The Ryanodine Receptors Gene Family: Expression and Functional Meaning

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Abstract
The family of ryanodine receptor (RyR) genes encodes three Ca^{2+} release channels: RyR1, RyR2 and RyR3. In addition to their well known role in regulating contraction in striated muscles, RyRs are expressed in many other cell types, where eventually multiple RyR isoforms can be co-expressed. Recent studies have revealed that several important regulatory mechanisms can modulate RyRs activity under normal and pathological conditions. In this review we shall summarise the most recent developments in this area of research.
Key words: brain, calcium channel, calcium channel binding protein, muscle, muscle disease.

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Ca^{2+} is one of the most primitive second messengers in biological systems. In resting cells, the intracellular Ca^{2+} concentration is usually kept below 200 nM [14, 32], but it can rise in the micromolar concentration in response to extracellular stimulation [13, 19]. Although Ca^{2+} can enter eukaryotic cells through channels located in the plasma membrane, specialized subcompartments of the endoplasmic reticulum which function as intracellular Ca^{2+} stores have been also developed. These stores represent an important source of Ca^{2+} for generating signals and are provided by specialized Ca^{2+} channels and Ca^{2+} transport systems. Intracellular Ca^{2+} release channels belong to two main distinct families: the Inositol Trisphosphate Receptor family ($InsP_3$) is activated by inositol 1,4,5 trisphosphate and the Ryanodine Receptor family (RyR), which has been identified by its ability to bind with high affinity the plant alkaloid ryanodine [51, 152, 201].

RyRs are tetramers with a molecular mass of approximately 2.3 million Daltons. In vertebrates, three different genes have been identified that encode three isoforms of RyRs (RyR1, RyR2 and RyR3). By contrast, in invertebrate species only one RyR isoform has been cloned [75, 121]. Mammalian RyR1, RyR2 and RyR3 show a high degree of homology, with an amino acid sequence identity of 67 to 70% [110, 123, 137]. In particular, amino acid sequence identities between the RyR3/RyR2, RyR3/RyR1 and RyR1/RyR2 isoforms in rabbit is 70%, 67% and 67%, respectively [74] Divergence among RyR isoforms can be restricted to three main regions named divergency (D) regions. With reference to the RyR1 sequence, region D1 spans amino acids 4254-4631, region D2 amino acids 1342-1403 and region D3, a glutamate rich sequence is localized between residues 1872 and1923 [189, 240]. The overall protein structure of RyRs is similar to that of $InsP_3Rs$, with a large cytosolic N-terminal region, a central modulatory domain and a C-terminal domain. Alignment of the amino acid sequences of RyRs and $InsP_3Rs$ reveals a certain degree of homology in the first 600 amino acids in the N-terminal region [57]. The central regions of RyRs and $InsP_3Rs$ are dissimilar in their sequence and are likely to contain domains with modulatory and transducing functions. In this region two internally repeated domains, referred to as RIH for "RyR and $InsP_3R$ Homology" have been described. The RIH domains lie between amino acid residues 466-643 and 2187-2364 in the human RyR1 and amino acid residues 199-677 and 1196-1356 in the human $InsP_3R1$ [155].

The C-terminal domain of both $InsP_3Rs$ and RyRs contains the transmembrane segments that form the Ca^{2+} channel pore. Twelve hydrophobic domains have been predicted in the COOH-terminal region of the molecule by Zorzato et al. [246]. Of these, only 4 (M5, M6, M8, M10) were predicted by Takeshima et al. [194] to be transmembrane sequences. The amino acid sequences forming the transmembrane domains are highly conserved between $InsP_3Rs$ and RyRs, with the exception of domains 3 and 4 (accordingly to the model proposed by Takeshima et al. [194]) that show the lowest degree of homology. Different evidence has shown that the carboxy terminal region of RyRs is important for the correct localization and functional activity of the channel [17, 18, 195]. It has been demonstrated that M2, M7 and M10 are involved in tetramer assembly and channel pore formation [40]. A conserved sequence (GVRAGGGIGD) in transmembrane domain 9 (M9) has been proposed to be part of the pore-forming segment of RyRs [42, 242].

Amino acids 4894 and 4899 in the rabbit RyR1 sequence have been proposed to be involved in channel conductance. Indeed mutations in these residues result in RyR1 channels displaying an altered K^+ conductance. Moreover, channels carrying mutations I4897A, I4897L, I4897V and D4917A show, in addition to a reduced K^+ conductance, lack of ryanodine binding and altered caffeine induced Ca^{2+} release [59].

Cryoelectron microscopy and three-dimensional reconstruction studies have confirmed the fourfold symmetry of the channels with a large cytoplasmic assembly and a small transmembrane region [164, 179, 180, 213]. Cryoelectron microscopy reconstructions of RyR2 have shown that much of the differences with RyR1 are located in the clamp domains that are thought to interact with DHPRs [180, 181]. In particular, the N-terminal domain has been found to be located at the corners within the clamp structure while the D1 region is adjacent to the calmodulin binding site in domain 3 [100, 101]. The D3 region has been recently mapped to domain 9 in the clamp structure, adjacent to the FKBP12 and FKBP12.6 binding sites [241].

The cytoplasmic assembly, corresponding to the N-terminal region of the RyRs is constructed from 10 or more domains that are loosely packed together and some of them consist of multidomain structures [164, 179, 180]. The large cytoplasmic domain represents the modulatory region of the receptor and contains several binding sites for nucleotide [74, 123, 194], calmodulin [128, 133, 134, 218, 231, 232, 241] FKBP12 [22, 23, 124], Mg^{2+} [247], as well as phosphorylation [38, 165, 166, 173, 174, 190, 192, 227] and glycosylation sites [18, 123]. High and low affinity binding sites for Ca^{2+} have been described in the C-terminal region of the channel [28, 31, 41, 141]. Cryoelectron microscopy and reconstruction analysis have allowed to identify the location of some RyR binding proteins on the three dimensional architecture of the channel. FKBP12 has been found to bind to the cytoplasmic region of RyR, near the face that would interact with the T-tubule system [213, 214]. The same studies have revealed that calmodulin can also bind the cytoplasmic assembly of RyR, in a region on the channel that faces the sarcoplasmic reticulum (SR) [213, 214]. Finally, Samso' et al. have investigated the three dimensional location of Imperatoxin A ($IpTx_a$), on RyR1 [178]. $IpTx_a$ is a peptide toxin that mimics a DHPR domain that triggers RyR1 opening and that has been found to bind RyR1 and affect its function in vitro [44, 45, 66, 72, 96, 136, 182, 187, 210]. Interestingly, the three dimensional binding site of $IpTx_a$ has been identified on the cytoplasmic assembly of RyR1, between the clamp and the handle domains, suggesting that this region may be involved in the excitation-contraction coupling transduction mechanism *in vivo* [178] (see below for a more detailed discussion on the excitation-contraction coupling mechanism). Leucine/isoleucine zipper motifs are present in RyR1 and RyR2 and bind to corresponding domains in adaptor proteins for kinases and phosphatases [118]. RyR2 forms a macromolecular complex that includes FKBP12.6, PKA and its targeting protein mAKAP, PP1 and its targeting protein spinophilin and PP2 with its targeting protein PR130 [112, 117, 120, 174]. Phosphorylation of RyRs by PKA was demonstrated to occur at Ser^{2843} and Ser^{2809} in the skeletal and cardiac isoforms of ryanodine receptors, respectively [118, 166, 173]. The specific regions in RyR2 that contain LIZ motifs have been identified: amino acids from 555-604 in RyR2 contain the LIZ motif that mediate targeting of spinophilin/PP1 to RyR2, while region 1603-1631 mediates binding of PR130/PP2A to RyR2; region 3003-3039 contains the LIZ motif that mediates targeting of mAKAP/PKA to RyR2. The LIZ motifs that mediate the targeting of PKA and PP1 to RyR2 are conserved among RyR isoforms, but only RyR2 contains the PP2A targeting motif [118].

Ryes Expression and Function

Ryanodine receptors have been first identified because of the pronounced actions of the plant alkaloid ryanodine on insects and vertebrate striated muscles. Ryanodine receptors have been subsequently detected in different species, from platyhelminthes to mammals, including nematodes, molluscs, arthropods, fish, reptiles, amphibians and birds [191].

In vertebrate, three isoforms of RyRs have been identified. In mammals, RyR1 is the major intracellular Ca^{2+} release channel in skeletal muscle and RyR2 is most abundant in cardiac muscle and brain [110, 137]. RyR3 is widely expressed in different vertebrate tissues [61, 62, 74]. By contrast, in most avian, amphibian and fish skeletal muscles, two isoforms of RyRs, named α and β, that correspond to mammalian RyR1 and RyR3 are expressed [3, 92, 146, 150, 151]. A third isoform, which is better recognised by antibodies against the mammalian RyR2 than against avian α and β isoforms and is likely to represent the homologous of mammalian RyR2, has been detected in chicken heart [151].

The function of RyRs has been extensively studied in muscle cells as their expression has been associated with this tissue since their first identification as the "foot structure"/Ca^{2+} channels of the sarcoplasmic reticulum. The preferential expression of RyRs in muscle tissues can be traced back to *C. elegans*, whose genome contains only one RyR gene [188]. Interestingly, the excitation-contraction coupling (E-C coupling) mechanism that regulates activation of muscle contraction through the coordinate activation of voltage-dependent Ca^{2+} channels and RyRs has been found to be a common feature of invertebrate and vertebrate striated muscles. Indeed, in *C. elegans*, the RyR-1 (unc-68) gene is expressed in adult body-wall muscles, pharyngeal muscle cells, neurons and other cells [175]. Unc-68 null mutants move poorly exhibiting an incomplete flaccid paralysis, yet have normal muscle ultrastructure. Pharyn-

geal pumping is weaker in mutants than in wild types, although electrical activity during pharyngeal muscle contraction is normal. Since contraction in unc-68 mutants is impaired but not eliminated, it seems that intracellular Ca^{2+} release is not essential for E-C coupling in *C. elegans* [122].

In mammals, RyR1 is the major intracellular Ca^{2+} release channel in skeletal muscle and RyR2 is most abundant in cardiac muscle, where they are closely associated with E-C coupling mechanisms that are specific for each muscle type. RyR3 has been found to be also expressed in mammalian skeletal muscle, although at levels that vary accordingly to the muscle type and the developmental stage [16, 36, 52]. In particular, expression studies on adult skeletal muscles from different mammalian vertebrates show detectable levels of RyR3 protein only in the diaphragm muscle [36], while in other muscles low to undetectable expression levels have been described. By contrast, during mouse muscle development, the RyR3 isoform is expressed in all muscles, from late embryonic stage and during the first two weeks after birth. RyR3 expression is down-regulated in most muscles starting from 2-3 weeks of post-natal life [16, 52]. The subcellular distribution of RyRs has been extensively studied in striated muscles. RyR1 and RyR2 display a precise localisation in skeletal and cardiac muscles to structures called triads and diads, respectively. These represent junctional complexes between the sarcoplasmic reticulum and the T tubule system that guarantee the direct interaction between dihydropyridine receptors and ryanodine receptors essential for activation of the E-C coupling mechanism [54, 161]. Recently, Felder and Franzini-Armstrong have shown that in skeletal muscle cells, RyR3 is likely localised in the parajunctional membranes immediately adjacent to the junctional region of skeletal muscles from toadfish and frog [48]. Although they do not unequivocally identify RyR3 as the main parajunctional channels in these muscles, the differential disposition of feet in the junctional and parajunctional domains of the sarcoplasmic reticulum and the typical disposition of tetrads in muscles expressing equal amount of RyR1 and RyR3 isoforms, suggest that RyR3 could be actually restricted to this area of the sarcoplasmic reticulum [48].

E-C coupling occurs with similar, but different mechanism in skeletal and cardiac muscles [95, 138, 156, 196, 197]. In skeletal muscle, a direct coupling model has been described. According to this model, RyR1s are physically coupled with DHPRs and open in relation to conformational changes of the DHPRs induced by membrane depolarization. In cardiac fibers, by contrast, RyR2s are not in physical association with DHPRs and are activated by a Calcium Induced Calcium Release (CICR) mechanism [15, 161]. Experiments with knockout animals have shown that RyR1 but not RyR2 can restore mechanical coupling in RyR1 deficient myotubes [139] and the skeletal muscle α_{1S} subunit, but not the cardiac α_{1c} subunit of DHPR can restore skeletal muscle E-C coupling in DHPR deficient mice confirming the close relationship between expression of a particular subset of genes (skeletal vs cardiac isoforms of RyR and DHPR) and generation of a specific function in muscle tissue [65]. Different studies have indicated the II-III loop of the α_{1S} subunit of DHPR as the region responsible for RyR1 channel opening [104, 200]. Inside this region, a peptide corresponding to residues 681-690 represents the minimal structural element that can activate skeletal-muscle specific excitation–contraction coupling [46, 47]. A second region inside the II-III loop has been identified between residues 725-742 [65, 139, 140]. Indeed, the two regions seem to have opposite effects on RyR1 activation: region 671-690 (also known as peptide A) was found to activate the channel, whereas region 724-760 (peptide C) was shown to antagonize the effect of the peptide A [45, 176]. However, physiological studies on dysgenic myotubes expressing chimeric DHPRs showed that the presence of regions corresponding to peptide C were important in determining the skeletal muscle-type of E-C coupling [65, 138]. In particular, expression of chimeric proteins where region 724-760 of the skeletal DHPR was replaced by the corresponding region from cardiac DHPR could not restore skeletal muscle type E-C coupling in dysgenic myotubes. In a different study, chimeric DHPRs composed by a rabbit skeletal α_{1S} subunit in which the sequences corresponding to the II-III loop were replaced by corresponding regions of the DHPR of Musca domestica, that show only a 19% identity with skeletal and cardiac mammalian DHPR, were expressed in myotubes from dysgenic mice [225]. The chimeric DHPRs were not able to restore skeletal muscle E-C coupling. However, when the region corresponding to residues 720-764 of rabbit DHPR was reintroduced into the musca II-III loop, a complete rescue of skeletal muscle E-C coupling could be observed, suggesting that this domain may be essential for the correct regulation of this mechanism [225]. Inside this region, the four amino acid residues Ala^{739}, Phe^{741}, Pro^{742} and Asp^{744} were found to be essential for skeletal type E-C coupling [91]. Interestingly, changes of any of the four residues to their cardiac counterpart led to an alteration of the predicted secondary structure of the adjacent domains that may be responsible for failure of functional interaction with RyR1 and activation of skeletal muscle E-C coupling [91]. Actually, using a surface plasmon resonance detection system, O'Reilly and co-workers demonstrated that only region 671-690 of the II-III loop of the DHPR can bind RyR1 [144]. Interestingly, the interaction between this region and RyR1 is strongly dependent on binding of the immunophilin FKBP12 to RyR1 (see below for discussion on FKBP12) [144]. Indeed, a previous study by Dulhunty et al., 1999, showed that activation of both native and lipid bilayer reconstituted RyR1 channels by peptide A

required FKBP12 binding to RyR1 [43]. In contrast, no binding to RyR1 could be detected for region 724-760, although it was previously proven to be essential for determining the type of E-C coupling [65, 91, 139].

