

# Physiology and pathophysiology of excitation–contraction coupling: the functional role of ryanodine receptor

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**Abstract** Calcium ( $\text{Ca}^{2+}$ ) release from intracellular stores plays a key role in the regulation of skeletal muscle contraction. The type 1 ryanodine receptors (RyR1) is the major  $\text{Ca}^{2+}$  release channel on the sarcoplasmic reticulum (SR) of myocytes in skeletal muscle and is required for excitation–contraction (E–C) coupling. This article explores the role of RyR1 in skeletal muscle physiology and pathophysiology.

**Keywords** Calcium · Excitation–contraction coupling · Muscular dystrophy · RyR1 · Skeletal muscle

## Introduction

Ryanodine receptors (RyRs) are intracellular calcium ( $\text{Ca}^{2+}$ ) release channels located on the endo/sarcoplasmic reticulum (ER/SR) (Flucher et al. 1993), a heterogeneous intracellular compartment consisting of a network of tubules (Chen et al. 2013; Brochet et al. 2005) representing the major  $\text{Ca}^{2+}$  reservoir within the cell. There are three subtypes of RyRs in mammalian tissues: RyR1 and RyR2 are required for skeletal muscle and cardiac excitation–contraction coupling (E–C coupling), respectively (Marks et al.

1989; Otsu et al. 1990), and are also expressed in non-muscle tissues (Awad et al. 1997); RyR3, originally identified in the brain (Nakashima et al. 1997), is also widely expressed (Zhang et al. 2011).

RyR1 facilitates the rapid and coordinated release of  $\text{Ca}^{2+}$  from SR stores to activate skeletal muscle contraction. EC coupling is the process that converts electrical signals and rising  $\text{Ca}^{2+}$  levels into mechanical output (muscle contraction). RyRs are highly regulated for precise control and  $\text{Ca}^{2+}$  plays the key signaling role in activating the channel and amplifying the signal (Endo et al. 1970). In this process, depolarization of the plasma membrane activates L-type voltage-gated calcium channels ( $\text{Ca}_v$ ), which signal RyRs located on the SR to gate open and release  $\text{Ca}^{2+}$  to activate muscle contraction (Rios and Brum 1987; Gordon et al. 2000; Tobacman 1996; des Georges et al. 2016). RyR is a 2.2 mega Dalton homotetramer, composed of four ~5000 residue protomers (Marks et al. 1989; Santulli and Marks 2015), making it the largest known ion channel (des Georges et al. 2016; Santulli and Marks 2015; Zalk et al. 2015). The narrow transmembrane core and larger cytoplasmic shell result in a mushroom shaped structure (des Georges et al. 2016; Zalk et al. 2015; Hwang et al. 2012). The large shell interacts with other receptors and forms much of the regulatory mechanism for the channel, allowing a range of stimuli to exert precise control over opening (Marks et al. 1989; des Georges et al. 2016; Santulli and Marks 2015; Zalk et al. 2015; Brillantes et al. 1994; Marx et al. 1998, 2000; Lehnart et al. 2005; Huang et al. 2006; Bellinger et al. 2009; Kushnir et al. 2010; Shan et al. 2010; Andersson et al. 2011; Lanner et al. 2010). The core of RyR houses the approximately 90 Å long pore responsible for passage of  $\text{Ca}^{2+}$  from the ER/SR to the cytoplasm (des Georges et al. 2016; Yan et al. 2015). This cation channel is actually poorly selective for  $\text{Ca}^{2+}$  (~7-fold selective

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for  $\text{Ca}^{2+}$  vs  $\text{K}^{+}$ ) and displays an exceptionally large single channel conductance (Santulli and Marks 2015).

