Chapter 6

INTRA-MOLECULAR DOMAIN-DOMAIN INTERACTION

A KEY MECHANISM FOR CALCIUM CHANNEL REGULATION OF RYANODINE RECEPTORS

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DOMAIN-DOMAIN INTERACTION FOR Ca^{2+} CHANNEL REGULATION

Introduction

E-C coupling in both skeletal muscle and cardiac muscle is mediated by a common mechanism as well as by tissue-specific mechanisms.[168] The most important common feature is that the ryanodine receptor plays a central role in this process.[169] Some tissue-specific differences may be ascribable to the fact that the RyR is expressed by different tissue-specific genes and that its structural arrangements with another important component DHPR are quite different (see Chapter 1).[170]

The skeletal RyR isoform (RyR1) and the cardiac RyR isoform (RyR2) show about 60% homology, and interestingly, homologous regions and non-homologous regions appear to be segregated along the RyR polypeptide chain. An early analysis identified the three major divergent (non-homologous) regions; the so-called D1, D2 and D3 regions as indicated in Fig. 6-1.[115] Fig. 6-1 also shows a heterogeneity map we constructed on the basis of the residue distance score of individual corresponding residues of the two isoforms. As seen (the peak height shows heterogeneity), there are several additional divergent regions in the RyR. It is quite reasonable to

assume that some of the tissue-specific differences in the RyR function mentioned above are ascribable to these divergent regions, and some common features may be ascribable to the homologous regions as discussed in the following parts.

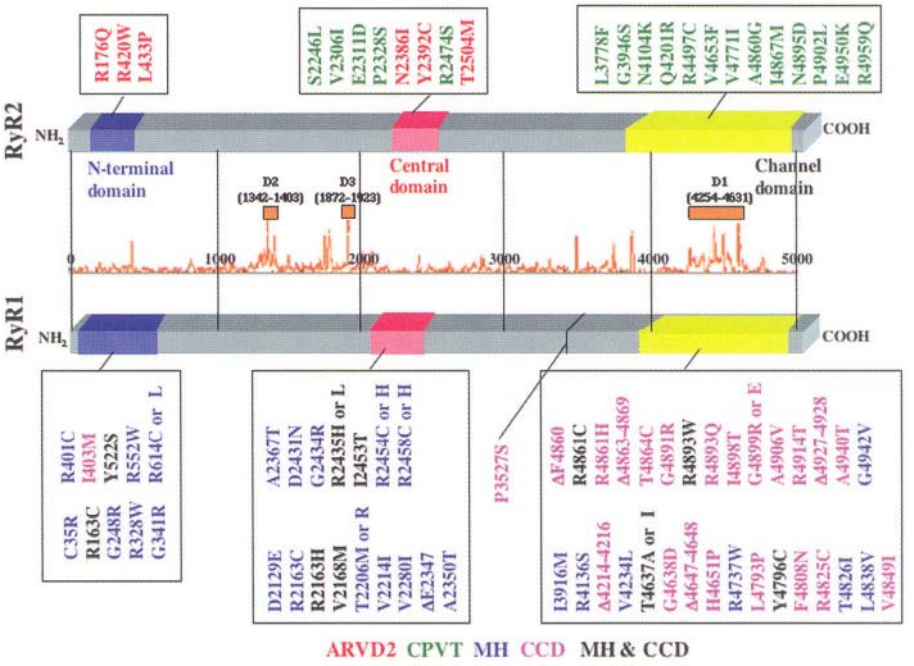

Figure 6-1. **The locations of MH/CCD mutation sites (RyR1) and cardiac disease mutation sites (RyR2).** Most of these mutations are located in the three well-definable regions, the N-terminal domain, central domain, and channel domain. As a reference, the heterogeneity map is indicated. Three highly divergent regions D1, D2 and D3 are shown. Note that the two hot-spot domains located in the cytoplasmic lobe of the RyR (i.e. N-terminal domain and central domain) are relatively homologous between RyR1 and RyR2. ARVD2, arrhythmogenic right ventricular dysplasia/cardiomyopathy type 2; CCD, central core disease; CPVT, catecholaminergic polymorphic ventricular tachycardia; MH, malignant hyperthermia.

