
c.10616G>A p.R3539H	c.14804–1G>T p.G4935.T4957>D fsX11	R3539H	50%	Reduced	Cores	**DHPR retrograde** (2659-3720)	[122]
c.11315G>A p.R3772E	c.5938ΔC p.Leu1980SerfsX1	R3772E	50%	Reduced	Cores	**S100A1**? (3773- 3873)	[122]
c.11315G>A p.R3772Q	c.11315G>A p.R3772Q	R3772Q	50%	-		**S100A1**? (3773- 3873)	[2]
c.11941C>T p.H3981Y	c.8342_8343ΔTA	H3981Y	50%	-	CNM	None known	[4]
c.12986C>A p.A4329D	Monoallelic	A4329D	50%	Reduced	Cores	**Ca^{2+}** (4245-4378)	[2]
c.13676G>A p.R4558Q	c.2455C>T p.R819X	R4558Q	50%	Reduced	Cores	**S100A1** (4426- 4622)	[122]
c.14126C>T p.T4709M	Monoallelic	T4709M	50%	Reduced	Cores		[2]
c.14126C>T p.T4709M	c.12541G>A p.D4181K	T4709M & D4181K	100%	-	Cores	**Ca^{2+}** (4245-4378)	[125]
c.14524C>A p.V4842M	c.10348C>T intronic splice variant	V4842M	50%	-	CNM	**Interdomain** (4821-4842)	[4]
c.10348–6C>G p.His3449ins 33aafsX54 c.14524G>A p.V4842M	c.7324–1G>T p.Leu2442_ Ala2454>ProfsX86	V4842M	50%	Reduced	Cores (severe)	**Interdomain** **zipper** (4821-4842)	[122]
c.14545G>A p.V4849I	c.1742.1743insTCA p.H581GlnX	V4849I	50%	Not known	Cores (severe)		[122]
c.14731G>A p.D4911K	c.7006C>T p.N2336C	D4911K & N2336C	100%	-		**Triadin** (4861- 4918)	[125]
c.10343C>T p.S3448F	c.14365-2A>T acceptor splice site	p.S3448F & p.S4789_ K4822del	50 – 100%	-	Cores	**DHPR $Cav_{1.1}$** (2642-3770)	[2]
c.8692+131G>A p.Gly2898Gly fsX36 +	c.8692+131G>A p.Gly2898GlyfsX36 +	20% wild type transcript		Reduced			[125]

ɔ.Gly2898Asp ʃsX54	p.Gly2898AspfsX54	expressed					

Abbreviations: c., coding sequence; p., protein sequence; X, stop codon; CNM, centronuclear myopathy; CFTD, congenital fiber type disproportion; MmD, multi-minicore disease; MHS, malignant hyperthermia susceptibility; rhabdo, rhabdomyolysis; FSD, fiber-size disproportion; DHPR, dihydropyridine receptor; CaM, calmodulin. -, data not available.

* compared with normal RyR1 expression levels. Monoallelic refers to when only one RYR1 allele was seen on analysis of cDNA in patient muscle. A second 'null' mutation likely exists in these patients but has not been identified.