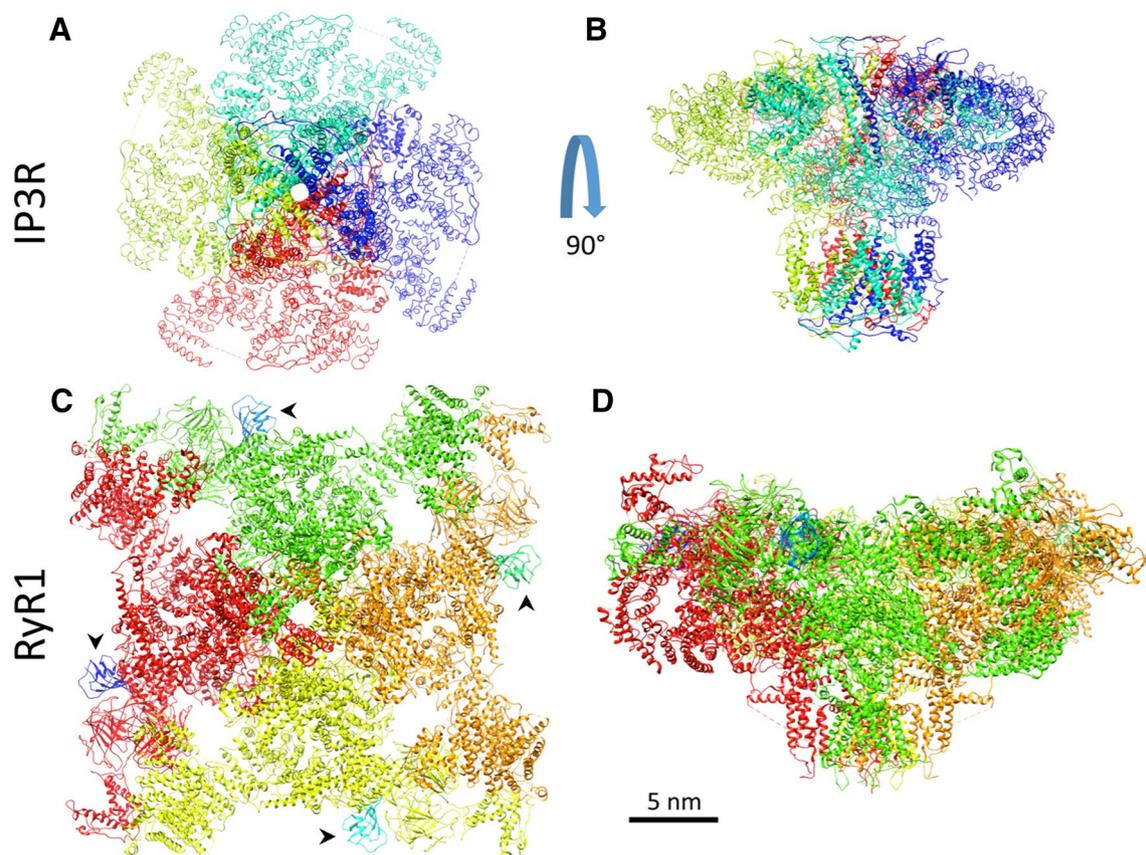
We recently solved the high-resolution structure of RyR1 using cryogenic electron microscopy (cryo-EM) (des Georges et al. 2016; Zalk et al. 2015), confirming that it adopts a fourfold symmetric mushroom-like superstructure, with the large ‘cap’ (about 80% of the mass) located in the cytosol and the ‘stalk’ embedded in the ER/SR membrane, with six transmembrane helices (S1–S6) per protomer surrounding the central pore (des Georges et al. 2016). Each protomer is built around an extended scaffold of alpha-solenoid repeats which include an aminoterminal, a bridging, and a core solenoid (des Georges et al. 2016; Zalk et al. 2015). At the extreme outer corners of the tetramer there are three SPRY domains and two pairs of RyR repeats, RY12 and RY34, the latter containing a regulatory protein kinase A (PKA) phosphorylation site (Marx et al. 2000). The RyR1 pore domain most closely resembles that of the voltage-gated sodium channel (NavAB) and presents a single cytosolic constriction in the ion conduction pathway, at the S6 bundle crossing (Zalk et al. 2015). Glycine residues in the pore-lining helices may operate as “hinges” to

facilitate the orientation of the cytoplasmic extension of S6 in order to modulate the aperture of the channel. In particular, Gly<sup>4934</sup> is conserved in all RyR isoforms and in the IP3R (Fan et al. 2015).

