Ryanodine Receptors: Structure, Expression, Molecular Details, and Function in Calcium Release

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Ryanodine receptors (RyRs) are located in the sarcoplasmic/endoplasmic reticulum membrane and are responsible for the release of Ca^{2+} from intracellular stores during excitation-contraction coupling in both cardiac and skeletal muscle. RyRs are the largest known ion channels (>2MDa) and exist as three mammalian isoforms (RyR 1–3), all of which are homotetrameric proteins that interact with and are regulated by phosphorylation, redox modifications, and a variety of small proteins and ions. Most RyR channel modulators interact with the large cytoplasmic domain whereas the carboxy-terminal portion of the protein forms the ion-conducting pore. Mutations in RyR2 are associated with human disorders such as catecholaminergic polymorphic ventricular tachycardia whereas mutations in RyR1 underlie diseases such as central core disease and malignant hyperthermia. This chapter examines the current concepts of the structure, function and regulation of RyRs and assesses the current state of understanding of their roles in associated disorders.

Intracellular Ca^{2+} is an important secondary messenger for signal transduction and is essential for cellular processes such as excitation-contraction coupling (E-C coupling). The major source of intracellular Ca^{2+} is the sarcoplasmic reticulum (SR) in striated muscle and the endoplasmic reticulum (ER) in other cell types. There are two major Ca^{2+} release channels localized in the SR/ER, the ryanodine receptors (RyRs) (Otsu et al. 1990) and inositol 1,4,5-triphosphate receptors (IP_3Rs) (Nixon et al. 1994). The present article reviews the structure, regulation, expression, and function of the RyRs. RyRs exist in three isoforms (RyR 1-3) and are named after the plant alkaloid ryanodine, which binds to RyRs with high affinity and specificity and displays preferential interactions with the open state of the channel allowing its usage to evaluate the functional state of the channel (Imagawa et al. 1987; Inui et al. 1987; Lai et al. 1988; Chu et al. 1990). Ryanodine at nanomole concentrations locks the channel in an open subconductance state and inhibits the channel at high concentrations (>100 μM) (Meissner et al. 1986; Lai et al. 1989; McGrew et al. 1989). RyRs are homotetamers with a total molecular mass of >2 MDa (each subunit is >550 kDa) (Inui et al. 1987; Lai et al. 1988). RyRs are modulated (see Fig. 1) directly or indirectly by the dihydropyridine receptor

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(Hayek et al. 1999). In addi
regions, the large cytoplasmic domain is the site
of both interaction with a large number of the
modulators of channel activity, and many of the
that underlie the RyR channelopa-
RyR protein is cytoplas-
membrane
RyRβ is expressed in various tis
skeletal and cardiac muscles, cerebellum, lungs,
and stomach (Oyamada et al. 1994). In *C. elegans* RyRs are found in body wall, vulval, anal,
and pharyngeal muscles (Hamada et al. 2002).
melanogaster a single isoform of RyR is
and nervous system

1993), multiminicore
et al. 2002), and at
(APP) (Zhou et al. 2
cause/are associated
polymorphic ventric
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et al. 1993, Magee e
300 mutations have b
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MH is an autoso
which genetically su
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of RyR1 mutations link
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to 614 and 2129 to 24
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ARVD2

Figure
cardiac muscle
CCD (63 mutations), and
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to MH is heat/exercise-induced exertional rhabdomyolysis; a clinical syndrome where heat/exercise-induced triggers breakdown of striated muscles that results in renal failure, hyperkalemia, and multi-organ failure. Approximately 26,000 cases are identified per year in United States (Capacchione et al. 2010).

CCD is a congenital myopathy in humans, and is characterized by hypotonia and muscle weakness of lower extremities leading to delayed attainment of motor skills (Dirksen and Avila 2002; Lueck and Dirksen 2004; Robinson et al. 2006). Slow-twitch (type I) skeletal muscles (e.g., soleus) of CCD of patients exhibit amorphous areas (central cores) that lack mitochondria and oxidative enzyme activity (Magee and Shy 1956; Shuaib et al. 1987). In some cases SR and t-tubules also degenerate and resting Ca^{2+} concentrations elevate with or without luminal store depletion (Tong et al. 1999). The majority of CCD causing mutations are in the pore-forming domain of RyR1 (Lynch et al. 1999; Monnier et al. 2000; Scacheri et al. 2000; Tilgen et al. 2001). Incidence of CCD is ~1 in 16,000 of total congenital myopathies and ~1 in 100,000 live births (Jungbluth 2007a).

MmD is an autosomal recessive myopathy characterized by weakness in axial and proximal limb muscles, hypoxia, and muscle biopsies showing characteristic mini cores due to lack of oxidative enzyme activity (Jungbluth 2007b; Sharma et al. 2007).