THE CONCEPT OF 'DOMAIN SWITCH'

Critical regulatory domains

Presumably, a number of domains and sub-domains of RyR are working in a coordinated manner to perform the necessary conformational control of RyR Ca^{2+} channels. In searching for such regulatory domains, Ikemoto and

his colleagues, as well as other investigators, have paid particular attention to the fact that the reported sites of MH/CCD mutations on RyR1 are not randomly distributed. As a matter of fact, they are localized to three rather restricted regions: the N-terminal, central, and the channel domains (Fig. 6-1). For further details of these mutations, the readers must refer to Chapters 22 and 23. The vast majority of MH mutations are located in the N-terminal and central domains. In contrast, most mutations conferring susceptibility to CCD, a rare myopathy linked to RyR1, are located in the C-terminal channel region.[171] These MH mutations cause aberrations in the RyR1 channel function, such as hyper-activation of the channel by, and hyper-sensitization to, various physiological and pharmacological agonists, resulting in a leaky Ca^{2+} channel and an elevated cytoplasmic Ca^{2+} level.[172-174] The studies on the Ca^{2+} release properties of heterologously[175] or homologously[176] expressed RyR1 channels containing randomly selected MH mutations from the N-terminal and central domains demonstrated that these channels in fact display MH-like hyper-activation and hyper-sensitization of RyR Ca^{2+} channels. However, the expressed RyR1 containing selected CCD mutations from the C-terminal channel domain displayed a different phenotype: that is, unlike RyR1 containing MH mutations, it showed normal response to pharmacological agonists, but it showed no response to the physiological stimulus ('EC uncoupling').[177] These facts suggest that mutations occurred in the N-terminal and central domains affect primarily upon the intra-molecular control of channel functions, while those in the C-terminal channel domain affect primarily upon the inter-molecular (DHPR-RyR1) signal transmission.

The primary structure of the RyR2 corresponding to both of the skeletal N-terminal and central domains are relatively well conserved (Fig. 6-1, heterogeneity map). This suggests that the cardiac domains corresponding to these N-terminal and central domains also play a key role. Recently several RyR2 mutations have been reported that are related to inheritable cardiac diseases[53,54,178]; for further details, see Chapters 25. Many of these mutations are located in either of the predicted N-terminal or central domain of the RyR2 (see blue and red coded regions, respectively, Fig. 6-1), although many more are located in the putative transmembrane channel region (yellow-coded). Of particular interest is that one of the cardiomyopathy (ARVD2) mutations in the N-terminal domain of the RyR2, Argl76Gln, corresponds exactly to the Arg163Cys human MH mutation of the RyR1. One must also note that the amino acid residues in RyR1 or RyR2 that are mutated in disease are usually ones that are identical in RyR1 and RyR2. Thus, it is very likely that the essentially identical sets of regulatory domains are operating for channel regulation in both RyR1 and RyR2.

Domain-domain interaction for Ca^{2+} channel regulation

Considering again the afore-mentioned properties of the expressed RyR1 channels containing randomly selected MH mutations[175,176], we notice a quite important feature. That is, wherever these mutations are, any of these mutations produces more or less identical MH-like effects (hyper-activation/hyper-sensitization) on Ca^{2+} channels, so far as those mutations were located in either N-terminal domain or central domain of RyR1. One of the most feasible explanations is that these hot domains (i.e. N-terminal and central domains) constitute the intra-molecular machinery that controls Ca^{2+} channel functions, hence mutations occurring in either domain will produce a global impact on the operation of the machinery, and in turn abnormal Ca^{2+} channel regulation.