### RyR macromolecular complex

The ER/SR of most cell types contains two types of intracellular  $\text{Ca}^{2+}$  release channels: the ryanodine receptors (RyRs) and the inositol 1,4,5-trisphosphate receptors (IP3Rs) (Santulli and Marks 2015; Go et al. 1995; Yuan et al. 2016; Santulli 2017). There is ~40% homology between the RyR and IP3R in the putative transmembrane regions (Marks et al. 1989, 1990; Santulli 2017), a sequence similarity sufficient to indicate that these two channels evolved from a common ancestral cation release channel in unicellular species. The structural homology between RyR1 and IP3R1 is depicted in Fig. 1.

RyR was named based on its purification using the high affinity plant alkaloid ryanodine (Rogers et al. 1948), an agent known to profoundly alter intracellular  $\text{Ca}^{2+}$  handling (Fairhurst and Hasselbach 1970). Indeed, when bound to



**Fig. 1** Structural homology between the intracellular  $\text{Ca}^{2+}$  release channels IP3R1 (*top*) and RyR1 (*bottom*). In **a**, **c** channels are viewed from the ER/SR lumen; in **c**, *arrowheads* indicate Calstabin. IP3R1

figure adapted from Protein Data Bank (PDB) ID: 3JAV (Fan et al. 2015) and RyR1 figure adapted from PDB ID: 5TA3 (des Georges et al. 2016)

RyR at low concentrations ryanodine locks the channel in a half open state, thereby resulting in depletion of  $\text{Ca}^{2+}$  from the SR and subsequent interruption of E–C coupling. This explains the historical use of extracts from the *Ryania* plant family by natives of South and Central America as poison for blow darts: the release of SR  $\text{Ca}^{2+}$  via the locked open RyRs causes tetany, and at high concentrations ryanodine blocks the channel (Rogers et al. 1948). RyR is normally closed at low cytosolic  $[\text{Ca}^{2+}]$  (~100–200 nM); at sub-micromolar cytosolic  $[\text{Ca}^{2+}]$   $\text{Ca}^{2+}$  binds to high-affinity binding sites on RyR increasing the open probability ( $P_o$ ) of the channel (Bezprozvanny et al. 1993). Channel activity is maximal at cytosolic  $[\text{Ca}^{2+}]$  ~10  $\mu\text{M}$  while elevating cytosolic  $[\text{Ca}^{2+}]$  beyond this point leads to a reduction in  $P_o$  (Bezprozvanny et al. 1993; Copello et al. 1997; Laver et al. 1995).

The large and complex structure of RyR contains function-modifying phosphorylation sites and protein-binding domains, providing an attractive target for disease intervention (des Georges et al. 2016; Santulli and Marks 2015; Zalk et al. 2015; Brillantes et al. 1994; Marx et al. 1998, 2000, 2001; Lehnart et al. 2005; Kushnir et al. 2010; Marks et al. 2002). RyRs are macromolecular signaling complexes, in which multiple proteins bind to a domain of the channel modulating its function (Marks et al. 1989, 2002). The  $\text{Ca}^{2+}$  stabilizing proteins calstabin1 (Calcium channel stabilizing binding protein, previously known as FKBP12) and calstabin2 (FKBP12.6) are peptidyl-propyl-*cis-trans* isomerases that associate via amphiphilic  $\beta$ -sheet structures with RyR1 and RyR2, respectively, such that one calstabin protein is bound to each RyR monomer (des Georges et al. 2016; Zalk et al. 2015; Jayaraman et al. 1992; Timmerman et al. 1993; Xin et al. 1995; Yuan et al. 2014), in order to modulate the channel gating through protein–protein interactions (Brillantes et al. 1994) and prevent pathological intracellular  $\text{Ca}^{2+}$  leak that cause diseases (Huang et al. 2006). Calstabin1 and calstabin2 differ at only 18 positions out of 108 residues. We identified the calstabin-binding loop as part of the aminoterminal subdomain of the bridging solenoid (Zalk et al. 2015). Calstabin binding may rigidify the interface between such a subdomain with SPRY1–2, thereby stabilizing the connection with the cytosolic regulatory domains and eventually altering the relative orientation of these domains (Zalk et al. 2015). Highly conserved leucine–isoleucine zipper motifs in RyR2 form binding sites for adaptor proteins that mediate binding of other proteins (Marx et al. 2001; Marks et al. 2002), including kinases (e.g. PKA) (Shan et al. 2010) CaMKII $\delta$  (Kushnir et al. 2010) and phosphatases (e.g. PP1 and PP2A). Specifically, the adaptor protein mAKAP mediates the binding of PKA and phosphodiesterase PDE43, whereas PP1 and PP2A are targeted to RyR2 via spinophilin and PR130, respectively (Marx et al. 2000; Lehnart et al. 2005). All of