Nevertheless, a clear determination of the regions of DHPR that are precisely involved in E-C coupling is still to be attained. Actually, deletion analysis of the II-III loop of the skeletal muscle DHPR indicated that loss of region 671-690 does not effect skeletal muscle E-C coupling and, furthermore, that deletion of both 671-690 and 720-765 domains do not completely abolish skeletal muscle type E-C coupling, suggesting that other regions may contribute to activation of RyR1 during E-C coupling [2]. In addition, a scrambled sequence in residues 681-690 did not alter skeletal muscle E-C coupling when expressed in skeletal muscle cells, indicating that integrity of this region is nor required for this mechanism [158]. Actually, recent studies showed that inside region 667-692, the secondary protein structure determined by alignment of a cluster of basic residues, more than the primary amino acid sequence, is important for RyR1 activation [9, 25, 243].

As to the sequences present in RyR1 responsible for E-C coupling, region from amino acid 1635 to amino acid 2636 has been found to play an important role in this mechanism [140]. Using *in vitro* interaction experiments between GST-fusion proteins of DHPR fragments corresponding to the II-III loop and *in vitro* translated RyR1 fragments, Leong and MacLennan showed that the 37 amino acid region spanning from Arg^{1076} to Asp^{1112} in RyR1 was able to bind the II-III loop from skeletal muscle but not from cardiac DHPR [98]. In addition, the presence of the D2 region in RyR1 (namely aa 1303-1356) is important for E-C coupling. Actually, deletions of this region from RyR1 channels abolish E-C coupling and transfection of RyR1 knockout myocytes with expression vectors carrying the RyR2 cDNA do not restore E-C coupling. However, when chimeric channels in which the D2 region of RyR1 was replaced by the corresponding sequences of RyR2 were expressed in knockout myocytes, skeletal muscle E-C coupling could be recovered, indicating that the D2 region alone does not determine the functional differences between RyR1 and RyR2 [233].

Recently, using a yeast two-hybrid approach, a second region in RyR1 corresponding to residues 1837-2168 has been proposed to bind to the portion 720-765 of the II-III loop of the α_{1s} subunit of DHPR, suggesting that more domains in RyR1 might be involved in RyR1/DHPR interaction [159]. Expression of different RyR1/RyR2 chimeras in dyspedic myotubes showed that replacement of region 1626-3686 of RyR2 with the corresponding region 1653-3720 of RyR1 (named chimera R4) restored almost completely the skeletal muscle type E-C coupling in transfected cells. Interestingly, at least two non-overlapping regions inside chimera R4, corresponding to residues 1635-2559 and 2659-3720 can partially restore skeletal muscle E-C coupling [161]. Similarly, expression of chimeric channels where regions 2508-3088 and 1798-2617 of RyR3 were replaced with the corresponding regions of RyR1 (namely residues 1924-2446 and 2644-3223 in RyR1) restored skeletal muscle E-C coupling in dyspedic myotubes, suggesting that full functional coupling may result from interaction of DHPR with multiple regions in RyR1 [153].

The physiological role of the different RyR isoforms in the regulation of intracellular Ca^{2+} signalling has been addressed by generation of knockout mice. Mice carrying a targeted disruption of the RyR1 gene show complete loss of the skeletal muscle E-C coupling and die perinataly due to respiratory failure [160, 196]. Skeletal myotubes from RyR1 knockout mice fail to respond to electrical stimulation, although they retain the ability to release Ca^{2+} in response to caffeine [196]. As the RyR3 isoform is expressed in skeletal muscles, it has been proposed that this residual Ca^{2+} release could be mediated by this isoform [36, 197]. RyR3 is expressed in all skeletal muscles in the late stages of fetal development and between 2-3 weeks after birth. Later on RyR3 levels progressively decrease and this isoform is no longer detected in adult mouse muscles with the exception of the diaphragm muscle. In agreement with this patter of expression, RyR3 knockout mice showed impairment of muscle contraction during the first weeks after birth. Tension developed following electrical stimulation was significantly lower in RyR3 knockout than in control mice, and an even stronger difference was observed when neonatal muscles were exposed to high caffeine concentrations [16]. By contrast, no significant difference between normal and RyR3 knockout mice was observed when analysing skeletal muscles from adult mice. The reduced contractility observed following electrical and caffeine stimulation in RyR3 knockout mice during the first weeks after birth is in agreement with a preferential expression of RyR3 during this developmental stage and suggests a qualitative contribution of RyR3-mediated Ca^{2+} release to regulation of contraction in neonatal skeletal muscles [16]. Interestingly, Yang et al., 2001 reported that the time required for diffusion of a Ca^{2+} signal following depolarisation from the membrane to the central region of a muscle fiber is higher in RyR3 knockout mice compared to control, suggesting that co-expression of RyR3 with RyR1 contributes to build a system of amplification which results in a more uniform and synchronous activation of Ca^{2+} release across the neonatal skeletal muscle fiber [236].

RyR1/RyR3 double mutant mice do not actively move and die after birth as was the case of RyR1 deficient mice [11, 78]. Double knockout mice confirm the functional data obtained from single knockout mice, showing a complete loss of E-C coupling and contraction in response to caffeine and ryanodine stimulation indicat-

ing the absence of all ryanodine/caffeine sensitive pathways of Ca^{2+} release. Morphological analysis of double knockout muscles shows a severe muscular damage with loss of myofibrillar protein content [11].

Similarly to what observed in RyR1 knockout mice, generation of mice carrying a targeted disruption of the RyR2 gene indicated a pivotal role of this isoform in cardiac E-C coupling and during myocardial development. RyR2 knockout mice die at embryonic day (E) 10 and show morphological abnormalities in the heart tube. Mutant cardiac myocytes loose functional channel activity and no residual caffeine response can be detected. In addition, cardiac myocytes present ultrastructural defects as large vacuoles in the sarcoplasmic reticulum and abnormal mitochondria. It has been proposed that these abnormalities may be due to excessive overload of intracellular Ca^{2+} stores and mitochondria indicating that during myocardial development, RyR2 is required for intracellular Ca^{2+} homeostasis in myocytes [199].

Studies on RyR3 knockout mice have been also extended to other tissues than skeletal muscle, such as the nervous system and smooth muscles, where this isoform has been found to be expressed. Behavior tests showed that RyR3 knockout mice display a higher speed of locomotion, defects of spatial working memory and learning, suggesting an involvement of RyR3 in mechanisms of behavior associated with hippocampal activity [8, 90, 198]. Further studies by Balshun et al., have investigated the eventual role of RyR3 in LTP, which is thought to mediate processes of learning and memory formation at the cellular level. Actually, while no differences between RyR3 knockout mice and control were present in LTP generated by a strong tetanization protocol, LTP induced by a weak tetanization protocol and depotentiation were markedly changed by RyR3 deletion, suggesting a role of RyR3 in certain types of hippocampal synaptic plasticity [8].

A final field of investigation regarding RyR function is represented by studies on smooth muscle. In particular, the contribution of RyR isoforms to generation of localized Ca^{2+} release events in smooth muscle has been investigated. In smooth muscle, depending on membrane potential, Ca^{2+} sparks can trigger activation of Ca^{2+}-activated K^+ channels (BK), causing generation of "spontaneous transient outward currents" (STOCs) or activation of Ca^{2+}-activated Cl^- channels (ClCa), causing generation of "spontaneous transient inward currents" (STICS) [245]. Smooth muscle cells express different combinations of the three RyR isoforms. In order to identify which isoform could be responsible for generation of Ca^{2+} sparks in rat portal vein myocytes, Mironneau and colleagues used an antisense oligonucleotide strategy. They found that inhibition of either RyR1 or RyR2 strongly reduced generation of spontaneous Ca^{2+} sparks in myocytes following membrane depolarization, suggesting that these elementary Ca^{2+} release events may results from activation of mixed Ca^{2+} release units that require the presence of both channel types [37]. By contrast, inhibition of RyR3 by means of isoform-specific antisense oligonucleotides did affect neither Ca^{2+} sparks generation nor caffeine-induced Ca^{2+} release, indicating that both RyR1 and RyR2, but not RyR3, were required for Ca^{2+} release during Ca^{2+} sparks [37]. In contrast, in skeletal muscle, both RyR1 and RyR3 were found to contribute equally to generation of Ca^{2+} sparks [34, 35, 185]. In further studies performed by the same authors on rat portal vein myocytes and non-pregnant mouse myometrial cells, RyR3 activation was observed only by conditions of increased SR Ca^{2+} load, suggesting the existence of isoform specific mechanisms for the regulation of RyRs [130, 131]. In partial agreement with the previously described data, Löhn and co-workers reported that in arterial smooth muscle cells, RyR3 is apparently not involved in Ca^{2+} sparks generation. In particular, they measured the spatial-temporal characteristics of Ca^{2+} sparks in cells prepared from RyR3 knockout mice compared to control mice. No difference in amplitude, width and duration of Ca^{2+} sparks could be observed in RyR3 knockout cells compared to control. However, analysis of BK current activation in RyR3 knockout mice revealed a significant increase in the STOCs frequency compared to control, suggesting that, at least in this cellular model, RyR1 and RyR2 may contribute to Ca^{2+} release underlying a single spark, while RyR3 channels may control the basal Ca^{2+} spark frequency, although through a not yet defined mechanism [102].

Interestingly, a recent study by Jiang et al., reported that most of smooth muscle tissues from rabbit express a splice variant of RyR3 that contains a deletion of 87 base pair encompassing a predicted transmembrane segment [80]. Expression of the corresponding cDNA in HEK293 cells revealed that RyR3 subunits coded by this splice variant transcript could not form functional channels. However, they were found to combine with wild type RyR3 channel subunits to form hetero-tetrameric proteins that display a reduced caffeine sensitivity compared to homo-tetrameric RyR3, suggesting that expression of splice variant RyR3 transcripts in smooth muscle tissues may contribute a novel mechanisms to regulate intracellular Ca^{2+} signaling in these cells [80].

RyR Binding Proteins

The functional activity of RyRs is regulated by association with multiple proteins that may interact with both the N-terminal/cytoplasmic regions of the receptors and with domains facing the lumen of the endoplasmic reticulum. RyRs have been described to form a multiprotein complex that includes calsequestrin, a high capacity Ca^{2+} binding protein located on the junctional SR, triadin and junctin that anchor calsequestrin to the inner face of the junctional SR membrane [109, 239]. The large cytoplasmic domain of RyRs has been found to bind several accessory proteins that include the

FK506 binding protein FKBP12 [22, 23, 124], calmodulin [128, 133, 134, 218, 231, 232, 241], protein kinases [38, 165, 166, 173, 174, 190, 192, 227], phosphatases [112, 117, 120, 174], S100 [208], sorcin [129] and homer proteins [77].

FKBP12

FKBP12 is a cis-trans prolyl isomerase, originally identified as the receptor for the immunosuppressant drugs FK506 and rapamycin. The FKBP family includes at least five members, with molecular masses from 12 to 52 kD. FKBP12 has been found to be tightly associated with the RyR1 Ca^{2+} channel [24, 81, 163]. Evidence for the association of FKBP12 and RyR1 came first from cloning and protein sequencing studies. Indeed, an additional peptide isolated from peptide mapping of purified skeletal muscle RyRs was identified as the N-terminal region of FKBP12 [33]. In addition, FKBP12 copurified with RyR1 during column chromatography and sucrose density centrifugation and anti-FKBP12 antibodies can immunoprecipitate RyR1 from purified preparations [237]. FKBP12 is localised in the terminal cisternae of the sarcoplasmic reticulum and not in the longitudinal tubules [237]. The stoichiometry of FKBP-RyR has been found to be one molecule of FKBP for a RyR protomer [203, 213, 214].

Numerous studies indicate that FKBP12 can regulate the ryanodine receptor activity. Incubation of muscle vesicles with FK506 or rapamycin removes FKBP12 from RyRs. The FKBP12 devoid channel is activated by lower concentration of Ca^{2+} or caffeine, displays longer mean open times and greater open probability and requires greater Mg^{2+} concentration for inactivation [Timerman 1995, 58, 125, 203, 219, 226]. In addition, recombinant RyR1 proteins expressed in non-muscle experimental models, such as Sf9 cells or Xenopus oocytes, as well as skeletal muscle channels treated with FK506, exhibit subconductance opening states to four distinct levels [1, 21, 29]. These effects can be reversed upon addition or co-expression of recombinant FKBP12 in Sf9 cells, suggesting that FKBP12 can enhance the cooperativity among the four subunits of RyRs, resulting in full conductance channels, with decreased open probability and stabilising the closed conformation of RyRs [21]. Other functions that can be mediated by FKBP12 are the "couple gating", that is the simultaneous gating of multiple channels [119, 147, 148], the "rectification" of RyR1, in which FKBP12 induces unidirectional block of Ca^{2+} currents from the cytosol to the SR lumen [30, 106] and the "adaptation", inducing the response of RyRs to repeated caffeine applications [73]. In addition, a more specific role of FKBP12 in the E-C coupling mechanism has been recently proposed by different authors [6, 43, 144]. In particular, it has been demonstrated that activation of RyR1 channels by peptides derived from the II-III loop of the skeletal DHPR can be substantially reduced after FKBP12 depletion [43, 144]. In addition, disruption of FKBP12 binding to RyR1 was shown to severely compromise voltage-gated SR Ca^{2+} release, suggesting that FKBP12 could gain E-C coupling in the skeletal muscle [6].

