The RyR1 SPRY2 domain binds to the DHPR α1S II-III loop and to the RyR1 binding site for the DHPR β1a subunit

H-S. Tae, M.G. Casarotto, P.G. Board and A.F. Dulhunty, Division of Molecular Bioscience, The John Curtin School of Medical Research, Australian National University, ACT 2601, Australia.

Excitation-contraction coupling (ECC) links electrical signals in muscle fibers to the massive release of Ca^{2+} from the sarcoplasmic reticulum (SR) that initiates muscle contraction. A physical interaction between the dihydropyridine-sensitive L-type Ca^{2+} channel (DHPR) and the SR-Ca^{2+} release channel; ryanodine receptor 1 (RyR1) are prerequisites for skeletal muscle ECC. This protein-protein interaction depends on a cytoplasmic loop in the DHPR α1S subunit, between the second and third transmembrane domains (II-III loop), that allows the receptor to communicate allosterically with RyR1. Although it is generally accepted that the II-III loop is involved in the physical interaction between the DHPR and RyR1, the location of its binding site on RyR1 remains elusive. Skeletal ECC is also strongly dependent on residues in the variably spliced ASI region of RyR1 (Thr3471 – Gly3500), which bind to the β1a subunit of the DHPR. The ASI region (β1a binding region) has a sequence of positively charged residues that very similar in structure to those of α1S II-III loop. Leong & Maclennan (1998) co-immunoprecipitated 35S-labeled RyR1 fragments with the DHPR II-III loop and identified a stretch of 37-RyR1 amino acid residues that were involved in the interaction. Most of the identified residues are located within a region of RyR1 known as SPRY2 domain. Based on these observations, we proposed that the second of three RyR1-SPRY domains (SPRY2) is the interaction site for the II-III loop. SPRY domains were initially identified in a fungal protein (SplA) and in ryanodine receptor (RyR) (Rhodes *et al.*, 2005) and hence named SPRY. So far, the RyR1-SPRY domains have been classified as domains of an unknown function (Ponting *et al.*, 1997). Our aims were therefore (a) to characterise binding of the RyR1-SPRY2 domain to the II-III loop and identify key regions of the II-III loop that are involved in the interaction and (b) to determine whether the AS1 region also bound to SPRY2. In order to achieve these aims, we have used nuclear magnetic resonance (NMR) and spectrofluorimetry techniques with the SPR2 domain and the II-III loop, as well as mutations in both proteins to identify the key regions involved in the interactions. Experimental results demonstrated that SPRY2 domain binds at different binding affinity to two 'critical regions' (A and C) in the N-terminal and central regions (defined by El-Hayek *et al.*, 1995) of the II-III loop at non-analogous binding sites. The binding affinity for the A region is 8.3 ± 0.3 μM, while the affinity for the C region is 20.6 ± 0.4 μM. Mutational analysis showed that a negatively charged domain of SPRY2, including most of the 37 residues identified by Leong & Maclennan (1998), binds to the positively charged motif of the II-III loop-A region. We find that a peptide corresponding to the ASI region also interacts with the SPRY2 domain with an affinity of 1.4 ± 0.1 μM. We have provided compelling evidence that the RyR1-SPRY2 domain is a functional domain, which binds to the DHPR II-III loop and to the ASI domain of RyR1.

Leong P & Maclennan DH. (1998) *Journal of Biological Chemistry*, **273:** 7791-4.

Rhodes DA, de Bono B & Trowsdale J. (2005) *Immunology*, **116:** 411–7.

Ponting C, Schultz J & Bork P. (1997) *Trends in Biochemical Sciences*, **22:** 193–4.

El-Hayek & Ikemoto Wang J, Hamilton SL & Ikemoto N. (1995) *Journal of Biological Chemistry*, **270:** 22116-8.

Cheng W, Altafaj X, Ronjat M & Coronado R. (2005) *Proceedings of the National Academy of Science USA*, **102:** 19225-30.