the above mentioned proteins regulate the phosphorylation-dephosphorylation of RyR2 in Ser<sup>2808</sup> (Shan et al. 2010) in response to stress (Andersson et al. 2011; Shan et al. 2010; Liu et al. 2012; Tester et al. 2007). Other channels are also regulated by stress signals including the voltage-gated  $\text{Ca}^{2+}$  channels (Maki et al. 1996). RyRs are also regulated by oxidation and nitrosylation (Shan et al. 2010; Andersson et al. 2011; Santulli 2017; Fauconnier et al. 2010). Other modulatory proteins complex directly and indirectly with RyR, including sorcin (Farrell et al. 2004), calmodulin (Meissner and Henderson 1987), homer (Feng et al. 2002), histidine-rich  $\text{Ca}^{2+}$  binding protein (Lee et al. 2001), triadin (Rossi et al. 2014), junctin (Zhang et al. 1997), and calsequestrin (Ohkura et al. 1998).

### Intracellular $\text{Ca}^{2+}$ leak

$\text{Ca}^{2+}$  finely regulates innumerable events as muscle contraction, secretion, and gene transcription (Santulli and Marks 2015; Santulli 2017; Ringer 1883; Zetterstrom and Arnhold 1958; Jayaraman and Marks 2000). Cytosolic  $\text{Ca}^{2+}$  signals are produced by rapidly increasing the concentration of free  $\text{Ca}^{2+}$  ions (Blaustein 1993) by opening channels permeable to  $\text{Ca}^{2+}$  either in the surface cell membrane or in the membranes of intracellular organelles containing high  $\text{Ca}^{2+}$  concentrations. Amplification of external stimuli by triggering the release of intracellular  $\text{Ca}^{2+}$  stores represents a common signaling mechanism in the cell. The key role of RyRs in the rapid and voluminous release of  $\text{Ca}^{2+}$  from the SR during E–C coupling is well known. Importantly, RyRs are also crucially involved in maintaining  $\text{Ca}^{2+}$  homeostasis in the cell under resting conditions. Stress-induced remodeling of RyRs results in leaky channels and the inappropriate release of  $\text{Ca}^{2+}$  from the intracellular stores into the cytosol, contributing to the pathophysiology of diverse disorders including heart failure, cardiac arrhythmias, muscular dystrophy, diabetes, and cognitive dysfunction (Brillantes et al. 1994; Marx et al. 1998, 2000, 2001; Lehnart et al. 2005; Huang et al. 2006; Bellinger et al. 2008, 2009; Kushnir et al. 2010; Shan et al. 2010; Andersson et al. 2011, 2012; Marks et al. 2002; Liu et al. 2012; Tester et al. 2007; Fauconnier et al. 2010; Ward et al. 2003; Umanskaya et al. 2014; Matecki et al. 2016; Santulli et al. 2015a, b, 2017; Xie et al. 2013, 2015).

### Skeletal muscle

E–C coupling is similar in skeletal and cardiac muscle but there are important differences (Santulli 2017). Briefly, whereas in the heart a depolarizing  $\text{Na}^+$  current activates  $\text{Ca}^{2+}$  influx via the L-type  $\text{Ca}^{2+}$  channel (LCC,  $\text{Ca}_v1.2$ ), which in turn activates the RyR2 isoform via  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release (Fabiato and Fabiato 1975), the depolarization