APP are dominant and genetically heterogeneous conditions characterized by muscle weakness and are divided into hypokalemic periodic paralysis and hyperkalemic periodic paralysis. Mutations on SCN4A and CASNAS1S gene that codes for α1s subunit of DHPR have been identified as general cause of hypokalemic- and hyperkalemic-periodic paralysis, respectively. Recently in a patient suffering from MmD the RyR1 mutation Arg2939Lys has been identified and clinical features of the patient are reminiscent of hyperkalemic periodic paralysis, suggesting a new RyR1-related form of periodic paralysis with additional myopathy features (Zhou et al. 2010).

Mutations in RyR2 produce altered Ca^{2+} homeostasis leading to ARVD2 (Dalla Volta et al. 1961; Marcus et al. 1982; Fontaine et al. 1984) and CPVT (Marks et al. 2002; Priori et al. 2002; Laitinen et al. 2003). ARVD2 an autosomal dominant cardiac disease characterized by replacement of myocytes with fibrofatty tissue leading to ventricular arrhythmias (Corrado et al. 2000). Mutations in RyR2 are detected at three regions that are homologous to the mutations on RyR1 associated with MH and CCD. Studies in ARVD2 suggest that Ca^{2+} leakage from myocardial SR via dysfunctional RyR2 is associated with development of ventricular arrhythmias (Tiso et al. 2001). The incidence of ARVD2 is ~1 in 10,000 adults in United States (Fontaine et al. 2001). CPVT is characterized by stress-induced ventricular tachycardia (Marks et al. 2002; Priori et al. 2002; Laitinen et al. 2003). Roles for protein kinase A (PKA) and Ca^{2+}/calmodulin dependent protein kinase II (CaMKII) phosphorylation and enhancement of RyR2 open probability in these arrhythmias has been suggested (Valdivia et al. 1995; Marx et al. 2000; Wehrens et al. 2004). In single channel recordings it has been shown that CPVT RyR2 mutation Arg4496Cys increases open probability at low Ca^{2+} concentrations (~5 nM) but not at higher concentrations (~150 nM) (Jiang et al. 2002; Wehrens and Marks 2003). In patients with CPVT increased PKA phosphorylation and leaky RyR2 channels was observed during β-adrenergic stress and exercise. Binding studies in vitro suggested that the mutant RyR2 associated with CPVT have lower affinity for FKBP12.6 (Wehrens et al. 2003). Later studies suggested that CPVT RyR2 expressing cells are more sensitive to β-adrenergic receptor stimulation (by either isoproterenol or forskolin) and have prolonged Ca^{2+} transients under these conditions. This sensitivity does not appear to be caused by differences either in RyR2 phosphorylation or loss of FKBP12.6 (George et al. 2003). Nonsense or missense mutations in the calsequestrin 2 gene have also been associated with autosomal recessive form of CPVT (Lahat et al. 2001; Postma et al. 2002).

RyR3 is the least studied ryanodine receptor, and consequently little is known of its function. Recently, RyR3 was suggested to play a role

in Alzheimer's disease, and up-regulation of RyR3 in cortical neurons is neuroprotective in TgCRND8 mouse model of Alzheimer's disease (Supnet et al. 2009).

ULTRASTRUCTURAL STUDIES ON RYANODINE RECEPTOR

The Si e Challenge

RyRs, the largest known ion channels (Takeshima et al. 1989; Nakai et al. 1990; Otsu et al. 1990; Zorzato et al. 1990; Hakamata et al. 1992), are large conductance channels (Smith et al. 1985; Smith et al. 1986b) capable of creating rapid transient increases of cytosolic Ca^{2+}. Analysis of the primary structure of RyRs reveals several functional motifs seen in other proteins; but the role of these motifs in RyRs function has not yet been elucidated (see review Hamilton and Serysheva 2009). The importance of RyRs in mammalian physiology and disease drives the need for high resolution structural information. The massive size, multiple modulators, and the dynamic nature of RyRs make their structural analysis a challenge. Advances in single-particle electron cryomicroscopy (cryo-EM) and crystal structures of small fragments (~200 amino acids) of the protein (Amador 2009; Lobo and Van Petegem 2009) are beginning to elucidate many important structural features.