In addition to FKBP12, another isoform of FKBP, the FKBP12.6 protein was found to be able to bind RyRs. Interestingly, the FKBP12.6 protein can bind selectively the RyR2 isoform of RyRs [22, 94, 124, 204, 205, 228], while RyR1 and RyR3 can bind both FKBP12 and FKBP12.6 [10, 23, 205]. As previously discussed, binding of FKBP12 to RyR1 can regulate channel activity. In contrast, the role of FKBP12.6 in the regulation of RyR2 is still controversial. Single channel recordings of RyR2 channels indicated that removal of FKBP12.6 from RyR2 increases the open probability of the channel and induces the appearance of long-lasting subconductance states [85, 226]. Conversely, Timerman et al. showed that depletion of FKBP12.6 from RyR2 channels did not significantly change the open probability of RyR2 nor the re-addition of FKBP12.6 to depleted channels alters the gating properties of RyR2 [205].

Recently, a direct correlation between dissociation of FKBP12.6 from RyR2 and development of heart failure has been proposed by Marks and co-workers [112, 117]. They reported that PKA phosphorylation of RyR2 can induce dissociation of FKBP12.6 from the channel, resulting in increased channel activity [112, 117]. Interestingly, hyperphosphorylation of RyR2 and depletion of FKBP12.6 has been reported in failing hearts [117, 149, 165, 167, 235]. Accordingly to the model proposed by Marx this condition may induce a defective channel function that may lead to delayed after depolarization and ventricular arrhythmias [111-114, 221, 222]. Interestingly, in heart failure, not only RyR2, but also RyR1 in skeletal muscles was found to be hyperphosphorylated by PKA [168, 216]. Hyperphosphorylation of RyR1 results in FKBP12 depletion from the channel that leads to an increased channel activity, impaired Ca^{2+} release from the sarcoplasmic reticulum due to appearance of leaky channels and early fatigue in skeletal muscle during heart failure [168]. Nevertheless, although intriguing, the role of phosphorylated RyR channels in muscle physiology is still controversial. Actually, other studies failed to detect dissociation of FKBP12.6 from phosphorylated RyR2 channels, suggesting that other mechanisms may be involved in altered channel function in failing hearts [227].

Additional insights on the role of FKBPs on RyRs activity *in vivo* come from studies on knockout mice for FKBP12 and FKBP12.6 genes [Shou et al., 1998, 229]. The majority of FKBP12 deficient mice die between E14.5 and birth because of severe dilated cardiomyophathy and ventricular septal defects. At E18.5 FKBP12 knockout mice show dramatically enlarged hearts with ventricular septal defects, increased cavity diameters, thinner left ventricular walls, hypertrophyc trabeculae, and deep intertrabeculae recesses. Mutant hearts also show diminished fractional shortening and ejection frac-

tion, compared to control, indicating a depression of contractile activity in the ventricular wall. Despite the role of FKBP12 in regulation of RyR1 activity, no evident skeletal muscle abnormalities could be observed in FKBP12 mutant mice. In addition, single channel recordings show increased open probability and subconductance states both for RyR1 and RyR2 channels. These data indicate that FKBP12 can alter the properties of both RyR1 and RyR2 and that FKBP12.6 cannot functionally replace FKBP12 in the heart [Shou et al., 1998].

In contrast, FKBP12.6 knockout mice grow normally and are fertile. Studies on cardiac myocytes derived from FKBP12.6 knockout showed a marked increase in calcium induced calcium release (CICR) gain, that is a much larger release from the sarcoplasmic reticulum following membrane depolarization. The increase in CICR gain was associated with a greater contraction of mutant ventricular myocytes compared to control. In addition, Ca^{2+} sparks in knockout cardiomycytes were increased in amplitude and size and were longer in duration compared to wild type cells, suggesting that depletion of FKBP12.6 from RyR2 may result in longer channel opening [229]. Interestingly, the Ca^{2+} overload that results from altered RyR2 activity has been found to be associated with cardiac hypertrophy, but only in male hearts, suggesting activation of different adaptation mechanisms in male and female that have been proposed to involve oestrogen receptor signalling [229].

Calsequestrin

Calsequestrin is a high capacity Ca^{2+} binding protein localised to the junctional face membrane of the sarcoplasmic reticulum [53, 108]. Anchoring to the membrane seems to be mediated by interaction with two integral membrane proteins, junctin and triadin [70, 83, 89, 183, 239]. In particular, an asp-reach region corresponding to amino acids 354-367 of calsequestrin has been found to bind triadin in a Ca^{2+} dependent manner [183]. The localisation of calsequestrin to the junctional membranes of the sarcoplasmic reticulum favours the accumulation of large amounts of Ca^{2+} ions in proximity of their release sites, the RyRs [54, 135]. Actually, a quaternary complex between calsequestrin, triadin, junctin and RyR was proposed to be localised to the inner face of the junctional sarcoplasmic reticulum [64, 239]. The effect of calsequestrin on RyRs activity is still controversial. [^{3}H]ryanodine binding to solubilized SR membranes was found to be potentiated by the addition of calsequestrin [145]. Similarly, the open probability of RyRs incorporated into lipid bilayers was increased when calsequestrin was added to the luminal side of the channel [86]. The effect of calsequestrin on RyRs activity has been proposed to be dependent on its phosphorylation state. Namely, dephosphorylated calsequestrin induced channel opening of purified RyRs in the presence of 1 mM Ca^{2+}, while phosphorylated calsequestrin had apparently no effect [193]. C2C12 cells overexpressing calsequestrin showed an enhancement of both caffeine and voltage-induced Ca^{2+} release, associated with an increase in Ca^{2+} storage in the sarcoplasmic reticulum [184]. Similarly, cardiac myocytes overexpressing calsequestrin showed an increase of caffeine induced Ca^{2+} release, but an impairment of I_{ca}-induced Ca^{2+} release [84] and a reduced frequency of Ca^{2+} sparks [215]. Likewise, Beard et al., reported that binding of calsequestrin suppresses RyR1 single channel activity in lipid bilayers whereas dissociation of calsequestrin was found to enhance channel opening [12]. It has still to be defined, however, whether the inhibitory effect of calsequestrin on RyRs activity is mediated by binding to other proteins, like triadin [12] and, in the case of cardiac myocytes overexpressing calsequestrin, by the increase in Ca^{2+} load of the sarcoplasmic reticulum that may itself exhibit a negative effect on RyR regulation [84].

Triadin and junctin

Junctin and triadin are integral membrane proteins localised to the terminal cisternae of the sarcoplasmic reticulum of both skeletal and cardiac muscles that share structural and amino acid sequence similarities [20, 26, 69, 71, 76, 83, 87, 88, 116, 224]. Both proteins contain a single membrane domain that is 62% identical, a short N-terminal cytoplasmic domain and a long C-terminal tail located in the SR lumen with a long run of alternating positively and negatively charged amino acids, rich in lysine and glutamic acid [71, 83, 87, 115]. Junctin can bind directly to calsequestrin, triadin and RyR and the site of interaction is localised in the C-terminal domain of junctin (residues 46-210 of canine cardiac junctin) [239]. Triadin is also able to bind junctin, calsequestrin and RyR via its luminal domain corrresponding to residues 69-264 of rabbit cardiac triadin [70, 99, 239]. These properties indicate a direct role of triadin and junctin as anchoring proteins to support the accumulation of calsequestrin to the junctional face membranes close to the RyRs.

High affinity binding sites for RyR1 have been located to the segment corresponding to amino acids 110-280 of the skeletal muscle triadin [27]. On the other hand, the specific binding sites for triadin are located in the second intraluminal loop of RyR1 at residues D4878, D4907 and E4908 and the interaction between these two proteins requires the presence of the KEKE motif (amino acids 200-232) in triadin [96, 97]. Interestingly, *in vitro* studies on purified RyR1 showed that the cytoplasmic region of triadin can modulate channel activity [67, 145]. Actually, [^{3}H]ryanodine binding to solubilized, but not native SR membrane was found to be significantly higher in triadin depleted membranes than in control [145]. In addition, application of triadin to the cytoplasmic side, but not to the luminal side of RyR1 channels reconstituted in lipid bilayers reduced the open probability of the receptors [145], Another study by Groh et al. pointed to the role of the cytoplasmic region

of triadin in RyR1 modulation [67]. Using a plasmon resonance spectroscopy, a specific cytoplasmic domain corresponding to residues 18-46 of triadin has been found to interact with RyR1 in a Ca^{2+} dependent manner. Antibodies directed against the region of triadin interacting with RyR1 were found to inhibit Ca^{2+} release from the SR and to decrease the open probability of RyR1 channels [67].

Calmodulin

Calmodulin (CaM) is a Ca^{2+} binding protein containing four EF-hand type Ca^{2+} binding motifs, in the N-terminal and in the C-terminal regions. Both the N-terminal and C-terminal lobes of CaM can bind RyR1 at either high and low Ca^{2+} concentrations and each lobe has two possible binding sites [171, 172, 230]. The effect of calmodulin on RyRs activity was shown to be relatively complex and to be dependent on different factors. In the presence of CaM, the threshold for activation of RyR1 in [^{3}H]ryanodine binding experiments was found to be shifted to lower Ca^{2+} concentrations, suggesting that CaM may increase the sensitivity of RyR1 to Ca^{2+} dependent activation. Actually, the effect of calmodulin on RyR1 activity was shown to be dependent on Ca^{2+} concentration. At nanomolar Ca^{2+} concentration, calmodulin activates RyR1 channels, while at micromolar Ca^{2+} concentrations, calmodulin was shown to inhibit channel activity [209]. In addition, the effect of calmodulin on RyR1 is also dependent on Ca^{2+} binding to calmodulin. In its Ca^{2+}-free state (ApoCaM), calmodulin enhances RyR1 activity, while in its Ca^{2+} bound state (CaCaM) it inhibits the channel (Rodney et al., 2000). In order to understand the molecular basis of RyR regulation by calmodulin, numerous attempts aimed to identify the specific binding sites on both molecules have been performed. Each RyR tetramer can bind four molecules of calmodulin both in the absence and in the presence of Ca^{2+} [7, 133]. In particular, ApoCaM and CaCaM bind to overlapping sites located between residues 3614-3643 of RyR1 and 3583-3603 of RyR2 [133, 231, 232]. Namely, CaCaM was found to bind synthetic peptides matching amino acids 3614-3643 and 3614-3634, whereas apoCaM binds only the first peptide [171]. Mutations of residues 3624 and 3620 result in loss of high-affinity binding of CaM to RyR1. Expression of L3624D mutated RyR1 channels in dyspedic myotubes completely restored voltage-gated SR Ca^{2+} release, indicating that binding of calmodulin to RyR1 is not essential for E-C coupling in skeletal muscle [143]. However, expression in dyspedic myotubes of RyR1 channels lacking region 3614-3643 resulted in a dramatic reduction in depolarization, caffeine and 4-chloro-m-cresol- induced Ca^{2+} release and in changes in conductance and channel gating, suggesting that this domain may be important in the modulation of channel activity [244]. In addition, recent studies by Zhang et al. showed that CaCaM and ApoCaM can also bind to region 1975-1999 of RyR1 [241]. Within region 3614-3643, cysteine 3635 was found form a disulphide bond with the corresponding cysteine on an adjacent subunit in the RyR1 tetramer. Interestingly, binding of either CaCaM or apoCaM to RyR1 can block the inter-subunit disulphide bond formation, suggesting that calmodulin could be involved in redox modulation of RyR1 [134, 241]. Using calmodulin mutants in either the C-terminal or N-terminal lobes, Rodney et al. proposed a model in which the binding of Ca^{2+} to the C-terminal lobe of calmodulin is responsible for its conversion from an activator to an inhibitor of RyR1. Indeed, calmodulin mutants in the C-terminal lobe increase channel activity, while mutants in the N-terminal lobe inhibits RyR1 channels [172]. More recently, a second model for calmodulin/RyR1 interaction was proposed by Xiang et al. Accordingly to this model, the N-terminal and C-terminal lobes have each two possible binding sites on RyR1 (C1-C2 and N1-N2). The binding site of the C-terminal lobe is located within region 3614-3643 of RyR1 and which of the two possible sites is occupied by the lobe depends on interaction of the C-lobe with Ca^{2+}. Namely, binding of Ca^{2+} induces an N-terminal shift in the site of interaction of the C-terminal lobe (C1 to C2). The N-terminal lobe can bind alternatively sites N1 and N2. Occupancy of one of the two N sites depends on the location of the C-terminal lobe. In particular, when Ca^{2+} is bound and the C2 site is occupied, the N2 site is favoured and the opposite occurs at low Ca^{2+}. Occupancy of the N1 binding site increases the activity of the channel, while occupancy of the N2 binding site would inhibit the channel [230]. In addition, previous studies indicated the presence of CaM binding site to residues 2937-3225, 3610-3629 and 4534-4552 of RyR1 [128].

As regards RyR isoforms other than RyR1, controversial results have been obtained. [^{3}H]ryanodine binding to RyR2 was found to be inhibited by CaCaM at Ca^{2+} concentrations lower than 10 μM, while no effect could be observed at 100 μM Ca^{2+} [7]. In addition, at difference with RyR1, apoCaM binding to RyR2 resulted in channel inhibition [7]. By contrast, results from Fruen et al., indicate that CaM has no significant effect on Ca^{2+} dependent activation of [^{3}H]ryanodine binding to RyR2 channels [55, 56]. As to RyR3, CaM has been shown to exhert both potentiating and inhibitory effects on CICR at low (pCa>6) and high (pCa<5.5) Ca^{2+} concentrations, respectively [79].

VES1/Homer

Homer proteins are a family of proteins that have been described to mediate clustering of metabotropic glutamate receptors [211]. The C-terminal tail of homer proteins contains a coiled–coil domain responsible for self-multimerization. The N-terminal region of homer proteins contains an Enabled/Vasp homology domain (EVH1) that is essential for binding to target proteins that contain a prolin rich motif (PPXXF) [211]. Known targets of homer proteins are the glutamate receptor

mGluR1a, mGluR5a/b [4, 169, 211], the inositol 1,4,5-trisphosphate receptors [177, 211] and the cytoplasmic Shank proteins [142, 212]. The prolin-rich motif responsible for binding to homer proteins is also present in the RyR1 sequence. Immunoprecipitation and GST pull-down experiments by Feng et al., showed that RyR1 could interact with homer1c, homer 2 and homer3, although with different affinities and depending on the SR preparations [49]. In contrast, pull-down experiments and immunofluorescence studies on mouse skeletal muscle fibers did not show any co-precipitation or co-localization between homer 1a and/or 1b isoforms and RyR1 [177].