of skeletal myocytes involves a protein–protein interaction (Rios and Brum 1987) across the junctional cleft between the dihydropyridine receptor ( $\text{Ca}_v1.1$ ) on specialized invaginations of the sarcolemma (transverse tubules) and RyR1 on the SR membrane (terminal cisternae), leading to  $\text{Ca}^{2+}$  release (Nelson et al. 2013). Both morphologic and electrophysiological data are consistent with the concept that four  $\text{Ca}_v1.1$ s interact with a single RyR1 tetramer (one  $\text{Ca}_v1.1$  binding to each RyR1 subunit). However, Franzini-Armstrong and Kish determined that a cluster of four  $\text{Ca}_v1.1$  overlies only every other RyR1 tetramer (Franzini-Armstrong and Kish 1995). Reconciling those findings, we have demonstrated coupled gating of RyR1 (Marx et al. 1998), which provides a mechanism by which RyR1 channels that are not associated with  $\text{Ca}_v1.1$  can be regulated. RyRs were initially observed in skeletal muscle, visualized in electron micrographs as large electron-dense masses located along the face of the SR terminal cisternae, which is closely apposed to transverse tubule membranes to form a structure named triad junction (Santulli 2017; Block et al. 1988). Therefore, the RyRs were initially termed triad junctional foot proteins (Wagenknecht et al. 1989; Brandt et al. 1990). Noda and colleagues provided the *in vivo* evidence for a functional role of RyR1 in E–C coupling, engineering a mouse lacking exon 2 of *RyR1* and demonstrating that such a mouse exhibits severe skeletal muscle abnormalities and dies perinatally due to respiratory failure (Takeshima et al. 1994). Subsequent ultrastructural studies of hind limb and diaphragm muscles demonstrated the absence of RyR1- $\text{Ca}_v1.1$  complexes (Takekura et al. 1995), which are essential for a proper E–C coupling in the skeletal muscle (Nakai et al. 1996).

RyR1 dysfunction has been described in both inherited and acquired muscle disorders (Bellinger et al. 2008; Andersson et al. 2012). Central core disease (CCD) and malignant hyperthermia (MH) represent the best examples of RyR1 channelopathies in the skeletal muscle.

### Central core disease (CCD)

CCD is a congenital myopathy first described in 1956 (Magee and Shy 1956), characterized by the presence of tissue cores with reduced oxidative activity in type I myofibers, resulting in progressive muscle weakness (Sewry et al. 2002). Common symptoms include hypotonia, delayed motor milestones, and skeletal abnormalities including congenital hip dislocation and scoliosis. Over 60 different RyR1 mutations have been linked to CCD, which presents during infancy as delayed motor development and hypotonia. CCD occurs in 1:100,000 live births, and comprises 16% of total congenital myopathies (Jungbluth 2007).

We now know that RyR1 mutations cause the disorder which should be reclassified as RyR1 myopathies. There

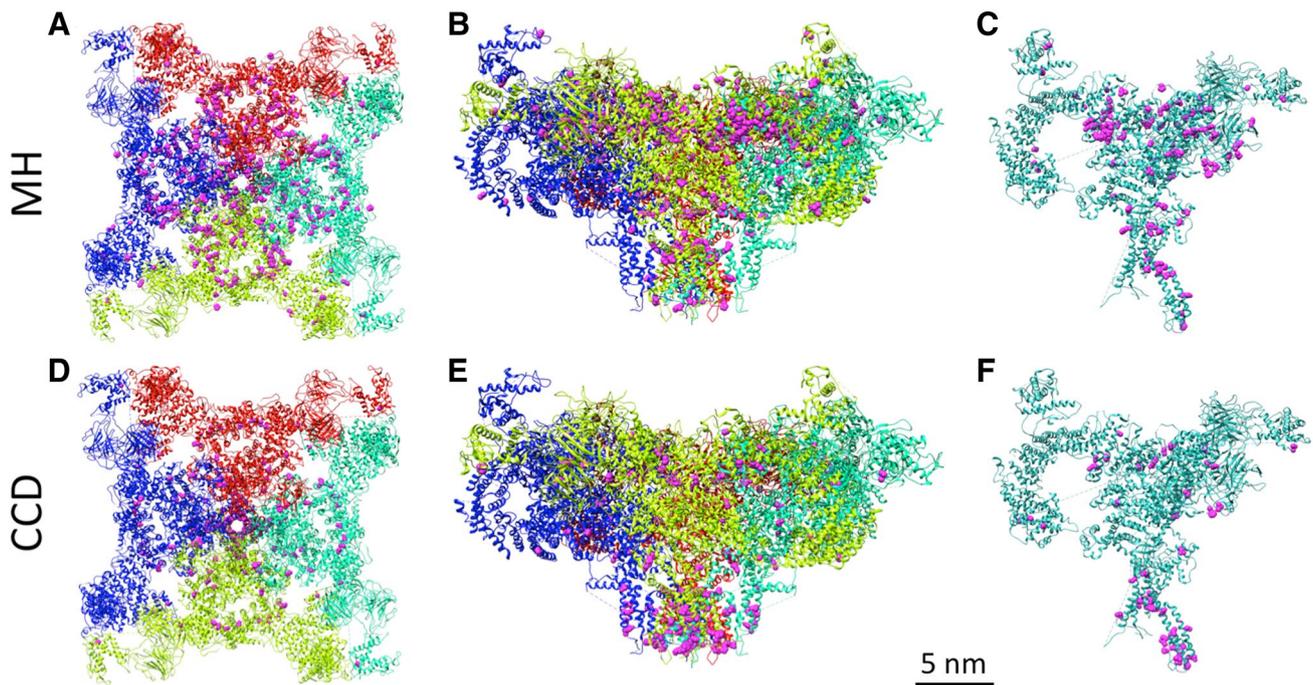
are no established therapeutics for RyR1 myopathies (Witherspoon and Meilleur 2016). The phenotypic presentation is quite variable ranging from near normal to neonatal death.