S l c l al S l die on R R

Most cryo-EM studies on RyRs, (Radermacher et al. 1992; Radermacher et al. 1994; Serysheva et al. 1995; Orlova et al. 1996; Sharma et al. 1998; Serysheva et al. 1999; Benacquista et al. 2000; Sharma et al. 2000; Ludtke et al. 2005; Samsó et al. 2005; Serysheva et al. 2005; Serysheva et al. 2008; Samsó et al. 2009) and all the subnanometer resolution analysis (Serysheva et al. 2008; Samsó et al. 2009) have focused on the RyR1, however, some progress has been made with RyR2 (Sharma et al. 1998; Liu et al. 2002) and RyR3 (Sharma et al. 2000; Liu et al. 2001). Overall, the structures of all three isoforms are similar, consistent with the high sequence homology (~65%). However, the small differences seen are important, because they reflect variations in the primary sequence and are likely to be related to the specialized functions of each isoform.

RyRs form homotetramers of square prism shape and are arrayed in the SR where they control the release of Ca^{2+}. The cytoplasmic area of the channel (280 Å × 280 Å × 120 Å) is connected with the transmembrane region (120 Å × 120 Å × 60 Å) (Fig. 3). The membrane region constitutes approximately one-fifth of the channel and is localized to the carboxy terminal of the protein and forms the ion-conducting pore. The cytoplasmic/sarcoplasmic area that is also called the "foot" is a huge area with cavities and micro-structures that facilitate interactions with solvent, small molecules, and protein modulators. The corners of the cytoplasmic area, also called "clamps" are connected through the "handle" domain that surrounds the "central rim" domain of the cytoplasmic area. This area is connected to the membrane region through the "column." These structural domains have been divided in to 15 subdomains. The clamps (Fig. 3, subdomains 5, 6, 7, 8, 9, 10) undergo major conformational changes during the opening and closing of the channel (Serysheva et al. 2008; Samsó et al. 2009), are likely to participate in intermolecular interactions with neighboring RyRs, and are the sites of interactions with modulators (Wagenknecht et al. 1994; Wagenknecht et al. 1996; Wagenknecht et al. 1997; Samsó and Wagenknecht 2002; Samsó et al. 2006; Sharma et al. 2006; Meng et al. 2009). Two of the areas of high divergence in the primary sequence of the RyR isoforms were mapped in the clamps (Zhang et al. 2003; Liu et al. 2004). At subnanometer resolution seven α-helixes and three β-sheets have been localized to the clamp domain (Serysheva et al. 2008).

The handle domain that is formed by subdomains 3 and 4 (Fig. 3) has been found to contain an expanded region of divergence (Liu et al. 2002), and a β-sheet mapped on the subdomain 4 (Serysheva et al. 2008). In total seven β-sheets and 36 α-helixes at various orientations have been mapped on the cytoplasmic region

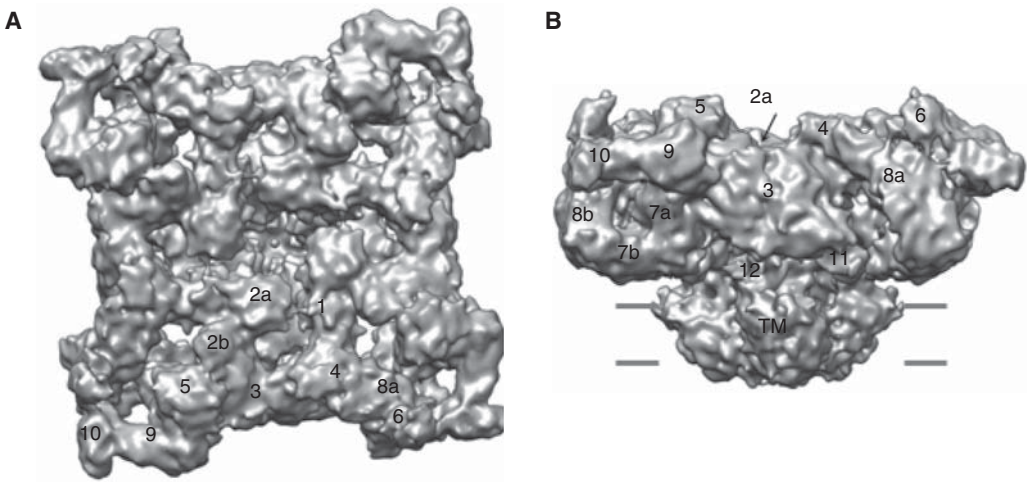

Figure 3. Cryo-EM reconstruction of RyR1 at 9.6 Å resolution. Cytoplasmic domain (*A*) and side view (*B*) of RyR1 with the different subdomains mapped by Irina Serysheva (See section "structural studies" for detailed information).

of the channel (Serysheva et al. 2008). These structures appear interconnected and merge toward the center of the molecule (Samsó et al. 2005). Two more β-sheets have been found in central rim (Fig. 3, subdomains 1,2) and one in the column (Fig. 3, subdomains 11,12), which also has eight α-helixes (Serysheva et al. 2008) that maintain the connection of the cytoplasmic and transmembrane regions (Samsó et al. 2005).