Based on the above consideration, Ikemoto and his colleagues have proposed a 'domain-switch' model (Fig. 6-2) that involves inter-domain interactions between the N-terminal and central domains of RyR serving as a key mechanism for Ca^{2+} channel regulation.[179,180] The model assumes that in the resting or non-activated state, the N-terminal and central domains make close contact at several as yet undetermined sub-domains (e.g. sub-domains x/y). The conformational constraints imparted by the 'zipped' configuration of these two domains stabilize the closed state of Ca^{2+} channel (Fig. 6-2, the left state of row a). The model proposes this conformation as the 'off' configuration of the implicit 'on/off switch' constituted by these two domains. Under usual stimulating conditions (EC coupling or pharmacological agonists), the inter-domain contacts are weakened leading to an 'unzipped' or 'on' configuration. This leads to Ca^{2+} channel opening (Fig. 6-2, the right state of row a). According to this model, if a mutation should occur in critical sub-domain *x* of the central domain for example, the interaction of this sub-domain with its mating sub-domain located in the N-terminal domain would weaken or be lost, causing a partial 'unzipping', and resulting in a lowering of the energy barrier necessary for channel opening (Fig. 6-2, the middle state of row b). Such a partially 'unzipped' domain pair is readily turned to its fully opened configuration by weaker-than-normal stimuli, causing the hyper-activation/hyper-sensitization effects seen in channels containing disease-causing mutations in both cases of skeletal and cardiac muscles (Fig. 6-2, the right state of row b).

This model has been tested by examining the effects of a family of synthetic peptides corresponding to the putative critical domains of RyR (designated as domain peptides, DP) on several aspects of channel function. The underlying assumption in rationalizing the use of synthetic domain peptides as a functional probe is that they are capable of mimicking native conformations in the *in vitro* solution. The strategy of the domain peptide