The histopathological appearance of CCD is most closely linked to dominant RyR1 mutations (often missense) clustered (Fig. 2) in disease causing “hot spots” in RyR1 (Quane et al. 1993; Zhang et al. 1993; Lynch et al. 1999; Monnier et al. 2000; Scacheri et al. 2000), whereas RyR1 mutations (often truncating) causing recessive RyR1-related myopathies, including multi-minicore disease, centronuclear myopathies, and congenital fiber-type disproportion, are evenly distributed throughout the entire RYR1 coding sequence (Amburgey et al. 2013; Klein et al. 2012).

### Malignant hyperthermia (MH)

MH is a pharmacogenetic disorder, inherited in an autosomal dominant fashion and causes inhaled anesthetic-induced deaths in otherwise healthy individuals (Censier et al. 1998). MH episodes are typically rapid and severe, reaching core body temperatures of 43 °C, leading to organ failure and death if not rapidly treated. Susceptibility can be determined *in vitro* by measuring the contractile response to caffeine or halothane in biopsied muscle fibers. Over 100 RyR1 mutations have been associated with MH, involving inappropriate activation of RyR1, which causes uncontrolled release of SR  $\text{Ca}^{2+}$  and muscle contractions. MH occurs at a rate of 1:50,000–100,000 adults and 1:15,000 children undergoing anesthesia; some studies have suggested a much more frequent rate of 1:5000 adults with MH susceptible mutations occurring at 1:3000 (Rosenberg et al. 2007; Monnier et al. 2002). The exact prevalence of MH susceptibility is difficult to determine since the syndrome only becomes apparent after exposure to triggering agents including volatile anesthetic agents such as halothane, isoflurane, sevoflurane, desflurane, enflurane and the neuromuscular blocking agent succinylcholine (Larach 2007). A related syndrome referred to as porcine stress syndrome is found in certain lines of domestic swine where stressed pigs undergo stress-induced hyperthermia (Nelson and Bee 1979). Alterations in  $^3\text{H}$ -ryanodine binding properties in porcine MH samples provided evidence linking RyR1 dysfunction to the disease (Mickelson et al. 1988), which was later confirmed by biophysical experiments (Fill et al. 1990).

Although dantrolene is an established therapeutic that quickly resolves MH episodes, mortality from this event remains at approximately 7% and a validated mechanism of action for dantrolene has yet to be reported (Paul-Pletzer et al. 2002; Zhao et al. 2001). This remains a concern for otherwise healthy individuals harboring these mutations (Fill et al. 1990). Mutations causing MH are autosomal