Current three-dimensional reconstructions at 8–10 Å resolution suggest five (Ludtke et al. 2005; Serysheva et al. 2008) or six α-helixes (Samsó et al. 2005) with different orientation in the transmembrane region of the closed channel. Although these studies (Ludtke et al. 2005; Samsó et al. 2005) were performed at similar conditions, they differ in interpretations, one study suggesting conformational similarity to the open K^+ channel (Ludtke et al. 2005) and the other suggesting a structure more similar to the closed K^+ channel (Samsó et al. 2005; Samsó et al. 2009). The conformation of the open RyR has so far only been proposed to resemble the conformation of the open K^+ channel (Samsó et al. 2009). The pore region has been predicted to consist of between 4 and 12 transmembrane segments (Takeshima et al. 1989; Zorzato et al. 1990; Tunwell et al. 1996; Du et al. 2002). Most of these models place both amino- and carboxy-termini in the cytoplasm. The ion conducting pore has been proposed to be located in the lumenal region and to include the GVRAGGGIGD amino acid sequence (4891–4900 human RyR1) (Zhao et al. 1999; Du et al. 2001) which is conserved between RyRs. The sequence GGIG has been proposed as the selectivity filter (Balshaw et al. 1999; Gao et al. 2000) based on the similarity with the consensus selectivity filter of K^+ channels. During channel opening, massive movements of cytoplasmic and transmembrane masses take place and result in a 4 Å increase of the ion gate (Samsó et al. 2009).

Crystal Structure of the Amino-Terminal Domain

The first crystal structures of RyRs are from the amino-terminal domain. The first 210 amino acids of the RyR1 structure (rabbit) was at 2.5 Å resolution (PDB ID code 3HSM) (Amador 2009), and a similar fragment (amino acids 9–205 of rabbit RyR1) was resolved at 2.9 Å resolution (PDB ID code 3ILA) (Lobo and Van Petegem 2009). The structure of the first 217 amino acids of mouse RyR2 was at 2.5 Å resolution (PDB ID code 3IM5) (Lobo and

Van Petegem 2009). These domains of RyR1 and RyR2 display the same overall fold, and consist of a β-trefoil domain flanked by a rigid α-helix. Furthermore, minor differences are seen in the loops connecting the various β-strands in the two isoforms (Amador 2009; Lobo and Van Petegem 2009).

Effect of Disease-Causing Mutation on the Structure of RyR1

An area rich in disease-associated mutations has been identified in the amino-terminal domain of RyR1 where mutations associated with MH and CCD have been located. Six are in a short loop between Gln156 and Asp167, and the rest either in different β-strands (Cys35, Arg178, and Tyr179) or loops (Leu14 and Arg45) (Amador 2009). Similar clustering of disease-causing mutations is seen in RyR2. Mutations found in RyR2 associated with CPVT and ARVD2, Arg169, and Arg176 (which correspond to Arg156 and Arg163 MH mutations of RyR1) are in a short loop with Pro164. Two more disease-causing mutations, Ala77 and Val186, are located in the rigid α-helix and in a small loop close to a β-strand, respectively (Lobo and Van Petegem 2009). These mutations in the amino-terminal of RyRs have been proposed to affect the interaction of RyR1 with modulators because they appear to cause only local changes in the structure (Amador 2009; Lobo and Van Petegem 2009). Another area of the amino-terminal region, amino acids 414–466 of RyR2 contains more than half of the amino-terminal mutations associated with cardiac arrhythmias and sudden death (Wang et al. 2007) and was mapped to a location between subdomains 5 and 9 using green fluorescent protein insertion into the primary structure and difference mapping of cryo-EM reconstructed structures (Wang et al. 2007). Notably, mutations at positions Glu161, Arg164, Arg402, and Ile404 of RyR1 and the mutations Arg169, Ile417, and Arg418 of RyR2 are located in the suggested FKBP binding pocket (Serysheva et al. 2008); whereas the Ile4898Thr CCD mutation appears to be located in the proposed selectivity filter in the pore region.

RYR REGULATION

RyRs, together with $Ca_v1.1/Ca_v1.2$, PKA, FKBP12 and 12.6, CaM, CaMKII, triadin, junction, and calsequestrin form the core of the macromolecular complex that regulates SR Ca^{2+} release. Thus, RyR structure, function and regulation are likely to be defined within this complex macromolecular set of interactions. Despite the ~65% sequence homology, the different RyR isoforms respond differently to some modulators. Most of the RyRs modulators interact with the cytoplasmic region of the channel, suggesting that they allosterically regulate channel gating. The locations of the binding sites for some of the modulators have been predicted from the primary structure, interactions with RyR1 fragments, and from difference mapping in the cryo-EM structures.

