calcium release playing a critical role in cardiac excitation-contraction coupling. Recently, we presented evidence that the RyR2 N-terminus self-associates into a tetrameric form, which stabilises the closed conformation of the channel. In order to identify the interacting domains mediating RyR2 N-terminus tetramerisation, we used truncated constructs in yeast two-hybrid and chemical cross-linking assays. RyR2 residues 1-418 remained in monomeric form, whereas residues 1-530 retained self-association ability although it was much weaker compared to longer fragments. Tetramerisation was not abolished by removal of the N-terminal 160 amino acids but further truncations were detrimental. A targeted approach for putative inter-subunit contact sites was also employed testing the effect of small internal deletions within RyR2 residues 1-906. We found that two small deletions (167-178 or 335-358 amino acids) severely compromised tetramerisation ability. These findings suggest that residues 167-178 and 335-358 are the primary interacting sites, whereas additional downstream determinants further strengthen the RyR2 N-terminus self-association.

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FRET-Based Trilateration of a Domain Peptide Bound within Functional Ryanodine Receptors in Cardiomyocytes

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We have used trilateration, the method of using distances to determine a location in space, simulated annealing calculations, and confocal FRET measurements in permeabilized rat myocytes to determine the topology of a modulatory interface within the ryanodine receptor (RyR) Ca^{2+} channel. A small peptide (~4kDa, termed DPc10), corresponding to a RyR central stretch, hypothetically destabilizes a key interaction between RyR N-terminal and central domains to promote the open channel. We used DPc10 labeled with a small fluorophore, HiLyte Fluor 647, as FRET acceptor. Five single-cysteine variants of the 12 kDa FK506-binding protein (FKBP) were labeled with FRET donor, Alexa Fluor 488 C5 maleimide, and targeted to RyR. Effective average positions of the donors were calculated from simulated annealing, constrained by the RyR cryo-EM map and by the FKBP atomic structure. FRET from the FKBP donors to the DPc10 acceptor was measured via confocal microscopy, and the calculated distances were used to trilaterate the acceptor location within the RyR 3D map. The trilateration method uses the distances determined by FRET from each donor's calculated average position, where the ranges of the distances were based on measurement precision for each donor labeling site. The DPc10-bound acceptor locus is found at a region in space matching four distance ranges from FRET, and was further constrained by FRET between donor-labeled calmodulin and F-DPc10. This locus is near the RyR N-terminal domain structure docked into the cryo-EM reconstructed map of RyR. Computational resources were provided by the Minnesota Supercomputing Institute. This work was supported by NIH grants R01HL092097 (to D.M.B. and R.L.C.), and R01GM27906 (to D.D.T.).

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Evolution, Structure and Function of Ryanodine Receptor Domains

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Ryanodine receptors (RyRs) control the Ca^{2+}-release from sarcoplamic reticulum (SR) and play an important role in excitation-contraction (EC) coupling. Misregulation of the channel by mutations or hyperphosphorylation can cause severe disease such as cardiac arrhythmias, heart failure and malignant hyperthermia. RyRs are the largest ion channels found in humans and consist of dozens of functional domains that may act as separate modules. Interestingly, a few domains are present multiple times in the channel due to the gene duplication. One of them, the so-called ''RYR domain'' is present four times in two tandem repeats, one near the N-terminus and the other in the central region. Previously we solved the crystal structure of the central repeat, which contains several major phosphorylation sites in RyR. By analyzing the sequences and structures of the different RYR domains from RyR and other proteins, we were able to gain interesting insights about the evolution of the domain. Some special structural features of the domain may be involved in the unique function of the channel. Some other repeating domains in RyRs were also studied and compared to their analogues from different protein families. Potential functions of these domains are proposed based on the evolutionary and structural relationships.

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Molecular Modeling and Structural Docking of a Ryanodine Receptor SPRY2 Domain

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Ryanodine receptors (RyRs) form a class of intracellular calcium release channels in various excitable tissues and cells such as muscles and neurons. SPRY domains are recognized as a protein interacting module and were so named because they were identified in a *Dictyostelium discoidueum*splA kinase and in the mammalian RyR. There are 3 SPRY domains present in all the RyR isoforms. A particular interest has been raised for the SPRY2 domain, as it has been identified as the binding partner for the II-III loop from the $\alpha 1_S$ subunit of the dihydropyridine receptor, an interaction that is thought to be crucial for skeletal excitation-contraction coupling. In the present study, we have generated pseudo-atomic structures for RyR1 fragment 1,071-1,208 and homologous RyR2 fragment 1,084-1,221, both of which contain a SPRY2 domain. Both modeled SPRY2 fragments contain multiple β-sheets. Our initial rigid-body docking model in the sub-nanometer resolution cryo-EM map of RyR has placed the modeled fragment into a cytoplasmic domain adjacent to the N-terminal and central mutation region of RyR. Based on the modeled SPRY2 structure, we have designed a RyR2-GFP construct, with GFP inserted after residue Arg-1084, the N-terminus of the SPRY2 domain, to test our docking position by a 3D cryo-EM study. Our preliminary 2D analysis result supported the docking position.

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A Regulatory Component of the Human Ryanodine Receptor 2 N-Terminus

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Human cardiac ryanodine receptor (hRyR2) is a channel mediating Ca^{2+} release from the sarcoplasmic reticulum during excitation-contraction coupling. The N-terminal (1-655) and central (2100-2500) regions of hRyR2 are thought to be involved in regulating channel gating. Mutations linked to several heart diseases are clustered within these two, as well as in the channel pore-containing C-terminal regions. High resolution structures of key regions involved in the regulation of RyR2 activity could further the understanding of the gating mechanism of hRyR2 and of its malfunction in disease. Here we present the structure of the $hRyR2^{1-606}$ N-terminal region; it shares a significant structural similarity with other solved N-terminal RyR structures (pdb code: 2XOA, 4J4K) but lacks the chloride binding site proposed for mouse RyR2 (4J4K). Structural data suggested high flexibility of the region starting at around residue 544 predicted to consist of helices forming an armadillo motif - a protein-protein interaction domain. Our SAXS data showed high flexibility and partial unfolding of the C-terminal part of $hRyR2^{1-606}$ beyond aa 544, suggestive of incompleteness of the C-terminus. Biochemical data showed a weak tendency of $hRyR2^{1-606}$ to form dimers. Prolonging the fragment to the end of the predicted RIH domain increased the propensity to dimerize. A mutation introduced to a specific spot of $hRyR2^{1-606}$ resulted in significant thermal destabilization and augmented dimerization. Docking of the $hRyR2^{1-606}$ structure into cryo-EM maps of RyR ruled out interactions of its C-terminus with neighbouring $hRyR2^{1-606}$ monomers. We interpret the formation of dimers as a surrogate for interaction of the N-terminal region with an armadillo motif of another part of RyR2, and propose the existence of a regulatory component at the secondary structure level controlling interaction power at inter-monomer interfaces within the RyR2 tetramer.

Supported by grants VEGA-2/0131/10 and APVV-0628-10.