**Fig. 2** RyR1 with localization of the reported mutations for CCD (**a–c**) and MH (**d–f**). **a** and **d** are the full tetramer viewed top down from the cytosol, while **b** and **e** are rotated 90° to show the narrow transmembrane core and the larger cytoplasmic shell (an additional 45° rotation along the vertical axis was also performed). In **c**, **f** one protomer is depicted (following a 60° rotation), demonstrating the

high proportion of interprotomer mutation sites (in pink). Interestingly, CCD mutations typically occur in the pore forming C-terminal domain, while MH mutations occur in central and N-terminal clusters. Figure adapted from Protein Data Bank (PDB) ID: 5TA3 (des Georges et al. 2016) and RyR1 mutations reported in UniProt

dominant and typically seen (Fig. 2) in the central and N-terminal clusters. Another MH mutation hotspot is at the inter-protomer contacts between the N-terminal domains A and B, which are disrupted in channel opening (Kimlicka et al. 2013).

Notably, there is no clear division between MH and RyR1 myopathies and some *RyR1* mutations have been linked to a combined MH and RyR1 myopathy phenotype (Zhou et al. 2007). Importantly, the mutated codons giving rise to MH and RyR1 myopathies tend to cluster in three specific regions of the *RyR1* gene (Fig. 2) corresponding to the following domains in the amino acid sequence: regions 1 (C35–R614) and 2 (D2129–R2458) reside in the myoplasmic foot domain of the protein, whereas region 3 (I3916–G4942) is located in the transmembrane/luminal region of the highly conserved carboxy-terminal domain, important for allowing  $\text{Ca}^{2+}$  flux through the channel (Zalk et al. 2015). Mutations in *RyR1* are also associated with other rare RyR1 related congenital myopathies including centronuclear myopathy, multi-minicore disease, Samaritan myopathy, heat/exercise induced exertional rhabdomyolysis, congenital fiber-type disproportion, late-onset axial myopathy, and atypical periodic paralyses (Bharucha-Goebel et al. 2013; Zvaritch et al. 2009; Ferreiro et al. 2002;

Capacchione et al. 2010; Zhou et al. 2010; Inui et al. 1987; Takeshima et al. 1989; Loseth et al. 2013).

### Intracellular $\text{Ca}^{2+}$ leak and muscular dystrophy

We recently demonstrated that intracellular  $\text{Ca}^{2+}$  leak via RyR1 represents an essential feature of different forms of muscular dystrophy (MD), including Duchenne muscular dystrophy (Bellinger et al. 2009) and limb-girdle (or Erb's) MD (Andersson et al. 2012). Specifically, RyR1 from a Duchenne muscular dystrophy murine model (*mdx* mouse) was excessively cysteine nitrosylated and the RyR1 complex was depleted of calstabin1, leading to increased spontaneous RyR1 openings and reduced specific muscle force (Bellinger et al. 2009). Similar findings were obtained when evaluating RyR1 in  $\beta$ -sarcoglycan-deficient mice, an established model of limb-girdle muscular dystrophy (Andersson et al. 2012). Thus, we demonstrated common mechanisms of stress-induced remodeling of RyR1, including post-translational modifications of the channel and dissociation of the stabilizing subunit calstabin1, in two major disorders that weaken the muscular system hampering locomotion and that remain without effective pharmacological treatment. We demonstrated in both cases that

stabilizing the RyR1-calstabin1 association using a novel small molecule Rycal called S107 improved muscle function (Bellinger et al. 2009; Andersson et al. 2012), thereby providing an innovative therapeutic target and potential options for the treatment of muscular dystrophy.

In conditions of strenuous muscular stress or in a disease such as heart failure, both of which are characterized by chronic activation of the sympathetic nervous system and increased production of reactive oxygen and nitrogen species (Santulli 2014; Dalla Libera et al. 2005; Santulli and Iaccarino 2016), skeletal muscle function is impaired, possibly due to remodeling of RyR1 and impaired E–C coupling. We have shown in both an animal model as well as in exercising humans that chronic  $\beta$ AR stimulation and depletion of calstabin1 from RyR1 plays a role in contractile failure and muscle fatigue, defined as a decline in ability of a muscle to generate force during sustained exercise (Bellinger et al. 2008). Consistent with these observations, we have demonstrated that the remodeling of RyR1 plays a role in sarcopenia or age-dependent loss of muscle function (Andersson et al. 2011) and we were able to reduce RyR1 dysfunction and improve skeletal muscle function in aged mice (2 years old) by genetically enhancing mitochondrial antioxidant activity (Umanskaya et al. 2014).