Ca 1.1 and 1.2

The voltage dependent Ca^{2+} channels ($Ca_V1.1$ and $Ca_V1.2$, skeletal and cardiac isoforms respectively), also known as dihydropyridine receptors (DHPRs), are composed of multiple subunits: α_1, α_2/δ, β and γ for $Ca_V1.1$. $Ca_V1.2$ has α_1, α_2/δ, and β but the γ subunit has not been identified. The α_1 subunit is both the voltage sensing and the pore forming subunit. DHPRs and RyRs are targeted to either side of the narrow junctional gap that separates the external and internal membrane systems in striated muscle. They are arranged so that bidirectional structural and functional coupling can occur between the proteins. The molecular mechanism of E-C coupling is fundamentally different between skeletal and cardiac muscle (Rios and Brum 1987; Bers and Stiffel 1993; Garcia et al. 1994; Lamb 2000). In skeletal muscle a physical interaction between $Ca_v1.1$ and RyR1 is required for E-C coupling and SR Ca^{2+} release; referred to as voltage-induced Ca^{2+} release (Lamb 2000). In contrast, RyR2 Ca^{2+} release in cardiac muscle is initiated by Ca^{2+} influx via $Ca_V1.2$, designated Ca^{2+} induced Ca^{2+}-release (see review Bers 2002). Thus, cardiac E-C coupling is dependent on extracellular Ca^{2+} and functional coupling between $Ca_v1.2$

and RyR2, which is assumed to rely on spatial proximity between the proteins rather than physical interaction. Because of the role of the direct physical interaction between $Ca_V1.1$ and RyR1 in skeletal muscle, E-C coupling can proceed for long periods in the absence of extracellular Ca^{2+} (Armstrong CM 1972; Dulhunty and Gage 1988). The $Ca_V1.1$ and RyR1 interaction in skeletal muscle is dependent on the strict geometrical alignments between the two proteins, which has been shown with electron microscopy in different muscle preparations (Takekura et al. 1994; Protasi et al. 1998) A critical determinant of E-C coupling in skeletal muscle is the α_{1S1S}

and adenine) also potentiate SR Ca^{2+} release but are less efficacious than ATP (Meissner 1984). In vitro studies have shown that skeletal muscle RyR1 can be activated by ATP in the absence of Ca^{2+}, but Ca^{2+} needs to be present for maximal activation (Meissner 1984; Meissner et al. 1986; Laver et al. 2001). Cardiac RyR is not activated by ATP in the absence of Ca^{2+}. However, ATP augments the Ca^{2+} induced activation of RyR2, but the effects are more modest than those seen with RyR1 (Xu et al. 1996; Kermode et al. 1998). In cells, most ATP is in complex with Mg^{2+}. Therefore, it is probable that under physiological conditions the MgATP complex rather than free ATP regulates Ca^{2+} release. The presence of high concentrations of free Mg^{2+} in cells and its inhibitory effects on RyR makes it difficult to determine the different effects of ATP and MgATP.

Calmodulin

CaM is a ubiquitously expressed 17-kDa Ca^{2+}-binding protein that regulates RyRs by direct binding. CaM is also known to bind to and regulate $Ca_v1.1$ and $Ca_v1.2$ (Tang et al. 2002; Ohrtman et al. 2008; Halling et al. 2009). CaM contains four EF-hand Ca^{2+} binding pockets (two in the carboxy-terminal domain and two in the amino-terminal domain of the protein) and binds to one site per RyR subunit (four per tetramer) (Moore et al. 1999a). All three RyR isoforms bind and are regulated by CaM both in its Ca^{2+}-free (apoCaM) and Ca^{2+}-bound (CaCaM) states (Tripathy et al. 1995; Yamaguchi et al. 2005). ApoCaM is a partial agonist whereas CaCaM is an inhibitor of RyR1 and SR Ca^{2+} release (Rodney et al. 2000). CaM binding site involves amino acids 3614–3643 of the RyR1 rabbit sequence (Takeshima et al. 1989; Moore et al. 1999b; Yamaguchi et al. 2003; Zhang et al. 2003). Cryo-EM difference mapping of the three-dimensional structures of RyR1 with and without added CaM has suggested that the CaCaM binding site is located in subdomain 3. The site seems to be displaced to ~33 Å in the presence of Ca^{2+} with respect to its position for apoCaM (Wagenknecht et al. 1994; Wagenknecht et al. 1997; Samsó and Wagenknecht 2002). This displacement could be caused by a movement of the CaM upon binding calcium and/or a movement of the CaM binding site when RyR1 binds Ca^{2+}. The structure of CaM bound to a RyR1 peptide (3614–3643) has been visualized by NMR residual dipolar coupling (Maximciuc et al. 2006). Amino acids 3615–3628 contact the carboxy lobe of CaM, whereas amino acids 3628–3637 bind the amino lobe of CaM. In cardiac muscle, CaM shifts the Ca^{2+}-dependence of RyR2 activation to higher Ca^{2+} concentrations and hence decreases the RyR2 opening at all Ca^{2+} concentrations (Balshaw et al. 2001; Yamaguchi et al. 2003). Recently reduced affinity for CaM binding to RyR2 with PKA phosphorylation was found in a CPVT-associated mouse model (Arg2474Ser), resulting in spontaneous local Ca^{2+} release events leading to lethal arrythmias (Xu et al. 2010). In addition to CaM, a number of other EF-hand containing proteins have been recognized to interact with and regulate RyR, including calumenin and S100A1 (Jung et al. 2006; Wright et al. 2008). S100A1 has been found to compete with CaM for the RyR binding site (Wright et al. 2008). The questions of which of these EF-hand proteins actually regulate RyRs in vivo have yet to be answered.