The effect of homer proteins of RyRs activity has not been completely defined. Single channel experiments performed in the presence of either homer1c and homer1 lacking the multimerization domains showed that homer proteins can enhance RyR1 channel open probability [49]. Furthermore, addition of homer proteins to skeletal muscle junctional membranes resulted in an increase in [^{3}H]ryanodine binding, suggesting that these proteins may act as physiological modulators of RyR1. This activity is conferred by the EVH1 domains and is enhanced by homer multimerization [49]. More recently, a differential activity of homer1c (or V-1L) and of its short form lacking the coiled-coil domain (V-1S) on RyR1 activity has been reported [77]. In particular, both forms of homer can bind RyR1, but only homer1c (V-1L) can increase RyR1 activity in single channel recordings and in Ca^{2+} release experiments on rat skeletal muscle microsomes, whereas no effect could be observed for the short form. Nevertheless, increasing concentrations of V-1S decreased the effect of V-1L, likely by competing for the same binding site on RyR1 [77]. An opposite effect of V-1L has been reported by Westhoff et al., on RyR2. Actually, V-1L was shown to bind RyR2 but, at difference with RyR1, it reduced the channel activity both in experiments performed in intact cells and in single channel recordings. Similarly to what observed for RyR1, however, the short form of homer1c, V-1S was found not to exert any significant effect on RyR2 [223].

Recent studies on frog skeletal muscle fibers showed that both V-1L and V-1S were effective in increasing Ca^{2+} sparks frequency, without altering other spark parameters, such as the temporal properties [217]. These effects were also observed in [^{3}H]ryanodine binding experiments and were counteracted by addition of homer proteins containing a mutation in the RyR-interacting domain (EVH1). On this bases the authors proposed a model in which the EVH1 domain is responsible for RyR activation by increasing RyR1 sensitivity for CICR. Interestingly, the effect of the two homer forms, long and short, was found to be additive, suggesting that, although the coiled-coil domain is not essential for RyR activation, it may be important to induce multimeric association of RyRs that promotes Ca^{2+} spark activation [217].

RyR and Human Diseases

The human RYR1 gene is located on chromosome 19q13.1 and is composed of 106 exons spanning a genomic region of approximately 158 Kb [154].

Mutations in the RYR1 gene have been demonstrated to be linked to three muscle genetic disorders, malignant hyperthermia (MH), central core disease (CCD) and multi-minicore disease (MmD) [50, 107, 126, 132, 238]. MH is an autosomal-dominant inherited disorder that causes spasm tachycardia and hyperthermia in susceptible patients when exposed to volatile anaesthetics and muscle relaxants. Clinical diagnosis of MH is carried out by in vitro contracture tests performed on muscle tissues obtained by biopsies exposed to halotane and caffeine. CCD is a rare non-progressive autosomal dominant congenital myophathy, characterized by hypotrophy and hypotonia in the infancy. The clinical diagnosis is performed by histological analysis of sample muscles that usually reveals the presence of abundant central “cores” characterised by mitochondria depletion and sarcomere disorganization in type I fibers. CCD is closely associated with MH susceptibility, while only a fraction of MH patients is affected by CCD. MmD are also congenital myophathies, but, unlike CCD, they are characterized by appearance of small regions with sarcomeric disruption and lack of mitochondria in both fiber types I and II [68, 132].

The causative molecular defect for both MH and CCD has been proposed to lie in an altered release of Ca^{2+} through RyR1 channels that may lead either to a rapid and high increase in the myoplasmic Ca^{2+} concentration in response to volatile anaesthetics in MH patients or to a chronic Ca^{2+} overload in muscles of CCD patients. So far, clusters of mutations linked to MH/CCD have been described in the myoplasmic domain of RyR1 and in the C-terminal region. In particular, three hot spots of mutations have been identified in the N-terminal region (amino acids 35-614), in the central region (amino acids 2117-2787) and in the C-terminal region (amino acids 4136-4973). The substituted residues at the mutated sites are highly conserved among RyR isoforms and across species [127].

In the last decade, a detailed investigation of the causal role of single RyR1 mutations in the development of CCD/MH has been performed by *in vitro* characterization of RyR1 channels expressed either in HEK293 cells or in muscle cells. In general, mutated RyR1 exhibit a higher sensitivity to channel activators like caffeine or 4-chloro-cresol [63, 207, 220, 236] or prolonged ion channel open time which causes a transient increase in cytosolic Ca^{2+} levels, that can cause glycogenolyisis, ATP depletion and muscle damage [103, 127, 186]. Interestingly, cells expressing CCD mutations show a lower intraluminal Ca^{2+} content as revealed by thapsigargin treatment, suggesting that the

mutated channels may behave as leaky channels that cause Ca^{2+} depletion from intracellular stores [202]. Accordingly, measurements of resting Ca^{2+} in HEK293 cells transfected with either MH or MH/CCD mutations showed that the latter display higher cytosolic Ca^{2+} concentrations and low luminal Ca^{2+} content compared to MH mutations, supporting the hypothesis that channels carrying mutations linked to CCD may cause a chronic increase in intracellular Ca^{2+} in muscle cells from affected patients [207]. Although studies on HEK293 cells have revealed to be a convenient model to study RyR1 functional activity, they have the disadvantage not to represent the physiological environment in which RyRs are expressed. Actually, more recent studies performed in muscle cells allowed to identify new effects of RyR1 channels carrying the mutation I4897T (identified in a patient affected by CCD) on voltage gated Ca^{2+} release and interaction with DHPRs. In HEK293 cells, the I4897T mutated channels were found to behave as leaky channels, leading to an increase of the resting Ca^{2+} concentration and a decrease in the Ca^{2+} store content [105]. However, expression of the same mutation in dyspedic myotubes did not induce any alteration in the resting Ca^{2+} concentration nor in the Ca^{2+} store content. Actually, electrophysiological studies on dyspedic myotubes expressing the I4897T mutated proteins revealed that these channels lacked voltage gated Ca^{2+} release indicating that they were uncoupled from sarcolemmal excitation and DHPR activation [5, 39, 105]. On this basis, although many advances have been performed in the last years in understanding the causative role of RyR1 mutations in the development of MH/CCD, a more comprehensive characterization of these diseases requires defining not only the functional properties of RyR1 channels but also understanding their interactions with accessory proteins, such as DHPR or FKBP12. Likewise, mutations in the RyR1 gene have been also found in patients that present with heterogeneous classes of myophathies characterized by the presence of multiple cores (MmD). Interestingly, some severe recessive forms of MmD with mutations in the RyR1 gene, associated with ophtalmoplegia have been also reported [68, 132], suggesting that existence of large genetic heterogeneity for distinct forms of myophaties.

More recently, missense mutations in the RyR2 gene have been identified in patients exhibiting catecholaminergic polymorphic ventricular tachycardia and arrhythmogenic right ventricual cardiomyophaty [60, 93, 157, 206]. The electrocardiograph pattern of this ventricular tachycardia closely resembles the arrhythmias associated with Ca^{2+} overload and the delayed afterdepolarizations observed during digitalis toxicity. Interestingly, these mutations lie in regions of RyR2 that correspond to those representing hot spots of mutation in the RyR1 gene, further suggesting that they may contain crucial domains for channel function. In particular the R176Q mutation found in some patients affected by arrhythmogenic right ventricular cardiomyophathy type 2 corresponds exactly to the Arg163Cys mutation found in the RyR1 gene in patients affected by malignant hyperthermia. As discussed in the "FKBP12" section, binding of FKBP12.6 to RyR2 appears to contribute to channel stabilization. In agreement with these observations, hyperphosphorylation of RyR2 by PKA and depletion of FKBP12.6 has been reported in failing hearts [117, 149, 165, 168, 235]. Interestingly, some mutations in the RyR2 channels found in patients affected by exercise-induced arrhythmias were found to reduce the channel affinity for FKBP12.6 and to induce an increase of channel activity, suggesting that leaky RyR2 channels may be responsible for triggering fatal cardiac arrhythmias [222]. Nevertheless, a precise causative role of RyR2 mutations and FKBP12.6 depletion from mutated channels in the development of cardiac defects is still to be completely elucidated. *In vitro* expression of RyR2 channels carrying the R4497C mutation in HEK293 cells showed that the mutated channels exhibit an increase in basal channel activity as revealed by single channel recordings and [^{3}H]ryanodine binding assays [80]. Conversely, RyR2 channels carrying mutations S2246L, N4104K and R4497C expressed in HL-1 cardiomyocityes did not show any increase in basal activity, suggesting that appropriate regulation of RyR2 may require interaction with accessory proteins that are not expressed in HEK293 cells [60]. However, β-adrenergic stimulation of RyR2 mutated channels expressed in HL-1 cells led to abnormal Ca^{2+} release and FKBP12.6 dissociation due to PKA hyperphosphorylation of RyR2. Nevertheless, the extent of FKBP12.6 dissociation from RyR2 was found to be equivalent for wild type and mutated channels, indicating that appearance of the disease phenotype may not be entirely due to differential phosphorylation or selective dissociation of FKBP12.6 from mutated channels.

In conclusion, increasing evidence indicate that RyRs are involved in different diseases of the striated muscle tissue. However, a more detailed understanding of the molecular interactions between RyR channels and accessory proteins and how these interactions can regulate RyRs activity is still required in order to better understand the mechanisms leading to altered Ca^{2+} homeostasis in muscle cells.

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References

[1] Ahern GP, Junankar PR, Dulhunty AF: Subconductance states in single-channel activity of skeletal muscle ryanodine receptors after removal of FKBP12. *Biophys J* 1997; 72: 146-162.

[2] Ahern CA, Bhattacharya D, Mortanson L, Coronado R: A component of excitation-contraction coupling triggered in the absence of the T671-L690 and L720-Q765 regions of the II-III loop of the dihydropyridine receptor α_{1s} pore subunit. *Biophys J* 2001; 81: 3294-3307.

[3] Airey JA, Beck CF, Murakami K, Tanksley S J, Deerinck TJ, Ellisman MH, Sutko JL: Identification and localization of two triad junctional foot protein isoforms in mature avian fast twitch skeletal muscle. *J Biol Chem* 1990; 265: 14187-14194.

[4] Ango F, Robbe D, Tu JC, Xiao B, Worley PF, Pin J, Bockaert J, Fagni L: Homer-dependent cell surface expression of metabotropic glutamate receptor type 5 in neurons. *Mol Cell Neurosc* 2002; 20: 323-329.

[5] Avila G, O'Brien JJ, Dirksen RT: Excitation-contraction uncoupling by a human central core disease mutation in the ryanodine receptor. *Proc Natl Acad Sci USA* 2001; 98: 4215-4220.

[6] Avila G, Lee EH, Perez CF, Allen PD, Dirksen RT: FKBP12 binding to RyR1 modulates excitation-contraction coupling in mouse skeletal myotubes. *J Biol Chem* 2002; 278: 22600-22608.

[7] Balshaw DM, Xu L, Yamaguchi N, Pasek DA, Meissner G: Calmodulin binding and inhibition of cardiac muscle calcium release channel (ryanodine receptor). *J Biol Chem* 2001; 276; 20144-20153.

[8] Balschun D, Wolfer DP, Bertocchini F, Barone V, Conti A, Zuschratter W, Missiaen L, Lipp HP, Frey U, Sorrentino V: Deletion of the ryanodine receptor type 3 (RyR3) impairs forms of synaptic plasticity and spatial learning. *EMBO J* 1999; 18: 5264-5273.

[9] Bannister ML, Williams AJ, Sitsapesan R: Removal of clustered positive charge from dihydropyridine receptor II-III loop peptide augments activation of ryanodine receptors. *Biochem Biophys Res Commu* 2004; 314: 667-674.

[10] Barg S, Copello JA, Fleischer S: Different interactions of cardiac and skeletal muscle ryanodine receptor with FK-506 binding protein isoforms. *Am J Physiol* 1997; 272: C1726-C1733.

[11] Barone V, Bertocchini F, Bottinelli R, Protasi F, Allen PD, Franzini Armstrong C, Reggiani C, Sorrentino V: Contractile impairment and structural alterations of skeletal muscles from knockout mice lacking type 1 and type 3 ryanodine receptors. *FEBS Lett* 1998; 422: 160-164.

[12] Beard NA, Sakowska MM, Dulhunty AF, Laver DR: Calsequestrin is an inhibitor of skeletal muscle ryanodine receptor calcium release channel. *Biophys J* 2002; 82: 310-320.

[13] Berridge MJ, Bootman MD, Lipp P: Calcium – a life and death signal. *Nature* 1998; 395: 645-648.

[14] Berridge MJ, Lipp P, Bootman MD: The versatility and universality of calcium signalling. *Nat Rev* 2000; 1: 11-21.

[15] Bers DM: Cardiac excitation-contraction coupling. *Nature* 2002; 415: 198-205.

[16] Bertocchini F, Ovitt CE, Conti A, Barone V, Schöler HR, Bottinelli R, Reggiani C, Sorrentino V: Requirement for the ryanodine receptor type 3 for efficient contraction in neonatal skeletal muscle. *EMBO J* 1997; 16: 6956-6963.

[17] Bhat MB, Zhao JY, Takeshima H, Ma J: Functional calcium release channel formed by the carboxy-terminal portion of ryanodine receptor. *Biophys J* 1997; 73: 1320-1328.

[18] Bhat MB, Ma J: The transmembrane segment of Ryanodine receptor contains an intracellular membrane retention signal for ca2+ release channel. *J Biol Che*m 2002; 277: 8597-8601.

[19] Bootman, MD, Collins TJ, Peppiatt CM, Prothero LS, MacKenzie L, De Smet P, Travers M, Tovey SC, Seo JT, Berridge MJ, Ciccolini F, Lipp P: Calcium signalling – an overview. *Sem Cell Dev Biology* 2001; 12: 3-9.

[20] Brandt NR, Caswell AH, Carl SA, Ferguson DG, Brandt T, Brunschwig JP, Bassett AL: Detection and localization of triadin in rat ventricular muscle. *J Membr Biol* 1993; 131: 219-228.

[21] Brillantes AB, Ondrias K, Scott A, Kobrinsky E, Ondriasova E, Moschella MC, Jayaraman T, Landers M, Ehrlich BE, Marks AR: Stabilization of calcium release channel (ryanodine receptor) function by FK506-binding protein. *Cell* 1994; 77: 513-523.