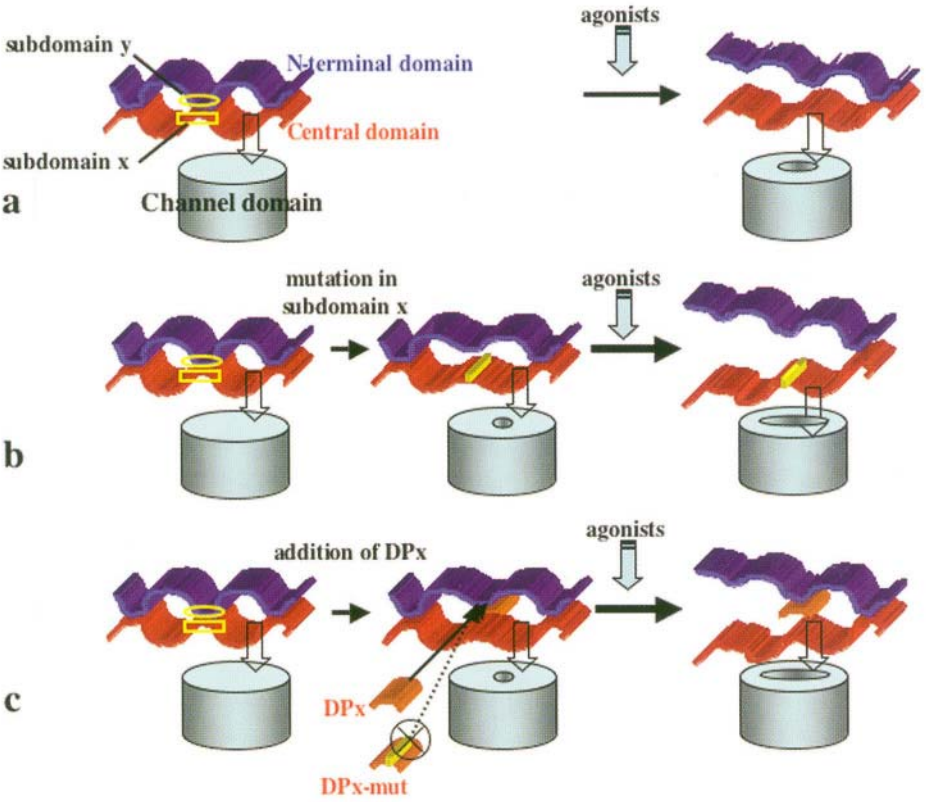

Figure 6-2. **Hypothetical model showing how the changes in the mode of interaction between the two key domains (N-terminal and central) control the state of the RyR Ca^{2+} channel. A.** Close contact between the N-terminal (blue) domain and the central domain (red, zipping) stabilizes the closed state of the Ca^{2+} channel. Upon activation of the RyR by adding the agonist, the close contact of the domain pair is removed (unzipping), then de-blocks the channel to open. **B**. Mutations in either of the N-terminal or the central domain (in this example, in sub-domain x of the central domain) weakens the interaction between sub-domain x and sub-domain y, causing a partial unzipping of the domain switch even before receiving the agonist signal. The activation by the agonist readily unzips domain switch even with lower than normal stimulus. This is manifested in the hyper-activation and hyper-sensitization effects seen in the channels of diseased muscle. **C**. Domain peptide (in this example, the peptide corresponding to sub-domain x of the central domain; namely domain peptide x or DPx) binds to its mating sub-domain: sub-domain y of the N-terminal domain. As a result of competition between DPx and sub-domain x for their binding to their mating sub-domain y, the interaction between sub-domains x and y (consequently, the interaction between the N-terminal and central domains) is weakened. This causes partial unzipping of the interacting domain pair and activation of the channel. Disease-causing mutation made in DPx (DPx-mut) abolishes its ability to bind to sub-domain y, resulting in the loss of the activating function of DPx. Thus, DPx-mut provides us with an excellent negative control.

approach is as shown in row c of Fig. 6-2. Addition of synthetic peptide DP*x*, corresponding to sub-domain *x,* to RyR results in the binding of the peptide to the N-terminal mating domain of sub-domain *x* (i.e sub-domain y), in competition with native sub-domain *x.* Resultant weakened native inter-domain interactions cause partial "unzipping" of RyR, thereby destabilizing the closed or 'off' conformation (Fig. 6-2, the middle state of row c).

An excellent negative control to test the physiological relevance of the observed activation effect of *DPx* is as follows. Since mutation in sub-domain *x* weakens the interaction between sub-domains *x* and *y as* mentioned above, the same mutation made in DP*x* (namely DP*x*-mut) will reduce the affinity of its binding to sub-domain *y,* causing a loss of the activating function that would have been present in the un-mutated peptide.

As an example of successful domain peptides, which worked exactly as predicted from the above hypothesis, Table 6-1 depicts the results obtained with DP4, which corresponds to the Leu^{2442}-Pro^{2477} region of the central domain of RyR1 (^{2442}LIQAGKGEALRIRAILRSLVPLDDLVGIISLPL-QIP^{2477}). DP4 enhanced ryanodine binding,[181] induced Ca^{2+} release from the SR,[181] induced contraction in skinned muscle fiber at an inhibitory Mg^{2+} concentration,[182] increased the sensitivity to caffeine,[181,182] increased the frequency of Ca^{2+} sparks in saponin-permeabilized fibers,[183] and increased the open probability of single channels.[183] DP4-mut, in which one mutation was made to mimic the Arg2458Cys or Arg2458His MH mutation, produced no appreciable effect on any of these parameters.

It has been shown that the central domain peptide DP4 binds to the N-terminal region of the RyR, as evidenced by the fact that the DP4-mediated site-directed probe labeling (see below) resulted in an exclusive fluorescence labeling of the ~150 kDa N-terminal segment of the RyR,[184] and according to more recent study in the 50 kDa segment starting from the N-terminus, that corresponds to the N-terminal domain.[185] This supports the view that the sub-domain of the central domain corresponding to DP4 interacts with the N-terminal domain. These data suggest that at least some of the synthetic domain peptides are capable of mimicking native conformations, and that experimental data obtained with them are physiologically relevant.