Calsequestrin

Calsequestrin (CSQ) is the major intra-SR Ca^{2+} buffer. There are two genes encoding CSQ; type 1 CSQ (CSQ1) expressed in skeletal muscle and type 2 CSQ (CSQ2) expressed in cardiac and low levels in slow-twitch skeletal muscle. In addition to functioning as a Ca^{2+} buffer, CSQ forms oligomers in the lumen and interacts with the RyR anchoring proteins junctin and triadin embedded in the SR membrane. Together these three proteins appear to regulate RyR activity. The molecular details underlying these interactions have not been elucidated in either skeletal or cardiac muscle (Beard et al. 2009; Györke et al. 2009).

CSQ1 and CSQ2 appear to have unique isoform-specific properties in skeletal and cardiac muscle. CSQ1 reduces the activity of

RyR1 whereas CSQ2 increases the open probability of RyR1 and RyR2 (Wei et al. 2009). CSQ1-mediated inhibition of Ca^{2+} release during a single action potential may tune RyR1 activation to stimulation frequency and maintain Ca^{2+} release with repeated stimulation. In cardiac muscle CSQ2 may facilitate high rates of Ca^{2+} efflux through RyR2 during systole resulting in fast activation of contraction.

Recently, a mutation in the CSQ2 gene was linked to exercise-induced cardiac death caused by CPVT, although under basal conditions the cardiac contractility is apparently normal in subjects lacking functional CSQ2 (Postma et al. 2002). Knollmann and coworkers showed that *Casq2*-null mice are viable and display normal SR Ca^{2+} release and contractile function under basal conditions. However, exposure to catecholamines in *Casq2*-null myocytes caused increased diastolic SR Ca^{2+} leak, resulting in premature spontaneous SR Ca^{2+} releases that triggered beats indicating that these mice are susceptible to catecholaminergic ventricular arrhythmias (Knollmann et al. 2006).

FK506-Binding P ǫ ein 12 and 12.6 (FKBP12 and FKBP12.6)

FKBPs are named according to their molecular mass and belong to the immunophilins, a family of highly conserved proteins that bind immunosuppressive drugs such as FK506 and rapamycin. FKBPs are expressed in most tissues and are involved in a number of biochemical processes such as protein folding, receptor signaling, protein trafficking, and transcription. FKBP12 and FKBP12.6 (also known as calstabin 1 and 2, respectively) physically interact with all three isoforms of RyR but have different expression levels and binding affinity in different tissues (Chelu et al. 2004). FKBP12 copurifies with RyR1 (Jayaraman et al. 1992; Brillantes et al. 1994) and FKBP12.6 copurifies with RyR2 (Timerman et al. 1995; Timerman et al. 1996; Barg et al. 1997; Jeyakumar et al. 2001; Masumiya et al. 2003). Although somewhat controversial, a component of the FKBP12 binding site appears to be located between amino acids 2458 and 2468 of RyR1 (Rabbit sequence, SwissProt accession #P11716). Mutation of the amino acid Val2461 abolishes the FKBP12 binding (Gaburjakova et al. 2001; Avila et al. 2003). The amino-terminal and the carboxy-terminal regions of RyR2 have also been suggested to interact with FKBP12.6 (Masumiya et al. 2003; Xiao et al. 2004; Zissimopoulos and Lai 2005). Difference mapping of three-dimensional reconstructions of RyR with and without FKBP12 or 12.6 places the FKBPs binding site between subdomains 3, 5, and 9 (Wagenknecht et al. 1996; Wagenknecht et al. 1997; Samsó et al. 2006; Sharma et al. 2006). In agreement with this localization, FRET studies have localized the FKBP12 (Cornea et al. 2009) and the FKBP12.6 (Cornea et al. 2010) binding site to the same area as the model from Samsó et al 2006. Furthermore, both FKBP12 and 12.6 bind RyR1 and RyR2 in the same orientation (Cornea et al. 2010). Comparison of this location with the docking of the IP3 homology model, which includes the suppressor domain and the IP3-binding core region both with high sequence similarity to RyR1 amino-terminus, suggests a binding pocket for FKBP12 formed by Glu161, Arg164, Arg402, and Ile404 (Serysheva et al. 2008).