[22] Bultynck G, Rossi D, Callewaert G, Missieaen L, Sorrentino V, Parys JB, De Smedt H: The conserved sites for the FK506-binding proteins in ryanodine receptors and inositol 1,4,5-trisphosphate receptors are structurally and functionally different. *J Biol Chem* 2001a; 276: 47715-47724.

[23] Bultynck G, De Smet P, Rossi D, Callewaert G, Missiaen L, Sorrentino V, De Smedt H, Parys JB: Characterization and mapping of the 12 kDa FK506-binding protein (FKBP12)-binding site on different isoforms of the ryanodine receptor and of the inositol 1,4,5-trisphosphate receptor. *Biochem* 2001b; 354: 413-422.

[24] Carmody M, Mackrill JJ, Sorrentino V, O'Neill C: FKBP12 associates tightly with the skeletal muscle type 1 ryanodine receptor, but not with other intracellular calcium release channels. *FEBS Lett* 2001; 505: 97-102

[25] Casarotto MG, Green D, Pace SM, Curtis SM, Dulhunty AF: Structural determinants for activation or inhibition of ryanodine receptors by basic residues in the dihydropyridine receptor II-III loop. *Biophys J* 2001; 80: 2715-2726.

[26] Caswell AH, Brandt NR, Brunschwig JP, Purkenson S: Localization and partial characterization of the oligomeric disulphide-linked molecular weight 95,000 protein (triadin) which binds the ryanodine and dihydropyridine receptors in skeletal muscle triadic vesicles. *Biochemistry* 1991; 30: 7507-7513.

[27] Caswell AH, Motoike HK, Fan H, Brandt NR: Location of ryanodine receptor binding site on skeletal muscle triadin. *Biochemistry* 1999, 38: 90-97.

[28] Chen SRW, Zhang L, MacLennan DH: Characterization of a Ca^{2+} binding and regulatory site in the Ca^{2+} release channel (ryanodine receptor) of rabbit skeletal muscle sarcoplasmic reticulum. *J Biol Chem* 1992; 267: 23318-23326.

[29] Chen SW, Vaughan DM, Airey JA, Coronado R, MacLennan DH: Functional expression of cDNA encoding the Ca^{2+} release channel (ryanodine receptor) of rabbit skeletal muscle sarcoplasmic in COS-1 cells. *Biochemistry* 1993; 32: 3743-3753.

[30] Chen SR, Zhang L, MacLennan DH: Asymmetrical blockade of the Ca^{2+} release channel (ryanodine receptor) by 12 kDa FK506 binding protein. *Proc Natl Acad Sci USA* 1994; 91: 11953-11957.

[31] Chen SRW, Ebisawa K, Xiaoli L, Zhang L: Molecular identification of the ryanodine receptor Ca^{2+} sensor. *J Biol Chem* 1998; 273: 14675-14678.

[32] Clapham DE: Calcium signaling *Cell* 1995; 80: 259-268.

[33] Collins JH: Sequence analysis of the ryanodine receptor: possible association with a 12KK, FK506-binding immunophilin/protein kinase C inhibitor. *Biochem Biophys Res Comm* 1991; 178: 1288-1290.

[34] Conklin MW, Barone V, Sorrentino V, Coronado R: Contribution of ryanodine receptor type 3 to calcium sparks in embryonic skeletal muscle. *Biophysical J* 1999; 77: 1394-1403.

[35] Conklin MW, Ahern CA, Vallejo P, Sorrentino V, Takeshima H, Coronado R: Comparison of Ca(2+) Sparks Produced Independently by Two Ryanodine Receptor Isoforms (Type 1 or Type 3). *Biophys J* 2000; 78: 1777-1785.

[36] Conti A, Gorza L, Sorrentino V: Differential distribution of ryanodine receptor type 3 (RyR3) gene product in mammalian skeletal muscles. *Biochem J* 1996; 316: 19-23.

[37] Coussin F, Macrez N, Morel J, Mironneau J: Requirement of ryanodine receptor subtypes 1 and 2 for Ca^{2+}-induced Ca^{2+} release in vascular myocytes. *J Biol Chem* 2000; 275: 9596-9630.

[38] Currie S, Loughrey CM, Craig M, Smith GL: Calcium/calmodulin-dependent protein kinase IIδ associates with the ryanodine receptor complex and regulates channel function in rabbit heart. *Biochem J* 2004; 377: 357-366.

[39] Dirksen RT, Avila G: Altered ryanodine receptor function in central core disease: leaky or uncoupled Ca2+ release channels? *Trends Cardiovasc Med* 2002; 12: 189-197.

[40] Du GG, MacLennan DH: Functional consequences of mutation of conserved, polar amino acids in trasmembrane sequences of the Ca^{2+} release channel (ryanodine receptor) of rabbit skeletal muscle sarcoplasmic reticulum. *J Biol Chem* 1998; 273: 31867-31872.

[41] Du GG, MacLennan DH: Ca^{2+} inactivation sites are located in the COOH-terminal quarter of recombinant rabbit skeletal muscle Ca^{2+} release channels (ryanodine receptors). *J Biol Chem* 1999; 274: 26120-26126.

[42] Du GG, Guo X, Khanna VK, MacLennan DH: Functional characterization of mutants in the predicted pore region of the rabbit cardiac muscle Ca^{2+} release channel (ryanodine receptor isoform 2). *J Biol Chem* 2001; 276: 31760-31771.

[43] Dulhunty AF, Laver DR, Gallant EM, Casarotto MG, Pace SZ, Curtis S: Activation and inhibition of skeletal RyR channels by a part of the skeletal DHPR II-III loop: effects of DHPR Ser^{687} and FKBP12. *Biophys J* 1999; 77: 189-203.

[44] Dulhunty AF, Curtis SM, Watson S, Cengia L, Casarotto MG: Multiple actions of imperatoxin A on ryanodine receptors: interactions with the II-III loop "A" fragment. *J Biol Chem* 2004; 279: 11853-11862.

[45] El-Hayek R, Lokuta AJ, Arevalo C, Valdivia HH: Peptide probe of ryanodine receptor function. *J Biol Chem* 1995a; 270: 28696-28704.

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

[47] El-Hayek R, Ikemoto N: Identification of the minimum essential region in the II-III loop of the dihydropyridine receptor a1 subunit required for activation of skeletal muscle-type excitation-contraction coupling. *Biochemistry* 1998; 37: 7015-7020.

[48] Felder E, Franzini-Armstrong C: Type 3 ryanodine receptors of skeletal muscle are segregated in a parajunctional position. *Proc Natl Acad Sci* 2002; 99: 1695-1700.

[49] Feng W, Tu J, Yang T, Vernon PS, Allen PD, Worley PF, Pessah IN: Homer regulates gain of ryanodine receptor type 1 channel complex. *J Biol Chem* 2002; 277: 44722-44730.

[50] Ferreiro A, Monnier N, Romero NB, Leroy J, Bönnermann C, Haenggeli C, Straub V, Voss WD, Nivoche Y, Jungblutch H, Lemainque A, Voit T, Lunardi J, Fardeau M, Guicheney P: A recessive form of central core disease, transiently presenting

as multi-minicore disease, is associated with a homozygous mutation in the ryanodine receptor type 1 gene. *Ann Neurol* 2002; 51: 750-759.

[51] Fill M Copello JA: Ryanodine receptor calcium release channel. *Phys Rev* 2002; 82: 893-922.

[52] Flucher BE, Conti A, Takeshima H, Sorrentino V: Type 3 and Type 1 ryanodine receptors are localized in triads of the same mammalian skeletal muscle fibers. *J Cell Biol* 1999; 146: 621-629.

[53] Franzini-Armstrong C, Kenney LJ, Varriano Marston E: The structure of calsequestrin in triads of vertebrate skeletal muscle: a deep-etch study. *J Cell Biol* 1987; 105: 49-56.

[54] Franzini-Armstrong C, Protasi F: Ryanodine receptors of striated muscles: a complex channel capable of multiple interactions. *Physiol Rev* 1997; 77: 699-729.

[55] Fruen BR, Bardy JM, Byrem TM, Strasburg GM, Louis CF: Differential Ca^{2+} sensitivity of skeletal and cardiac muscle ryanodine receptors in the presence of calmodulin. *Am J Physiol Cell Physiol* 2000; 279: C724-C733.

[56] Fruen BR, Black DJ, Bloomquist RA, Bardy JM, Johnson JD, Louis CF, Balog EM: Regualation of the RYR1 and RYR2 Ca^{2+} release channel isoforms by Ca^{2+}-insensitive mutants of calmodulin. *Biochemistry* 2003; 42: 2740-2747.

[57] Furuichi T, Yoshikawa S, Miyawaki A, Wada K, Maeda N, Mikoshiba K: Primary structure and functional expression of the inostiol 1,4,5- trisphosphate-binding protein P_{400}. *Nature*, 1989; 342: 32-38.

[58] Gaburjakova M, Gaburjakoca J, Reiken S, Huang F, Marx SO, Rosemblit N, Marks AR: FKBP12 binding modulates ryanodine receptor channel gating. *J Biol Chem* 2001; 276: 16931-16935.

[59] Gao L, Balshaw D, Xu L, Tripathy A, Xin C, Meissner G: Evidence for a role of the lumenal M3-M4 loop in skeletal muscle Ca^{2+} release channel (ryanodine receptor) activity and conductance. *Biophys J* 2000; 79: 828-840.

[60] George CH, Higgs GV, Lai FA: Ryanodine receptor mutations associated with stress-induced ventricular tachycardia mediate increased calcium release in stimulated cardiomyocytes. *Circ Res* 2003; 93: 531-540.

[61] Giannini G, Clementi E, Ceci R, Marziali G, Sorrentino V: Expression of a ryanodine receptor-Ca^{2+} channel that is regulated by TGF-β. *Science* 1992; 257: 91-94.

[62] Giannini G, Conti A, Mammarella S, Scrobogna M, Sorrentino V: The ryanodine receptor/Calcium release channel genes are widely and differentially expressed in murine brain and peripheral tissues. *J Cell Biol* 1995; 128: 893-904.

[63] Girard T, Cavagna D, Padovan E, Spagnoli G, Urwyler A, Zorzato F, Treves S: B-lymphocytes from malignant hyperthermia-subsceptible patients have an increased sensitivity to skeletal muscle ryanodine receptor activators. *J Biol Chem* 2002; 276: 48077-48082.

[64] Glover L, Culligan K, Cala S, Mulvey C, Ohlendieck K: Calsequestrin binds to monomeric and complexed forms of key calcium-handling proteins in native sarcoplasmic reticulum membranes from rabbit skeletal muscle. *Biochim Biophys Acta* 2001; 1515: 120-132.

[65] Grabner M, Dirksen RT, Suda N, Beam KG: The II-III loop of the skeletal muscle dihydropyridine receptor is responsible for the bi-directional coupling with the ryanodine receptor. *J Biol Chem* 1999; 31: 21913-21919.

[66] Green D, Pace S, Curtis SM, Sakowska M, Lamb GD, Dulhunty AF, Casarotto MG: The three-dimensional structural surface of two β-sheet scorpion toxins mimics that of an α-helical dihydropyridine receptor segment. *Biochem J* 2003; 370: 517-527.

[67] Groh S, Marty I, Ottolia M, Prestipino G, Chapel A, Villaz M, Ronjat M: Functional interaction of the cytoplasmic domain of triadin with the skeletal ryanodine receptor. *J Biol Chem* 1999; 274: 12278-12238.

[68] Guis S, Figarella-Branger D, Monnier N, Bendahan D, Kozak-Ribbens G, Mattei JP, Lunardi J, Cozzone PJ, Pellissier JF: Multiminicore disease in a family susceptible to malignant hyperthermia: histology, in vitro contracture tests, and genetic characterization. *Arch Neurol* 2004; 61: 106-113.

[69] Guo W, Jorgensen AO, Campbell KP: Characterization and ultrastructure localization of a novel 90-kDa protein unique to skeletal muscle junctional sarcoplasmic reticulum. *J Biol Chem* 1994; 269: 28359-28365.

[70] Guo W, Campbell KP: Association of triadin with the ryanodine receptor and calsequestrin in the lumen of the sarcoplasmic reticulum. *J Biol Chem* 1995; 270: 9027-9030.

[71] Guo W, Jorgensen AO, Jones LR, Campbell KP: Biochemical characterization and molecular cloning of cardiac triadin. *J Biol Chem* 1996; 271: 458-465.

[72] Gurrola GB, Arevalo C, Sreekumar R, Lokuta AJ, Walker JW, Valdivia HH: Activation of ryanodine receptors by imperatoxin A and a peptide segment of the II-III loop of the dihydropyridine receptor. *J Biol Chem* 1999; 274: 7879-7886.

[73] Gyorke S, Dettbarn C, Palade P: FK-506 influences adaptive behaviour of the SR ryanodine receptor. *Biophys J* 1994; 66: A225.

[74] Hakamata Y, Nakai J, Takeshima H, Imoto K: Primary structure and distribution of a novel ryanodine receptor/calcium release channel from rabbit brain. *FEBS Lett* 1992; 312: 229-235.

[75] Hasan G, Rosbash M: Drosophila homologues of two mammalian intracellular Ca^{2+}-release channels: identification and expression patterns of the inositol 1,4,5-trisphosphate and the ryanodine receptor genes. *Development* 1992; 116: 967-975.

[76] Hong C, Ji J, Kim JP, Jung DH, Kim DH: Molecular cloning and characterization of mouse cardiac triadin isoforms. *Gene* 2001; 278: 193-199.

[77] Hwang S, Wei J, Westhoff JH, Duncan RS, Ozawa F, Volpe P, Inokuchi K, Koulen P: Differential functional interaction of two Ves1/Homer protein isoforms with ryanodine receptor type 1: a novel mechanism for control of intracellular calcium signalling. *Cell Calcium* 2003; 34: 177-184.

[78] Ikemoto T, Komazaki S, Takeshima H, Nishi M, Noda T, Iino M, Endo M: Functional and morphological features of skeletal muscle from mutant mice lacking both type 1 and type 3 ryanodine receptors. *J Physiol* 1997; 501: 305-312.

[79] Ikemoto T, Takeshima H, Iino M, Endo M: Effect of calmodulin on Ca^{2+}-induced Ca^{2+} release of skeletal muscles from mutant mice expressing either ryanodine receptor type 1 or type 3. *Eu J Physiol* 1998; 437: 43-48.

[80] Jiang D, Xiao B, Li X, Wayne Chen SR: Smooth muscle tissues express a major dominant negative splice variant of the type 3 Ca^{2+} release channel (ryanodine receptor). *J Biol Chem* 2003; 278: 4763-4769.