Several other domain peptides have also been used to test the domain switch hypothesis. For instance, DP1 corresponding to the Leu^{590}-Cys^{609} region of the N-terminal domain of RyR1 (^{590}LDKHGRNHKVLDVLCSLCVC^{609}) produced MH-like hyper-activation/hyper-sensitization effects on RyR1 channels.[186] Importantly, this peptide contains the binding site for dantrolene, the drug that is used to treat MH (see Chapter 24).[187] Moreover, DP1 was recognized by mAb anti-RyR1 raised to native rabbit RyR1, and this antibody inhibits dantrolene binding to

RvR1,[187] indicating that the drug-binding site is located within the Leu^{590}-Cys^{609} region of the N-terminal domain.

Table 6-1. **A central domain peptide DP4 produces MH-like hyper-activation effects on the RyR Ca^{2+} channel as seen in various systems**: from the level of the single channel to the level of the whole cell. Single mutation in the peptide abolishes its activating function. (+):increase, (-):no change.

System	Function	DP4	DP4-mut
Triad	Ryanodine binding	+	-
	SR Ca^{2+} release	+	-
	Apparent affinity to agonist	+	-
Skinned fiber	Force response to caffeine	+	-
	Force response to sub-max depolarization	+	n.d.
Permeabilized fiber	Frequency of Ca^{2+} sparks	+	-
Single channel	Po	+	-

N.d.: not determined.

As described above, many mutations related to the inheritable cardiac myopathies occur in the regions of the RyR2 corresponding to the N-terminal and central MH domains of the RyR1 (Fig. 6-1). This suggests that these domains and their inter-domain interactions also play an important role in cardiac Ca^{2+} channel regulation. To test this hypothesis, a cardiac RyR domain peptide DPc10 corresponding to the Gly^{2460}-Pro^{2495} region (a portion of the central domain of RyR2 (^{2460}GFCPDHKAAMVLFLDRV-YGIEVQDFLLHLLEVGFLP^{2495}) was used. This peptide was found to enhance the ryanodine binding activity and increased the sensitivity of the RyR2 to activating Ca^{2+}: the effects that resemble the typical phenotypes of cardiac diseases.[188] A single Arg-to-Ser mutation made in DPc10, mimicking the recently reported Arg2474Ser mutation in the patient with polymorphic ventricular tachycardia,[53] abolished all of these effects that would have been produced by non-mutated DPc10. Furthermore, both skeletal domain peptides DP4 and DP1 activated RyR2 as they activated RyR1, again supporting the concept that the cardiac Ca^{2+} channel is controlled by the basically identical mechanism as in the RyR1. However, the site of DPc10 binding has not yet been identified.

MONITORING THE OPERATION OF THE DOMAIN SWITCH

All of these findings described above are consistent with the hypothesis that zipped and unzipped states of the domain switch constituted by the two regulatory domains (viz. N-terminal and central domains) are directly involved in down-regulation and up-regulation of the RyR Ca^{2+} channel, respectively. The next important question is how one can monitor such actions of the domain switch. Fig. 6-3 illustrates two independent approaches that have been used favorably for this purpose. The important first step for both approaches is to label the conformation sensitive fluorescence probe, MCA, to the designated site of the RyR in a site-directed manner. Site-specific fluorescent labeling of the domain peptide binding site of RyR was performed using the cleavable heterobifunctional cross-linking reagent, sulfo-succinimidyl-3-((2-(7-azido-4-methylcoumarin-3-acetamido)-ethyl)dithio)propionate (SAED) in the following way. First, the selected domain peptide is incubated with SAED to form peptide-SAED conjugate. The peptide-SAED conjugate, after purification, is mixed with RyR and photolyzed to cross-link the conjugate *via* the azido group, followed by the treatment with reducing reagent to cleave the disulfide bond of SAED and the domain peptide used as a site-direction carrier is removed. As illustrated in row a of Fig. 6-3, the agonist-induced domain unzipping decreases the fluorescence intensity of the MCA probe attached to either the N-terminal domain or the central domain of the domain switch, because a more hydrophobic environment of the MCA attachment site, that has prevailed in the zipped configuration, becomes less hydrophobic upon domain unzipping. This method was suitable to follow a rapid process of domain unzipping. The other method involves the determination of the accessibility of the attached MCA probe to a bulky fluorescence quencher (QSY conjugated with BSA: QSY-BSA). As shown in row b of Fig. 6-3, the MCA that is attached to the designated sub-domain of the domain switch is relatively inaccessible to the fluorescence quencher QSY-BSA in the zipped configuration of the domain switch, because the QSY-BSA is excluded from the gap between the interacting domains. Upon unzipping of the interacting domains, the attached MCA becomes accessible to the QSY-BSA, causing an appreciable decrease in the fluorescence intensity of the attached MCA probe.