In mammals FKBP12 and 12.6 bind to RyRs with a stoichiometry of four FKBPs per RyR homotetramer (Jayaraman et al. 1992; Timerman et al. 1993; Qi et al. 1998). Under physiological conditions (i.e., the absence of immunosuppressive drugs), FKBPs are though to bind to RyRs with high affinity and stabilize the closed state of the channel (Ahern et al. 1994; Brillantes et al. 1994; McCall et al. 1996; Ahern et al. 1997; Marx et al. 1998; Marx et al. 2001). Removal of FKBP12, by preventing rebinding with an immunosuppressive drug or as the result of a genetic FKBP deficiency leads to greater open probability of the channel and longer mean open times (Ahern et al. 1997; Marx et al. 1998; Shou et al. 1998). Furthermore, FKBP12 displacement in skeletal muscle alters the coupling between RyR1 and $Ca_V1.1$. The consequences of these changes are dependent on muscle type and activity (Tang et al. 2004). In cardiac muscle, FKBP12 deficiency results in cardiomyopathy and ventricle septal defects

that mimic human congenital heart disorder (Shou et al. 1998).

PKA and CaMKII Phosphorylation

The importance of RyR phosphorylation in modulation of Ca^{2+} release from SR was first established in the heart (Takasago et al. 1989). The functional consequences of phosphorylation on RyR function and the identity of the enzymes involved have been the focus of considerable debate. RyRs have several potential phosphorylation sites in their cytoplasmic domains. PKA, CaMKII, and cGMP-dependent kinase (PKG) have all been shown to phosphorylate RyR isoforms (Rodriguez et al. 2003; Wehrens et al. 2004; Xiao et al. 2006; Huke and Bers 2007).

The "fight or flight" response is a classic physiological stress pathway that involves activation of the sympathetic nervous system (SNS) that among other effects results in larger and faster Ca^{2+} transients and subsequently stronger and faster muscle contractions (Bers 2002). SNS activation causes β-adrenergic stimulation of the muscle, which via an intracellular signaling cascade results in activation of PKA. SNS-activated PKA phosphorylates RyR, altering its gating properties, but also phosphorylates several other key proteins involved in Ca^{2+} handling such as troponin I and phospholamban (Valdivia et al. 1995; Li et al. 2000; Kentish et al. 2001; Reiken et al. 2003). Modified RyR function is associated with increased SR Ca^{2+} leak in heart, which could contribute to reduced contractile function and increased propensity to arrhythmias. Altered phosphorylation of RyR2 has been suggested as one possible explanation for RyR dysfunction. Marks and colleagues propose that a hyper-adrenergic state that occurs in heart failure or during extreme stress, including exercise, leads to hyperphosphorylation of RyR serine residues (Ser2030, Ser2809 in RyR2 and Ser2843 in RyR1). They also suggest that hyperphosphorylation causes FKBPs to dissociate from RyRs, producing "leaky channels" (i.e., channels prone to open at rest). Such leaky channels could underlie increased risk for arrhythmias in heart failure and contribute to decreased muscle force production by reducing SR Ca^{2+} store content (Marx et al. 2000; Reiken et al. 2003). However, other groups have not found PKA-dependent hyperphosphorylation in failing hearts (Xiao et al. 2005). In addition, other laboratories suggest that CamKII-dependent phosphorylation of RyR2 is involved in enhanced SR Ca^{2+} leak and reduced SR Ca^{2+} load in heart failure and may contribute to arrhythmias and contractile dysfunction (Ai et al. 2005; Chelu et al. 2009; Curran et al. 2010; Neef et al. 2010). Phosphorylation of other targets of these kinases (troponin I, sarcolemmal Ca^{2+} channels, and phospholamban) could also alter the Ca^{2+} handling in cardiac and skeletal muscle.