[81] Jayaraman T, Brillantes AMB, Timerman AP, Erd-Jument-Bromage H, Fleischer S, Tempst P, Marks AR: FK506 binding protein associated with calcium release channel (ryanodine receptor) *J Biol Chem* 1992; 267: 9474-9477.

[82] Jiang D, Xiao B., Zhang L., Wayne Chen SR: Enhanced basal activity of a cardiac Ca^{2+} release channel (ryanodine receptor) mutant associated with ventricular thachycardia and sudden death. *Circ Res* 2002; 91: 218-225.

[83] Jones RL, Zhang L, Sanborn K, Jorgensen AO, Kelley J: Purification, primary structure and immunological characterization of the 26-kDa calsequestrin binding protein (junctin) from cardiac junctional sarcoplasmic reticulum. *J Biol Chem* 1995; 270: 30787-30796.

[84] Jones LR, Suzuki YJ, Wang W, Kobayashi YM, Ramesh V, Franzini-Armstrong C, Cleemann L, Morad M: Regulation of Ca^{2+} signalling in transgenic mouse cardiac myocytes overexpressing calsequestrin. *J Clin Inv* 1998; 101: 1385-1393.

[85] Kaftan E, Marks AR, Ehrlich BE: Effects of rapamycin on ryanodine receptor/Ca^{2+}-release channels from cardiac muscle. *Circ Res* 1996; 78: 990-997.

[86] Kawasaki T, Kasai M: Regulation of calcium channel in sarcoplasmic reticulum by calsequestrin. *Biochem Biophys Res Comm* 1994; 199: 1120-1127.

[87] Knudson CM, Stang KK, Jorgensen AO, Campbell KP: Biochemical characterization and ultrastructure localization of a major junctional sarcoplasmic reticulum glycoprotein (triadin). *J Biol Chem* 1993; 268: 12637-12645.

[88] Kobayashi YM, Jones LR: Identification of triadin 1 as the predominant triadin isoform expressed in mammalian myocardium. *J Biol Chem* 1999; 274: 28660-28668.

[89] Kobayashi YM, Alseikhan BA, Jones LR: Localization and characterization of the calsequestrin-binding domain of triadin 1. *J Biol Chem* 2000; 275: 17639-17646.

[90] Kouzu Y, Moriya T, Takeshima H, Yoshioka T, Shibata S: Mutant mice lacking ryanodine receptor type 3 exhibit deficits of contextual fear conditioning and activation of calcium/calmodulin-dependent protein kinase II in the hippocampus. *Mol Brain Res* 2000; 76: 142-150.

[91] Kugler G, Weiss RG, Flucher BE, Grabner M: Structural requirements of the dihydropiridine receptor a1s II-III loop for skeletal-type excitation-contraction coupling. *J Biol Chem* 2004; 279: 4271-4728.

[92] Lai FA, Liu Q-L, Xu L, El-Hashem A, Kramarcy NR, Sealock R, Meissner G: Amphibian ryanodine receptor isoforms are related to those of mammalian skeletal or cardiac muscle. *Am J Physiol* 1992; 263: C365-C372.

[93] Laitinen PJ, Brown KM, Piippo K, Swan H, Devaney JM, Brahmbhatt B, Donarum EA, Marino M, Tiso N, Viitasalo M, Toivonen L, Stephan DA, Kontula K: Mutations in the Cardiac Ryanodine Receptor (RyR2) Gene in Familiar Polymorphic Ventricular Tachycardia. *Circulation* 2001; 103: 7-12.

[94] Lam E, Martin MM, Timerman AP, Sabers C, Fleischer S, Lukas T, Abraham RT, O'Keefe SJ, O'Neill EA, Wiederrecht GJ: A novel FK506 binding protein can mediate the immunosuppressive effects of FK506 and is associated with the cardiac ryanodine receptor. *J Biol Chem* 1995; 270: 26511-26522.

[95] Lamb GD: Excitation-contraction coupling in skeletal muscle: comparisons with cardiac muscle. *Clin Exp Pharmacol Physiol* 2000; 27: 216-224.

[96] Lee CW, Lee EH, Takeuchi K, Takashi H, Shimada I, Sato K, Shin SY, Kim DH, Kim JI: Molecular basis of the high-affinity activation of type 1 ryanodine receptors by imperatoxin A. *Biochem J* 2004a; 377: 385-394.

[97] Lee JM, Rho SH, Shin DW, Cho C, Park WJ, Eom SH, Ma J, Kim do H: Negatively charged amino acids within the intraluminal loop of ryanodine receptor are involved in the interaction with triadin. *J Biol Chem* 2004b; 279: 6994-7000.

[98] Leong P, MacLennan DH: A 37-amino acid sequence in the skeletal muscle ryanodine receptor in-

teracts with the cytoplasmic loop between domains II and III in the skeletal muscle dihydropyridine receptor. *J Biol Chem* 1998; 273: 7791-7794.

[99] Liu G, Pessah IN: Molecular interaction between ryanodine receptor and glycoprotein triadin involves redox cycling of functionally important hyperreactive sulphydryls. *J Biol Chem* 1994; 269: 33028-33034.

[100] Liu Z, Zhang J, Sharma MR, Li P, Chen SRW, Wagenknecht T: Three-dimensional reconstruction of the recombinant type 3 ryanodine receptor and localization of its amino terminus. *Proc Natl Acad Sci* 2001; 98: 6104-6109.

[101] Liu Z, Zhang J, Li P, Chen SRW, Wagenknecht T: Three-dimensional reconstruction of the recombinant type 2 ryanodine receptor and localization of its divergent region 1. *J Biol Chem* 2002; 277: 46712-46719.

[102] Löhn M, Jessner W, Fürstenau M, Wellner M, Sorrentino V, Haller H, Luft FC, Gollasch M: Regulation of calcium sparks and spontaneous transient outward currents by RyR3 in arterial vascular smooth muscle cells. *Circ Res* 2001; 89: 1051-1057.

[103] Loke J, MacLennan DH: Malignant hyperthermia and central core disease: disorders of Ca^{2+} release channels. *Am J Med* 1998; 104: 470-486.

[104] Lu X, Xu L, Meissner G: Activation of the skeletal muscle calcium release channel by a cytoplasmic loop of the dihydropyridine receptor. *J Biol Chem* 1994; 269: 6511-6516.

[105] Lynch PJ, Tong J, Lehane M, et al: A mutation in the transmembrane/luminal domain of the ryanodine receptor is associated with abnormal Ca^{2+} release channel function and severe central core disease. *Proc Natl Acad Sci USA* 1999; 96: 4164-4169.

[106] Ma J, Bhat MB, Zhao J: Rectification of skeletal muscle ryanodine receptor mediated by FK506 binding protein. *Biophys J* 1995; 69: 2398-2404.

[107] MacLennan DH, Duff C, Zorzato F, Fujii J, Phillips M, Korneluk RG, Frodis W, Britt A, Worton RG: Ryanodine receptor gene is a candidate for predisposition to malignant hyperthermia. *Nature* 1990; 343: 559-561.

[108] MacLennan DH, Wong PT: Isolation of a calcium sequestering protein from sarcoplasmic reticulum. *Proc Natl Acad Sci* 1971; 68: 1231-1235.

[109] Mackrill JJ: Protein-protein interactions in intracellular Ca2+-release channel function. *Biochem J* 1999; 337: 345-361.

[110] Marks AR, Tempst P, Hwang KS, Taubman MB, Inui M, Chadwick C, Fleisher S, Nadal-Ginard B: Molecular cloning and characterization of the ryanodine receptor/junctional channel complex cDNA from skeletal muscle sarcoplasmic reticulum. *Proc Natl Acad Sci USA* 1989; 86: 8683-8687.

[111] Marks AR: Cardiac intrcellular calcium release channels. Role in heart failure. *Circ Res* 2000; 87: 8-11.

[112] Marks AR, Marx SO, Reiken S: Regulation of ryanodine receptors via macromolecular complexes: a novel role for leucine/isoleucine zippers. *Trends Card Med* 2002; 12: 166-170.

[113] Marks AR: Ryanodine receptor, FKBP12, and heart failure. *Front Biosci* 2002; 7: 970-977.

[114] Marks AR: Calcium and the heart: a question of life and death. *J Clin Inv* 2003; 111: 597-600.

[115] Marty I, Robert M, Ronjat M, Bally I, Arlaud G, Villaz M: Localization of the N-terminal and C-terminal ends of triadin with respect to the sarcoplasmic reticulum membrane of rabbit skeletal muscle. *Biochem J* 1995; 307: 769-774.

[116] Marty I, Thevenon D, Scotto C, Groh S, Sainnier S, Robert M, Grunwald D, Villaz M: Cloning and characterization of a new isoform of skeletal muscle triadin. *J Biol Chem* 2000; 275: 8206-8212.

[117] Marx SO, Reiken S, Hisamatsu Y, Jayaraman T, Burkhoff D, Rosemblit N, Marks AR: PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts. *Cell* 2000; 101: 365-376.

[118] Marx SO, Reiken S, Hisamatsu Y, Gaburjakova M, Gaburjakova J, Yang Y, Rosemblit N, Marks AR: Phosphorylation-dependent regulation of ryanodine receptors: a novel role for leucine/isoleucine zippers. *J Cell Biol* 2001a; 153: 699-708.

[119] Marx SO, Gaburjakova J, Gaburjakova M, Henrikson C, Ondrias K, Marks AR: Coupled gating between cardiac calcium release channels (ryanodine receptors). *Circ Res* 2001b; 88: 1151-1158.

[120] Marx S: Ion channel macromolecular complexes in the heart. *J Mol Cell Cardiol* 2003; 35: 37-44.

[121] Maryon EB, Coronado R, Anderson P: *unc-68* encodes a ryanodine receptor involved in regulating *C. elegans* body-wall muscle contraction. *J Cell Biol* 1996; 134: 885-893.

[122] Maryon EB, Saarl B, Anderson P: Muscle-specific functions of ryanodine receptor channels in *Caenorhabditis elegans*. *J Cell Science* 1998; 111: 2885-2895.

[123] Marziali G, Rossi D, Giannini G, Charlesworth A, Sorrentino V: cDNA cloning reveals a tissue specific expression of alternatively spliced transcripts of the ryanodine receptor type 3 (RyR3) calcium release channel. *FEBS Lett* 1996; 394: 76-82.

[124] Masumiya H, Wang R, Zhang J, Xiao B, Wayne Chen SR: Localization of the 12.6-kDa FK506-binding protein (FKBP12.6) binding site to the NH_2-terminal domain of the cardiac Ca^{2+} release channel (ryanodine receptor). *J Biol Chem* 2003; 278: 3786-3792.

[125] Mayrleitner M, Timerman AP, Wiederrecht G, Fleischer S: The calcium release channel of sarcoplasmic reticulum is modulated by FK-506 binding protein: effect of FKBP12 on single channel activity of the skeletal muscle ryanodine receptor. *Cell Calcium* 1994; 15: 99-108.

[126] McCarthy TV, Healy JMS, Heffron JJA, Lehane M, Deufel T, Lehmann-Horn F, Farrall M, Johnson K: Localization of the malignant hyperthermia locus to human chromosome 19q12-13.2. *Nature* 1990; 343: 562-564.

[127] McCarthy TV, Quane KA, Lynch PJ: Ryanodine Receptor Mutations in Malignant Hyperthermia and Central Core Disease. *Human Mutation* 2000; 15: 410-417.

[128] Menegazzi P, Larini F, Treves S, Guerrini R, Quadroni M, Zorzato F: Identification and characterization of three calmodulin binding sites of the skeletal muscle ryanodine receptor. *Biochemistry* 1994; 33: 9078-9084.

[129] Meyers MB, Pickel VM, Sheu S, Sharma VK, Scotto KW, Fishman GI: Association of sorcin with the cardiac ryanodine receptor. *J Biol Chem* 1995; 270: 26411-26418.

[130] Mironneau J, Coussin F, Jeyakumar LH, Fleisher S, Mironneau C, Macrez N: Contribution of ryanodine subtype 3 to calcium responses in Ca^{2+} -overloaded cultured rat portal vein myocytes. *J Biol Chem* 2001; 276: 11257-11264.

[131] Mironneau M, Macrez N, Morel JL, Sorrentino V, Mironneau C: Identification and function of ryanodine receptor subtype 3 in non-pregnant mouse myometrial cells. *J Physiology* 2002; 538: 707-716.

[132] Monnier N, Ferreiro A, Marty I, Labarre-Vila A, Mezin P, Lunardi J: A homozygous splicing mutation causing a depletion of skeletal muscle RYR1 is associated with multi-minicore disease congenital myopathy with ophtalmoplegia. *Hum Mol Gen* 2003; 12: 1171-1178.

[133] Moore CP, Rodney G, Zhang J, Santacruz-Toloza L, Strasburg G, Hamilton SL: Apocalmodulin and Ca^{2+} calmodulin bind to the same region of the skeletal muscle Ca^{2+} release channel. *Biochemistry* 1999a; 38: 8532-8537.

[134] Moore CP, Zhang J, Hamilton SL: A role of cysteine 3635 of RYR1 in redox modulation and calmodulin binding. *J Biol Chem* 1999b; 274: 36831-36834.

[135] Murray BE, Ohlendieck K: Complex formation between calsequestrin and the ryanodine receptor in fast- and slow-twith rabbit skeletal muscle. *FEBS Lett* 1998; 429: 317-322.

[136] Nabhani T, Zhu X, Simeoni I, Sorrentino V, Valdivia HH, Garcia J: Imperatoxin A enhances Ca^{2+} release in developing skeletal muscle containing ryanodine receptor type 3. *Biophys J* 2002; 82: 1319-1328.

[137] Nakai J, Imagawa T, Hakamata Y, Shigekawa M, Takeshima H, Numa S: Primary structure and functional expression from cDNA of the cardiac ryanodine receptor/calcium release channel. *FEBS Lett* 1990; 271: 169-177.

[138] Nakai J, Dirksen RT, Nguyen HT, Pessah IN, Beam KG, Allen PD: Enhanced dihydropyridine receptor channel activity in the presence of ryanodine receptor. *Nature* 1996; 380: 72-76.

[139] Nakai J, Tanabe T, Konno T, Adams B, Beam KG: Localization in the II-III loop of the dihydropiridine receptor of a sequence critical for excitation-contraction coupling. *J Biol Chem* 1998a; 273: 24983-24986.