As described in above, DP4 binds to the N-terminal domain of RyR1. Hence, MCA is introduced to the N-terminal domain in a site-directed manner if DP4 is used as a carrier. Recent fluorescence quenching studies showed that all agents known to produce channel hyper-activation and hyper-sensitization, such as DP4 and DP1,[184] produced domain unzipping, as

evidenced by a significant increase in the accessibility of the N-terminal domain-attached MCA to the QSY-BSA quencher. These findings indicate that MH-like Ca^{2+} channel dysfunction (hyper-activation and hyper-sensitization) is produced by domain unzipping as predicted from the domain switch concept (Fig. 6-2).

According to the recent fluorescence quenching study by Yano *et al.* (see also Chapter 26), it appears that the activation and sensitization of RyR2 channels by cardiac domain peptide DPc10 are produced also by domain unzipping, although it has not yet been confirmed that the DPc10-mediated MCA labeling takes place in the putative domain switch of RyR2.[189]

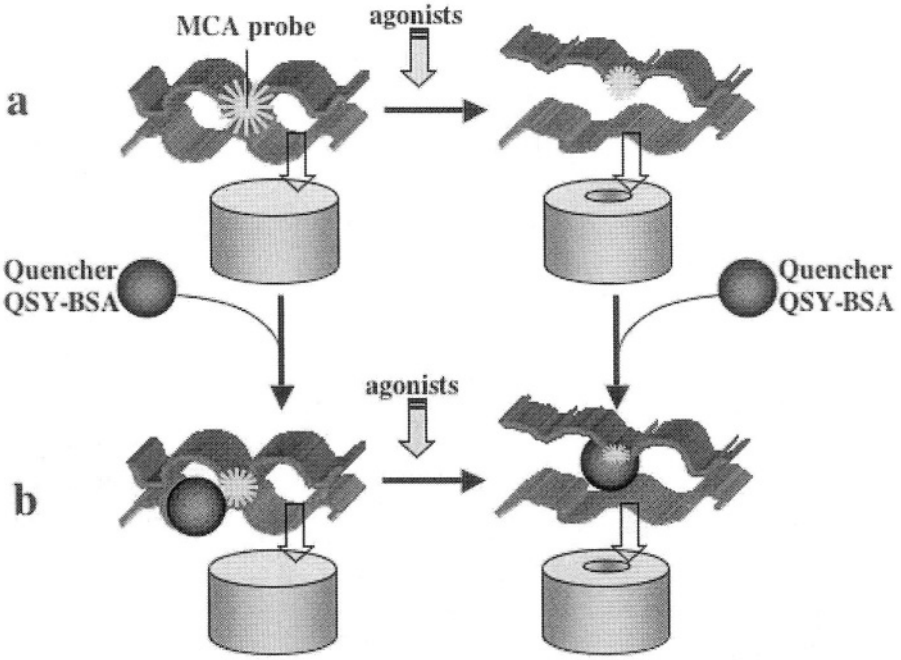

Figure 6-3. **Schematic illustration of the principle of the methods to monitor the process of domain unzipping. A**. In the zipped state, the fluorescence intensity of the attached MCA is high because of a more hydrophobic environment of the probe attachment site. Upon addition of agonists, the fluorescence intensity of the MCA decreases because the environment becomes less hydrophobic due to unzipping. **B**. A bulky fluorescence quencher, QSY-BSA, cannot enter the gap between the interacting domains in the zipped configuration, but can enter the widened inter-domain gap after the agonist has induced domain unzipping. Thus, in the zipped state, the quencher has only slight effect on the MCA fluorescence; after unzipping, the MCA fluorescence shows a considerable decrease because of the conferred access to the quencher.