CaMKII is modulated by changes in intracellular Ca^{2+} ($[Ca^{2+}]_i$), although little is still known quantitatively about the role of dynamic $[Ca^{2+}]_i$ fluctuations in the activation of CaMKII (Huke and Bers 2007; Aydin et al. 2007). CaMKII phosphorylates the same residues on RyR1 as PKA and also phosphorylates several other proteins such as troponin I, sarcolemmal Ca^{2+} channels, and phospholamban. Ser2808 on RyR2 was first described as a CaMKII phosphorylation site, but it was later shown that both PKA and PKG also phosphorylate this site (Witcher et al. 1991; Rodriguez et al. 2003; Wehrens et al. 2004; Xiao et al. 2006; Huke and Bers 2007). Ser2814, however, appears to only be phosphorylated by CaMKII whereas Ser2030 is only phosphorylated by PKA (Wehrens et al. 2004; Xiao et al. 2006).

Reactive Oxygen Species and Reactive Nitrogen Species

Sulfhydryl groups (SH, also called thiol) of cysteine (Cys) residues are potential targets for reduction/oxidation (redox) modifications of proteins. Alteration in the redox state of SH groups of two neighboring cysteine residues can lead to formation or breaking of disulfide bonds, which can modify both the structure and function of proteins. Low concentrations of redox active molecules (reactive oxygen species/reactive nitrogen species; ROS/RNS) constitute a basal endogenous redox buffering system that reversibly interacts with proteins.

Both RyR1 and RyR2 have nitric oxide (NO) covalently bound to cysteines (i.e., *S*-nitrosylation) and this posttranslational modification is reversible (Xu et al. 1998; Eu et al. 2000; Sun et al. 2008). High levels of ROS/RNS are able to irreversibly modify and even damage proteins in cardiac ischemia-reperfusion injury (Ferdinandy and Schulz 2003).

RyR is an established redox-sensitive channel and alterations in its redox state can result in either activation (Stoyanovsky et al. 1997; Eager and Dulhunty 1998) or inactivation (Boraso and Williams 1994; Marengo et al. 1998). Other key components of Ca^{2+} regulation and E-C coupling, e.g., SERCA and Ca_v's, are also redox modulated. RyR has ~100 cysteines per subunit and ~20 of them have been estimated to be free for redox modifications by oxidation, nitrosylation, or alkylation by the redox active molecule glutathione (Zable et al. 1997; Xu et al. 1998). A number of redox-sensitive cysteines have been identified in both the open and closed state of the channel and appear to be distributed across the primary structure of cytoplasmic region (Voss et al. 2004; Aracena et al. 2006). Several of these sites have been mapped to the clamp domains like Cys36 and Cys315 (Liu et al. 2005; Amador 2009; Hamilton and Serysheva 2009; Lobo and Van Petegem 2009), whereas the Cys3635 is located in the subdomain 3 in the CaM binding site (Moore et al. 1999b; Sun et al. 2001). *S*-nitrosylation of Cys3635 has been shown to reverse the CaM inhibition on RyR1 and to activate the channel (Moore et al. 1999b). The *S*-nitrosylation of Cys3635 appears to occur only at physiological tissue O_2 tension (pO_2; ~10 mm Hg) and facilitates muscle contraction (Eu et al. 2003). Moreover, increased *S*-nitrosylation-induced RyR1 activity is suggested to sensitize RyR1 to environmental heat stress and MH crises (Durham et al. 2008). Increased RyR1 nitrosylation has also been observed in muscle dystrophy and is thought to contribute to muscle weakness by increased SR Ca^{2+} leak (Bellinger et al. 2009). In comparison to RyR1, no specific redox sensitive cysteine residues have yet been identified for RyR2. RyR2 is also pO_2-responsive but is not activated or S-nitrosylated directly by NO; instead activation and *S*-nitrosylation of RyR2 requires *S*-nitrosoglutathione (Sun et al. 2008).

CONCLUDING REMARKS

Primary sequence and location of several mutations are identified for RyR, but unanswered questions and debated topics still remain regarding the tertiary structure, the macromolecular interactions, and the regulation of RyR. The large size of RyR makes it more challenging to study, but new insights into the detailed structure of RyR are emerging with the continuous improvement and refinement of technologies such as cryo-EM and FRET-based assays. Resolution of the structure of RyR is progressing steadily, and ultimately we will have a map including carbon backbones, side-chains, membrane spanning regions and binding sites of interacting molecules. Along with our understanding of RyRs structure, it is likely that the number of known modulators that interact with RyR will also increase. Although the basic role and function of RyR in E-C coupling in skeletal and cardiac muscle is well established, further refinement of our understanding of the many modulators of RyR will be important in the development of therapeutics for treatment of cardiac and skeletal muscle diseases.

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