[140] Nakai J, Sekiguchi N, Rando TA, Allen PD, Beam KG: Two regions of the ryanodine receptor involved in coupling with L-type Ca2+ channels. *J Biol Chem* 1998b; 273: 13403-13406.

[141] Nakai J, Gao L, Xu L, Xin C, Pasek DA, Meissner G: Evidence for a role of C-terminus in Ca^{2+} inactivation of skeletal muscle Ca^{2+} release channel (ryanodine receptor). *FEBS Lett* 1999; 459: 154-158.

[142] Naisbitt S, Kim E, Tu JC, Xiao B, Sala C, Valtschanoff J, Weinberg RJ, Worley PF, Sheng M: Shank, a novel family of postsynaptic density proteins that binds to the NMDA receptor/PSD-95/GKAP complex and cortactin. *Neuron* 1999; 23: 569-582.

[143] O'Connell KMS, Yamaguchi N, Meissner G, Dirksen RT: Calmodulin binding to the 3614-3643 region of RyR1 is not essential for excitation-contraction coupling in skeletal myotubes. *J Gen Physiol* 2002; 120: 337-347.

[144] O'Reilly FM, Robert M, Jona I, Szegedi C, Albrieux M, Geib S, De Waard M, Villaz M, Ronjat M: FKBP12 modulation of the binding of the skeletal ryanodine receptor onto the II-III loop of the dihydropyridine receptor. *Biophys J* 2002; 82: 145-155.

[145] Ohkura M, Furukawa K, Fujimori H, Kuruma A, Kawano S, Hiraoka M, Kuniyasu A, Nakayama H, Ohizumi Y: Dual regulation of the skeletal muscle ryanodine receptor by triadin and calsequestrin. *Biochemistry* 1998; 37: 12987-12993.

[146] Olivares EB, Tanksley SJ, Airey JA, Beck CF, Ouyang Y, Deerinck TJ, Ellisman MH, Sutko JL: Multiple foot protein isoforms in amphibian, avian and piscine skeletal muscles. *Biophys J* 1991; 59: 1153-1163.

[147] Ondrias K, Marks AR: A role for FKBP in coupled gating of the recombinant ryanodine receptor/calcium release channel. *Circulation* 1995; 92: I-235.

[148] Ondrias K, Marx SO, Marks AR: FKBP12 modulates ryanodine receptor gating. *Biophys J* 1998; 74: A340.

[149] Ono K, Yano M, Ohkusa T, Khono M, Hisaoka T, Tanigawa T, Kobayashi S, Khono M, Matsuzaki M: Altered interaction of FKBP12.6 with ryanodine receptor as a cause of abnormal Ca^{2+} release in heart failure. *Cardiovasc Res* 2000; 48: 323-331.

[150] Ottini L, Marziali G, Conti A, Charlesworth A, Sorrentino V: □ and □ isoforms of ryanodine receptors from chicken skeletal muscle are the homologues of mammalian RyR1 and RyR3. *Biochem J* 1996; 315: 207-215.

[151] Oyamada H, Murayama T, Takagi T, Iino M, Iwabe N, Miyata T, Ogawa Y, Endo M: Primary structures and distribution of ryanodine-binding protein isoforms of the bullfrog skeletal muscle. *J Biol Chem* 1994; 269: 17206-17214.

[152] Patel S, Joseph SK, Thomas AP: Molecular properties of inositol 1,4,5 trisphosphate receptors. *Cell Calcium* 1999; 25: 247-264.

[153] Perez CF, Voss A, Pessah IN, Allen PD: RyR1/RyR3 chimeras reveal that multiple domains of RyR1 are involved in skeletal-type E-C coupling. *Biophys J* 2003; 84: 2655-2663.

[154] Phillips MS, Fujii J, Khanna VK, DeLeon S, Yokobata K, De Jong P, MacLennan D: The Structural Organization of the Human Skeletal Muscle Ryanodine Receptor (RYR1) *Gene Genomics* 1996; 34: 24-41.

[155] Ponting CP: Novel repeats in ryanodine and IP3 receptors and protein 0-mannosyl-transferases. *Trends Biol Sci* 2000; 25: 48-50.

[156] Powell JA, Petherbridge L, Flucher BE: Formation of triads without the dihydropyridine receptor alpha subunits in cell lines from dysgenic skeletal muscle. *J Cell Biol* 1996; 134: 375-387.

[157] Priori SG, Napolitano C, Tiso N, Memmi M, Vignati G, Bloise R, Sorrentino V, Danieli GA: Mutations in the Cardiac Ryanodine Receptor Gene (hRyR2) Underlie Catecholaminergic Polymorphic Ventricular Tachycardia. *Circulation* 2000; 102: 49-53.

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

[159] Proenza C, O'Brien J, Nakai J, Mukherjee S, Allen PD, Beam KG: Identification of a region of RyR1 that participates in allosteric coupling with the α1S ($Ca_V1.1$) II-III loop. *J Biol Chem* 2002; 277: 6530-6535.

[160] Protasi F, Franzini-Armstrong C, Allen PD: Role of ryanodine receptors in assembly of calcium release units in skeletal muscle. *J Cell Biol* 1998; 140: 831-842.

[161] Protasi F: Structural interaction between RyRs and DHPRs in calcium release units of cardiac and skeletal muscle cells. *Front Biosci* 2002; 7: d650-d658.

[162] Protasi F, Paolini C, Nakai J, Beam KG, Franzini-Armstrong C, Allen PD: Multiple regions of RyR1 mediate functional and structural interactions with α1s-dihydropiridine receptors in skeletal muscle. *Biophys J* 2002; 83: 3230-3244.

[163] Qi Y, Ogunbunmi EM, Freund EA, Timerman AP, Fleischer S: FK-binding protein is associated with the ryanodine receptor of skeletal muscle in vertebrate animals. *J Biol Chem* 1998; 273: 34813-34819.

[164] Radermacher M, Rao V, Grassucci R, Frank J, Timerman AP, Fleischer S, Wagenknecht T: Cryo-electron microscopy and three-dimensional reconstruction of the calcium release channel/ryanodine receptor from skeletal muscle. *J Cell Biol* 1994; 127: 411-423.

[165] Reiken S, Gaburjakova M, Guatimosim S, Gomez AM, D'Armiento J, Burkhoff D, Wang J, Vassort G, Lederer WJ, Marks AR: Protein kinase A phosphorylation of the cardiac calcium release channel (ryanodine receptor) in normal and failing hearts. Role of phosphatase and response to isoprotenol. *J Biol Chem* 2003a; 278: 444-453.

[166] Reiken S, Lacampagne A, Zhou H, Kherani A, Lehnart SE, Ward C, Huang F, Gaburjakova J, Rosemblit N, Warren MS, He K, Yi G, Wang J, Burkoff D, Vassort G, Marks AR: PKA phosphorylation activates the calcium release channel (ryanodine receptor) in skeletal muscle: defective regulation in heart failure. *J Cell Biol* 2003b; 160: 919-928.

[167] Reiken S, Gaburjakova M, Gaburjakova J, He KH, Weherens MD, Vest JA, Barbone A, Klotz S, Mancini D, Burkhoff D, Marks AR: β-blockers restore calcium release channel function and improve cardiac muscle performance in human heart failure. *Circulation* 2003c; 107: 2459-2466.

[168] Reiken S, Lacampagne A, Zhou H, Kherani A, Lehnart SE, Ward C, Huang F, Gaburjakova J, Rosemblit N, Warren MS, He K, Yi G, Wang J, Burkhoff D, Wassort G, Marks AR: PKA phosphorylation activates calcium release channel (ryanodine receptor) in skeletal muscle: defective regulation in heart failure. *J Cell Biol* 2003d; 160: 919-928.

[169] Roche KW, Tu JC, Petralia RS, Xiao B, Wenthold RJ, Worley PF: Homer 1b regulates the trafficking of group 1 metabotropic glutamate receptors. *J Biol Chem* 1999; 274: 25953-25957.

[170] Rodney GG, Williams BY, Strasburg GM, Beckingham K, Hamilton SL: Regulation of RYR1 activity by Ca^{2+} and calmodulin. *Biochemistry* 2000; 39: 7807-7812.

[171] Rodney GG, Moore CP, Williams BY, Zhang J, Krol J, Pedersen SE, Hamilton SL: Calcium binding to calmodulin leads to an N-terminal shift in its binding site on the ryanodine receptor. *J Biol Chem* 2001a; 276: 2069-2074.

[172] Rodney GG, Krol J, Williams B, Bechingham K, Hamilton SL: The carboxy-terminal calcium binding sites of calmodulin control calmodulin's switch from an activator to and inhibitor of RYR1. *Biochemistry* 2001b; 40: 12430-12435.

[173] Rodriguez P, Bhogal MS, Colyer J: Stoichiometric phosphorylation of cardiac ryanodine receptor on serine 2809 by calmodulin-dependent kinase II and protein kinase A. *J Biol Chem* 2003; 278: 38593-38600.

[174] Ruehr ML, Russel MA, Ferguson DG, Bhat M, Ma J, Damron DS, Scott JD, Bond M: Targeting of protein kinase A by muscle A kinase-anchoring protein (mAKAP) regulates phosphorylation and function of the skeletal muscle ryanodine receptor. *J Biol Chem* 2003; 278: 24831-24836.

[175] Sakube Y, Ando H, Kagawa H: An abnormal ketamine response in mutants defective in the ryanodine receptor gene ryr1 (*unc-68*) of Caenorhabditis elegans. *J Mol Biol* 1997; 267: 849-864.

[176] Saiki Y, El-Hayek R, Ikemoto N: Involvement of the Glu^{724}-Pro^{760} region of the dihydropyridine receptor II-III loop in skeletal muscle-type excitation-contraction coupling. *J Biol Chem* 1999; 274: 7825-7832.

[177] Salanova M, Priori G, Barone V, Intravaia E, Flucher B, Ciruela F, McIlhinney RAJ, Parys JB, Mikoshiba K, Sorrentino V: Homer proteins and $InsP_3$ receptors co-localise in the longitudinal sarcoplasmic reticulum of skeletal muscle fibres. *Cell Calcium* 2002; 32: 193-200.

[178] Samso' M, Trujillo R, Gurrola GB, Valdivia HH, Wagenknecht T: Three-dimensional location of the imperatoxin A binding site on the ryanodine receptor. *J Cell Biol* 1999; 146: 493-499.

[179] Serysheva I, Orlova EV, Chiu W, Sherman MB, Hamilton SLH, van Heel M: Electron cryomicroscopy and angular reconstitution used to visualize the skeletal muscle calcium release channel. *Nature Struct Biol* 1995; 2: 18-24.

[180] Sharma MR, Penczek P, Grassucci R, Xin H, Fleischer S, Wagenknecht T: Cryoelectron microscopy and image analysis of the cardiac ryanodine receptor. *J Biol Chem* 1998; 273: 18429-18434.

[181] Sharma MR, Jeyakumar LH, Fleischer S, Wagenknecht T: Three-dimensional structure of ryanodine receptor isoform three in two conformational states as visualized by cryo-electron microscopy. *J Biol Chem* 2000; 275: 9485-9491.

[182] Shtifman A, Ward CW, Wang J, Valdivia HH, Schneider MF: Effects of imperatoxin A on local sarcoplasmic reticulum Ca^{2+} release in frog skeletal muscle. *Biophys J* 2000; 79: 814-827.

[183] Shin DW, Ma J, Kim DH: The asp-rich region at the carboxy-terminus of calsequestrin binds to Ca^{2+} and interacts with triadin. *FEBS Lett* 2000; 486; 178-182.

[184] Shin DW, Pan Z, Kim EK, Lee JM, Bhat MB, Parness J, Kim DH, Ma J: A retrograde signal from calsequestrin for the regulation of store-operated Ca^{2+} entry in skeletal muscle. *J Biol Chem* 2003; 278: 3286-3292.

[185] Shirokova N, Shirokov R, Rossi D, Garcia J, Sorrentino V, Rios E. Spatially segregated control of Ca^{2+} release in developing skeletal muscle. *J Physiology (Lond)* 1999; 521: 483-495.

[186] Shomer NH, Louis CF, Fill M, Litterer LA, Mickelson JR: Reconstitution of abnormalities in malignant hyperthermia-susceptible pig ryanodine receptor. *Am J Physiol* 1993; 264: C125-C135.

[187] Shou W, Aghdasi B, Armstrong DL, Gou Q, Bao S, Charng M, Mathews LM, Schneider MD, Hamilton SL. Matzuk MM: Cardiac defects and altered ryanodine receptor function in mice lacking FKBP12. *Nature* 1998; 391: 489-492.

[188] Simeoni I, Rossi D, Zhu X, Garcia J, Valdivia HH, Sorrentino V: Imperatoxin A ($IPTx_a$) from *Pandinus imperator* stimulates [^{3}H]ryanodine binding to RyR3 channels. *FEBS Lett* 2001; 508: 5-10.

[189] Sorrentino V, Barone V, Rossi D: Intracellular Ca^{2+} release channels in evolution. *Curr Opin Gen Dev* 2000; 10: 662-667.

[190] Sorrentino V, Volpe P: Ryanodine receptors: how many, where and why? *Trends in Pharmacol Sci* 1993; 14: 98-103.

[191] Stange M, Xu L, Balshaw D, Yamaguchi N, Meissner G: Characterization of recombinant skeletal muscle (Ser-2843) and cardiac muscle (Ser-2809) ryanodine receptor phosphorylation mutants. *J Biol Chem* 2003; 278: 51693-51702.

[192] Sutko JL, Airey JA: Ryanodine receptor Ca2+ release channels: does diversity in form equal diversity in function. *Physiol Rev* 1996; 76: 1027-1071.

[193] Suko J, Fogy-Maurer I, Plank B, Bertel O, Wyskovsky W, Hohenegger M, Hellmann G: Phosphorylation of serine 2843 in ryanodine receptor-calcium release channel of skeletal muscle by cAMP, cGMP-and CaM-dependent protein kinase. *Biochim Biophys Acta* 1993; 1175: 193-206.

[194] Szegedi C, Sarkozi S, Herzog A, Jona I, Varsanyi M: Calsequestrin: more than 'only' a luminal Ca^{2+} buffer inside the sarcoplasmic reticulum. *Biochem J* 1999; 337: 19-22.