Therapeutic drugs directed to the domain switch

Dantrolene is a hydantoin derivative that is widely used to treat malignant hyperthermia (MH) (see Chapter 24). One of dantrolene derivatives, azumolene, is also effective for the treatment of MH. However, virtually no other pharmacological reagents are known that are effective for the treatment of skeletal muscle disease. Since MH mutations in either N-terminal domain or central domain of RyR1 produce severe aberrations of channel function, one might expect that the pharmacological effect of dantrolene is directed to the domain switch and its operation. Most important in this context is the recent finding that dantrolene binds to the Leu^{590}-Cys^{609} region of RyR1, which is located in the C-terminal portion of the N-terminal domain (see Chapter 24).[187] Thus, the drug binds to a well-defined site located within the domain switch. According to the recent experiment in the author's laboratory (Kobayashi *et al.,* unpublished data), dantrolene decreased significantly the magnitude of agonist-induced domain unzipping, as determined by the fluorescence quenching technique described in above. This is particularly important because the increased tendency of domain unzipping causes MH-linked Ca^{2+} channelopathies. Thus, the evidence accumulated to this date suggests that the actual mechanism of drug action of dantrolene is to stabilize the zipped configuration of domain switch and prevent unwanted domain unzipping caused by mutations.

According to general consensus, dantrolene has no effect or much less effect (if any) on cardiac muscle and RyR2. Since RyR2 appears to have a potential drug binding region in the N-terminal domain as RyR1 does,[187] the site of drug binding may be occluded due to conformational constraint in the native RyR2, although it might become partially accessible in diseased conditions. Recently, Yano *et al.* have found that a new compound, the 1,4-benzothiazepine derivative JTV519, prevents heart failure by stabilizing RyR2 (see Chapter 26).[189] Although the binding site of JTV519 has not yet been identified, it is tempting to speculate that the pharmacological action of this drug may also be to stabilize the zipped state of domain switch of RyR2, as dantrolene does for RyR1.

DOMAIN-DOMAIN INTERACTION IN E-C COUPLING

Domains involved in the DHPR-to-RyR communication

Voltage-dependent activation of skeletal muscle-type E-C coupling is mediated by physical interaction between the DHPR and the RyR, presumably by mediation of the cytoplasmic loops of the DHPR $\alpha 1$

subunit[190] and β subunit.[191] Then, which portions of the RyR are involved in such a physical interaction in the case of the RyR1? Studies by several groups, yielding rather controversial results, have addressed this important question. There are many regions implicated in the coupling: e.g. residues 1635-2636,[192] a short 1076-1112 segment,[193] and the residues 1303-1406 D2 region.[194] Interestingly, according to the recent studies of immuno-localization of anti-D2 antibody in the 3D image, the site of antibody reaction is located in the so-called clamp region, which is regarded as the area for the interaction with the DHPR.[195] According to more recent information by Perez *et al.,* the residues 1-1,680 containing the D2 region is critical for RyR1-DHPR coupling.[196] Thus, the critical regions suggested in the literature are spread in a wide region of the primary structure encompassing residues 1-2636.

The fact that the DHPR-binding regions are distributed in widespread areas of the RyR polypeptide chain would indicate that the putative DHPR-interaction domain of RyR is constructed by a number of sub-domains derived from different regions of the RyR chain. Binding regions of some peptides corresponding to the DHPR II-III loop were localized within the RyR primary structure. Using the peptide-mediated site-directed probe-labeling technique, the conformation-sensitive fluorescence probe MCA was introduced into the binding sites of peptide A and peptide C (the peptides corresponding to the Thr^{671}-Leu^{690} and the Glu^{724}-Pro^{760} regions of the II-III loop, respectively) on the RyR. The A site and C site were localized at different sides of the major calpain cleavage site (residue #1400, which is located in the D2 region, which is regarded as the area for the interaction with the DHPR as described above).[197,198] Together with the accumulated information in the literature (see above), it is tentatively proposed that the putative DHPR-RyR signal transmission port of the RyR consists of several non-covalently but tightly associated domains flanking the D2 region.

