Possible contribution of SPRY2 and ASI regions of RyR1 to interdomain interaction

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Excitation-contraction (EC) coupling is the process that links an action potential to muscle contraction. A physical interaction between the surface membrane Ca^{2+} channel, the dihydropyridine receptor (DHPR), and the sarcoplasmic reticulum (SR) ryanodine receptor (RyR1) Ca^{2+} release channel facilitates skeletal muscle EC coupling. However, the mechanism and sites of interaction between the DHPR and RyR1 are not understood. RyR1 contains 3 SPRY domains in the N-terminal one third of each of its four subunits. Generally, SPRY domains function as protein-protein interaction domains and were identified in *Dictyostelium discoideum* tyrosine kinase spore lysis A (**Sp**lA) and the mammalian **Ry**R. The 2nd of the 3 domains (SPRY2, S^{1085}-V^{1208}) overlaps with the part of the RyR1 that support skeletal EC coupling and is a binding partner for the N-terminal residues in the II-III loop of the α_{1s} subunit of the DHPR as well as Imperatoxin A (IpTxA). As IpTxA and the II-III loop peptide increase the activity of RyR1 channels isolated from rabbit skeletal muscle, SPRY2 may contribute to an inhibitory regulatory module within the RyR1.

The SPRY2 domain may bind to a region within RyR1 which contains residues that exhibit close structural and functional similarity to the DHPR II-III loop N-terminal residues. This region (T^{3471}-G^{3500}) contains the alternatively spliced (ASI) residues (D^{3483}-G^{3487}), present in the adult RyR1 (ASI(+)RyR1) but excluded from the juvenile ASI(−)RyR, and a neighbouring basic sequence. ASI(−)RyR1 is upregulated in adults with myotonic dystrophy (Kimura *et al.* 2005). The ASI residues contribute to a regulatory module in the RyR1 and mutations of these residues or the adjacent basic residues affect skeletal EC coupling (Kimura *et al.* 2007; Kimura *et al.* 2009; Cheng *et al.* 2005). The positively charged residues in the ASI/basic region are aligned along one surface of an α-helix (Kimura *et al.* 2009). As a negatively charged F loop of the SPRY2 domain between residues P^{1170}-A^{1121} bind to the ASI/basic region (unpublished), these regions may interact to form the inhibitory module that is disrupted by IpTxA DHPR II-III loop N-terminal residue binding to the ASI/basic region. (Kimura *et al.* 2009). We predict that there may be different interactions between the F loop and ASI(−)RyR1 and that mutation of residues in the F-loop that bind to the ASI/basic region would prevent endogenous F-loop binding to the ASI region. To test this hypothesis, we have to first established that the characteristics of the interaction between the F-loop peptide and the recombinant RyR1.

Rabbit ASI(−)RyR1 was expressed in HEK293 cells. Cells were harvested 48h after transfection, homogenised, ASI(−)RyR1 partially purified and incorporated into an artificial lipid bilayer (phosphatidylethanolamine: phosphatidylserine: phosphatidylcholine; 5:3:2) with symmetrical 250mM cesium methanesulphonate solutions with 100nM cytoplasmic Ca^{2+} and 1mM luminal Ca^{2+}.

ASI(−)RyR1 expressed well and was visualised on Western Blots at the expected 500kDa. When incorporated into bilayers it had a single channel conductance of 364±20 pS. The relative open probability after adding 1μM F-loop peptide was 2.8±0.3 times greater than control, or after adding 100μM F-loop peptide fell to 0.56±0.07. These changes in P_o are similar to changes recorded in RyR1 from rabbit skeletal muscle. The action of the F loop peptide on a mutant ASI(−)RyR1 in which three residues in the F loop of the SPRY2 domain that bind to the isolated ASI region have been mutated will be examined next to test the prediction that the F loop peptide will either not affect the mutant RyR1, or will inhibit rather than activate the channel.

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