[195] Takeshima HS, Nishimura S, Matsumoto T, Ishida H, Kangawa K, Minamino N, Matsuo H, Ueda M, Hanaoka M, Hirose T, Numa S: Primary structure and expression from complementary DNA of skele-

tal muscle ryanodine receptor. *Nature* 1989; 339: 439-445.

[196] Takeshima H: Primary structure and expression from cDNAs of the ryanodine receptor. *Ann NY Acad Sci* 1993; 707: 165-177.

[197] Takeshima H, Iino M, Takekura H, Nishi M, Kuno J, Minowa O, Takano H, Noda T: Excitation-contraction uncoupling and muscular degeneration in mice lacking functional skeletal muscle ryanodine-receptor gene. *Nature* 1994; 369: 556-559.

[198] Takeshima H, Yamazawa T, Ikemoto T, Takekura H, Nishi M, Noda T, Iino M: Ca^{2+}-induced Ca^{2+} release in myocites from dyspedic mice lacking the type-1 ryanodine receptor. *EMBO J* 1995; 14: 2999-3006.

[199] Takeshima H, Ikemoto T, Nishi M, Nishiyama N, Shimuta M, Sugitani Y, Kuno J, Saito I, Saito H, Endo M, Iino MM, Noda T: Generation and Characterization of Mutant Mice Lacking Ryanodine Receptor Type 3. *J Biol Chem* 1996; 271: 19649-19652.

[200] Takeshima H, Komazaki S, Hirose K, Nishi M, Noda T, Iino M: Embryonic lethality and abnormal cardiac myocytes in mice lacking ryanodine receptor type 2. *EMBO J* 1998; 17: 3309-3316.

[201] Tanabe T, Beam KG, Adams BA, Niidome T, Numa S: Regions of the skeletal muscle dihydropyridine receptor critical for excitation-contraction coupling. *Nature* 1990; 346: 567-569.

[202] Taylor CW, Laude AJ: IP3 receptors and their regulation by calmodulin and cytosolic Ca^{2+}. *Cell Calcium* 2002; 32: 321-334.

[203] Tilgen N, Zorzato F, Halliger-Keller B, Muntoni F, Sewry C, Palmucci LM, Schneider C, Hauser E, Lehmann-Horn F, Muller CR, Treves S: Identification of four novel mutations in the C-terminal membrane spanning domain of the ryanodine receptor 1: association with central core disease and alteration of calcium homeostasis. *Hum Mol Genet.* 2001; 10: 2879-2887.

[204] Timerman AP, Ogunbumni E, Freund E, Wiederrecht G, Marks AR, Fleischer S: The calcium release channel of sarcoplasmic reticulum is modulated by FK-506-binding protein. *J Biol Chem* 1993; 268: 22992-22999.

[205] Timerman AP, Jayaraman T, Wiederrecht G, Onoue H, Marks AR, Fleischer S: The ryanodine receptor from canine heart sarcoplasmic reticulum is associated with a novel FK-506 binding protein. *Bioche Biophys Res Comm* 1994; 198: 701-706.

[206] Timerman AP, Onoue H, Xin H, Barg S, Copello J, Wiederrecht G, Fleischer S: Selective binding of FKBP12.6 by the cardiac ryanodine receptor. *J Biol Chem* 1996; 271: 20385-20391.

[207] Tiso N, Stephan DA, Nava A, Begattin A, Devaney JM, Stanchi F, Larderet G, Brahmbhatt B, Brown K, Bauce B, Muriago M, Basso C, Thiene G, Danieli GA, Rampazzo A: Identification of mutations in the cardiac ryanodine receptor gene in families affected with arhythmogenic right ventricular cardiomyophathy type 2 (ARVD2) *Hum Mol Gen* 2001; 10: 189-194.

[208] Tong J, McCarthy TV, MacLennan DH: Measurement of resting cytosolic Ca^{2+} concentrations and Ca^{2+} store size in HEK293 cells transfected with malignant hyperthermia or central core disease mutant Ca^{2+} release channels. *J Biol Chem* 1999; 274: 693-702.

[209] Treves S, Scutari E, Robert M, Groh S, Ottolia M, Prestipino G, Ronjat M, Zorzato F: Interaction of S100A1 with the Ca^{2+} release channel (ryanodine receptor) of skeletal muscle. *Biochemistry* 1997; 36: 11496-11503.

[210] Tripathy A, Xu L, Mann G, Meissner G: Calmodulin activation and inhibition of skeletal muscle Ca^{2+} release channel (ryanodine receptor). *Biophys J* 1995; 69: 106-119.

[211] Tripathy A, Resch W, Xu L, Valdivia HH, Meissner G: Imperatoxin A induces subconductance states in Ca^{2+} release channels (ryanodine receptor) of cardiac and skeletal muscle. *J Gen Physiol* 1998; 111: 679-690.

[212] Tu JC, Xiao B, Yuan JP, Lanahan AA, Leoffert K, Li M, Linden DJ, Worley PF: Homer binds a novel proline-rich motif and links group 1 metabotropic glutamate receptors with IP3 receptors. *Neuron* 1998; 21: 717-726.

[213] Tu JC, Xiao B, Naisbitt S, Yuan JP, Petralia RS, Brakeman P, Doan A, Aakalu VK, Lanahan AA, Sheng M, Worlay PF: Coupling of mGluR/Homer and PSD-95 complexes to the shank family of postsynaptic density proteins. *Neuron* 1999; 23: 583-592.

[214] Wagenknecht T, Radermacher M, Grassucci R, Berkowitz J, Xin HB, Fleisher S: Locations of calmodulin and FK506-binding protein on the three-dimensional architecture of the skeletal muscle ryanodine receptor. *J Biol Chem* 1997; 272: 32463-32471.

[215] Wagenknecht T, Samsò M: Three-dimensional reconstruction of ryanodine receptors. *Front Biosci* 2002; 7: 1464-1474.

[216] Wang W, Cleemann L, Jones LR, Morad M: Modulation of focal and global Ca^{2+} release in calsequestrin-overexpressing mouse cadiomyocytes. *J Physiol* 2000; 524: 399-414.

[217] Ward CW, Reiken S, Marks AR, Marty I, Vassort G, Lacampagne A: Defects in ryanodine receptor calcium release in skeletal muscle from post-myocardial infarct rats. *FASEB J* 2003; 17: 1517-1519.

[218] Ward CW, Feng W, Tu J, Pessah IN, Worley PK, Schneider MF: Homer protein increases activation of Ca^{2+} sparks in permeabilized skeletal muscle. *J Biol Chem* 2004; 279: 5781-5787.

[219] Wayne Chen SR, MacLennan DH: Identification of calmodulin, Ca^{2+}-, and ruthenium red-binding domains in the Ca^{2+} release channel (ryanodine receptor) of rabbit skeletal muscle sarcoplasmic reticulum. *J Biol Chem* 1994a; 269: 22698-22704.

[220] Wayne Chen SR, Zhang L, MacLennan DH: Asymmetrical blockade of the Ca^{2+} release channel (ryanodine receptor) by 12-kDa FK506 binding protein. *Proc Natl Acad Sci USA* 1994b; 91: 11953-11957.

[221] Wehner M, Rueffert H, Koenig F, Neuhaus J, Olthoff D: Increased sensitivity to 4-chloro-m-cresol and caffeine in primary myotubes from malignant hyperthermia susceptible individuals carrying the RyR1 Thr2206Met (C6617T) mutation. *Clin Genet* 2002; 62: 135-146.

[222] Wehrens XH, Marks AR: Altered function and regulation of cardiac ryanodine receptors in cardiac disease. *Trends Biochem Sci* 2003; 12: 671-678.

[223] Wehrens XHT, Lehnart SE, Huang F, Vest JA, Reiken SR, Mohler PJ, Sun J, Guatimosim S, Song L, Rosemblit N, D'Armiento JM, Napolitano C, Memmi M, Priori SG, Lederer WJ, Marks AR: FKBP12.6 deficiency and defective calcium release channel (ryanodine receptor) function linked to exercise-induced sudden cardiac death. *Cell* 2003; 113: 829-840.

[224] Westhoff JH, Hwang S, Duncan RS, Ozawa F, Volpe P, Inokuchi K, Koulen P: Ves1/Homer proteins regulate ryanodine receptor type 2 function and intracellular calcium signaling. *Cell Calcium* 2003; 34: 261-269.

[225] Wetzel GT, Ding S, Chen F: Molecular cloning of junctin from human and developing rabbit heart. *Mol Gen Metab* 2000; 69; 252-258.

[226] Wilksen CM, Kasielke N, Flucher BE, Beam KG, Grabner M: Excitation-contraction coupling is unaffected by drastic alteration of the sequence surrounding residues L720-L764 of the α1s II-III loop. *Proc Natl Acad Sci USA* 2001; 98: 5982-5987.

[227] Xiao R, Valdivia HH, Bogdanov K, Valdivia C, Lakatta EG, Cheng H: The immunophilin FK506-binding protein modulates Ca^{2+} release channel closure in rat heart. *J Physiol* 1997; 502: 343-354.

[228] Xiao B, Sutherland C, Walsh M, Wayne Chen SR: Protein kinase A phosphorylation at serine-2808 of the cardiac Ca2+ release channel (ryanodine receptor) does not dissociate 12.6-kDa FK506-binding protein (FKBP12.6). *Circ Res* 2004; 94.

[229] Xin H, Rogers K, Qi Y, Kanematsu T, Fleischer S Three amino acid residues determine selective binding of FK506-binding protein 12.6 to the cardiac ryanodine receptor. *J Biol Chem* 1999; 274: 15315-15319.

[230] Xin H, Senbonmatsu T, Cheng D, Wang Y, Copello J, Ji G, Collier ML, Deng K, Jeyakumar LH, Magnuson MA, Inagai T, Kotikoff MI, Fleischer S: Oestrogen protects FKBP12.6 null mice from cardiac hypertrophy. *Nature* 2002; 416: 334-337.

[231] Xiong L, Newman RA, Rodney GG, Thomas O, Zhang J, Persechini A, Shea MA, Hamilton SL: Lobe-dependent regulation of ryanodine receptor type 1 by calmodulin. *J Biol Chem* 2002; 277: 40862-40870.

[232] Yamaguchi N, Xin C, Meissner G: Identification of apocalmodulin and Ca^{2+}-calmodulin regulatory domain in skeletal muscle Ca^{2+} release channel, ryanodine receptor. *J Biol Chem* 2001; 276: 22579-22585.

[233] Yamaguchi N, Xu L, Pasek DA, Evans KE, Maissner G: Molecular basis of calmodulin binding to cardiac muscle Ca^{2+} release channel (ryanodine receptor). *J Biol Chem* 2003; 278: 23480-23486.

[234] Yamazawa T, Takeshima H, Shimuta M, Iino M: A region of the ryanodine receptor critical for excitation-contraction coupling in skeletal muscle. *J Biol Chem* 1997; 272: 8161-8164.

[235] Yang D, Pan Z, Takeshima H, WC, Nagaraj NJ, Ma J, Cheng H: RyR3 amplifies RyR1-mediated Ca^{2+}-induced Ca^{2+} release in neonatal mammalian skeletal muscle. *J Biol Chem* 2001; 276: 40210-40214.

[236] Yano M, Ono K, Ohkusa T, Suetsugu M, Khono M, Hisaoka T, Kobayashi S, Hisamatsu Y, Yamamoto T, Khono M, Noguchi N, Takasawa S, Okamoto H, Matsuzaki M: Altered stoichiometry of FKBP12.6 versus ryanodine receptor as a cause of abnormal Ca^{2+} leak through ryanodine receptor in heart failure. *Circulation* 2000; 102: 2131-2136.

[237] Yang T, Anh Ta T, Pessah IN, Allen PD: Functional defects in six ryanodine receptor isoform-1 (RyR1) mutations associated with malignant hyperthermia and their impact on skeletal excitation-contraction coupling. *J Biol Chem* 2003; 278: 25722-25730.

[238] Yayaraman T, Brillantes A, Timerman AP, Fleischer S, Erdjument-Bromage H, Tempst P, Marks AR: FK506 binding protein associated with the calcium release channel (ryanodine receptor). *J Biol Chem* 1992; 267: 9474.

[239] Zhang Y, Chen HS, Khanna VK, De Leon S, Phillips M, Schappert K, Britt BA, Brownell KW, McLennan DH: A mutation in the human ryanodine receptor gene associated with central core disease. *Nature Gen* 1993; 5: 46-51.

[240] Zhang L, Kelley J, Schmeisser G, Kobayashi YM, Jones LR: Complex formation between junctin, triadin, calsequestrin and the ryanodine receptor. *J Biol Chem* 1997; 272: 23389-23397.

[241] Zhang J, Liu Z, Masumiya H, Wang R, Jiang D, Li F, Wagenknecht T, Chen SWR: Three-dimensional localization of divergent region 3 of the ryanodine receptor to the clamp-shaped structures adjacent to the FKBP binding sites. *J Biol Chem* 2002; 278: 14211-14218.

[242] Zhang H, Zhang J, Danila CI, Hamilton SL: A non-contiguous, intersubunit binding site for calmodulin on the skeletal muscle Ca^{2+} release channel. *J Biol Chem* 2003; 278: 8348-8355.

[243] Zhao M, Li P, Li X, Zhang L, Winkfein RJ, Wayne Chen SR: Molecular identification of the ryanodine receptor pore-forming segment. *J Biol Chem* 1999; 274: 25971-25974.

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

[245] Zhu X, Ghanta J, Walker JW, Allen PD, Valdivia HH: The calmodulin binding region of the skeletal ryanodine receptor acts as a self-modulatory domain. *Cell Calcium* 2004; 35: 165-177.

[246] ZhuGe R, Sims SM, Tuft RA, Fogarty KE, Walsh JV: Ca2+ sparks activate K+ and Cl- channels, resulting in spontaneous transient currents in guinea-pig tracheal myocytes. *J Physiol* 1998; 513: 711-718.

[247] Zorzato F, Fujii J, Otsu K, Phillips M, Green NM, Lai FA, Meissner G, MacLennan DH: Molecular cloning of cDNA encoding human and rabbit forms of the Ca^{2+} release channel (ryanodine receptor) of skeletal muscle sarcoplasmic reticulum. *J Biol Chem* 1990; 265: 2244-2256.

[248] Zucchi R, Ronca-Testoni S: The sarcoplasmic reticulum Ca^{2+} channel/ryanodine receptor: modulation by endogenous effectors, drugs and disease states. *Pharmac Rev* 1997; 49: 1-51.