Role of domain switch in E-C coupling

MH mutation causes hyper-activation and hyper-sensitization effects on depolarization-induced Ca^{2+} release.[199] This suggests that the domain switch unzipping mechanism is used also for the depolarization-induced activation of Ca^{2+} channels.[182] This idea was tested by monitoring the changes in the fluorescence intensity of the MCA probe attached to the N-terminal domain of the RyR moiety of the triad after depolarizing the T-tubule moiety.[184] It was found that T-tubule depolarization produces a rapid decrease of the MCA fluorescence at a rate significantly higher than the Ca^{2+} release rate. This suggests that the environment of the domain switch, to which the MCA probe is attached, has become less hydrophobic, indicative of domain unzipping produced by T-tubule depolarization (cf. row a of Fig. 6-3). Thus,

it appears that the domain switch is used for the activation of RyR1 Ca^{2+} channels in the skeletal muscle-type E-C coupling.

CONCLUDING REMARKS

Recent structure-function studies of the ryanodine receptor (RyR) have led us to the concept that inter-domain interaction within the RyR serves as a key mechanism in the process of channel gating. Of such regulatory domains of the RyR known so far, three domains (designated as N-terminal domain, central domain and transmembrane channel domain) are particularly important when we consider their role in channel regulation, because disease-linked mutations that have occurred in these domains cause severe problems in Ca^{2+} channel regulation (e.g. malignant hyperthermia and central core disease in skeletal muscle, and inheritable cardiac diseases). Evidence accumulated to this date suggests the hypothesis that the N-terminal and central domains constitute, at least partly, the interacting domain pair, which serves as the implicit on/off switch for the channel operation (domain switch). Namely, unzipping and zipping of such domain pair cause opening and closing of Ca^{2+} channels, respectively. Several domains located in widely spread regions of the RyR polypeptide chain have been identified as the putative sites for RyR's interaction with the DHPR, suggesting that these domains come together to constitute the putative DHPR-to-RyR signal transmission port. Recent studies with an *in vitro* E-C coupling model indicated that the domain switch mediates the voltage-dependent activation of RyR Ca^{2+} release channels. The RyR Ca^{2+} channel can be regulated by a variety of pharmacological and immunological agents and proteins. Most important physiological regulators among these are the two satellite proteins of RyR: calmodulin and FKBP. Their binding domains on the RyR have been characterized, but the important question whether the domain switch is also involved in the satellite protein-mediated channel regulation is left as an important subject for future studies.

As shown in the mutation map (Fig. 6-1), disease-causing RyR mutations are located in three regions (Regions 1, 2 and 3) in both skeletal and cardiac muscle systems. The concept of domain switch described in this chapter was born out from the consideration of the fact that MH mutations are located chiefly in Region 1 (N-terminal domain) and Region 2 (central domain), while CCD mutations are located chiefly in Region 3 (channel domain). Since the phenotypes of MH and CCD are different, it is reasonable to assume that Region 3 may be involved in a mechanism other than the domain switch mechanism. However, it is anticipated that there is an intimate interaction between the domain switch (Region 1 plus Region 2)

and Region 3. Furthermore, those mutations causing inheritable cardiac diseases are also located in the three regions of RyR2 corresponding to the three hot regions of RyR1. To elucidate the details of inter-domain interactions among these three regions in both RyR1 and RyR2 will be one of the most important tasks in the future study. Clear understanding of the channel regulation mechanism mediated by these key domains will immediately provide us a valuable clue for the understanding of the pathogenic mechanism of channel-linked skeletal and cardiac muscle diseases, because these domains are the very places where those problems are originated from. Some therapeutic drugs, such as dantrolene, are targeted to the domain switch, as described in this chapter. This finding has hinted us a new guideline for the development of therapeutic drugs for channel-linked skeletal and cardiac diseases; that is to screen a group of reagents that bind to the domain switch and stabilize the zipped configuration of the domain switch.

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