Since skeletal muscle dysfunction, as observed in HF or muscular disorders, remains without effective treatment, drugs that restore RyR  $\text{Ca}^{2+}$  release function represent promising candidates. In this sense, Rycal treatment could be ideal in conditions that impair both cardiac and skeletal muscle function. Indeed, as well as muscular RyR1 undergoes post-translational modifications in HF (Ward et al. 2003), remodeling of the cardiac RyR2 has been also reported in murine models of Duchenne muscular dystrophy, triggering ventricular arrhythmias (Fauconnier et al. 2010).

### RyR1 mutations: clinical significance and structural effects

Over 300 mutations have been mapped to RyRs that are implicated in human diseases and 200 more that do not result in modified channel function. The disease causing mutations are most often found in hotspots, including the N-terminal (~1–600), the central (~2000–2600) and the C-terminal (~4000–5000) regions. High-resolution cryo-EM reconstructions have recently become available making it possible to see how these hotspots are localized, some in the channel pore and others in the inter-protomer and inter-domain interfaces (Tung et al. 2010). The phosphorylation domain is another hotspot for disease causing mutations (Yuchi et al. 2012).

Proper post-translational modifications and interaction with other proteins are also critical for RyR function.

Several human disorders are linked to improper phosphorylation or oxidation of RyRs including ventilator-induced diaphragmatic dysfunction (VIDD) and Duchenne muscular dystrophy (DMD). VIDD involves diaphragm muscle weakness after extended mechanical ventilation and has been linked to oxidation of RyR (Matecki et al. 2016). RyR1 cysteine-nitrosylation has been shown to have a role in DMD (Bellinger et al. 2009). An age-dependent increase in cysteine-nitrosylation occurs with dystrophic changes in the muscle, depleting the RyR1 macromolecular complex of calstabin1 resulting in  $\text{Ca}^{2+}$  leak. This finding links muscle inflammation and  $\text{Ca}^{2+}$  leak in the pathogenesis of DMD (Tidball and Villalta 2009). Indeed, in inflamed tissues there is an increased expression of inducible nitric oxide synthase (iNOS), which binds to RyR1 leading to  $\text{Ca}^{2+}$  leak and eventually to the activation of  $\text{Ca}^{2+}$ -dependent proteases (calpains) that promote muscle damage and wasting.

These alterations affect the function of RyRs, but the direct impact on the tetrameric assembly has yet to be shown in structural studies. Due to the critical requirement of the channel for proper muscle function, mutations that severely destabilize or significantly alter the channel structure most likely lead to non-viable embryos. These mutations most often lead to changes in the open probability of the channel, leading to  $\text{Ca}^{2+}$  leak. This hypersensitive activation can come from mutations on either the luminal or the cytosolic side of the receptor (Tong et al. 1997; Jiang et al. 2004). One potential explanation is that defects at the interface between the central and N-terminal regions would weaken the interactions stabilizing the receptor in the closed state, leading to increased susceptibility to stimuli (Tateishi et al. 2009; Suetomi et al. 2011). Albeit many disease-associated RyR1 mutations do increase the open probability of the channel, this is far from certain for all RyR1 mutations, in particular with regards to recessive RyR1-related myopathies associated with reduction of the RyR1 protein. Therefore, compounds enhancing the closed probability of the channel would have limited application in conditions where the RyR1 mutations result in reduced rather than enhanced  $\text{Ca}^{2+}$  conductance, or where the precise functional consequences of the specific RyR1 mutations are not known.

Adjacent RyRs are known to signal cooperatively as paracrystalline arrays in checkerboard patterns, allowing for simultaneous opening of multiple channels (coupled gating) in response to a stimulus (Marx et al. 1998; Cabra et al. 2016). This provides a mechanism by which RyR channels can effect the rapid and coordinated SR  $\text{Ca}^{2+}$  release (via mechanically triggering neighboring channels) that is required for EC coupling. Thus, RyRs act as both signal amplifiers and integrators by triggering neighboring

channels both physically and chemically with  $\text{Ca}^{2+}$  (Endo et al. 1970; Fabiato 1983).

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