Molecular recognition of the disordered dihydropyridine receptor II-III loop by a conserved domain in the type 1 ryanodine receptor

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Conformational coupling between the dihydropyridine receptor (DHPR) and the skeletal ryanodine receptor (RyR1) is essential for excitation-contraction (EC) coupling in skeletal muscle. The II-III loop of the DHPR α_{1s} subunit is central to this coupling process, but neither its structure nor its mode of binding to RyR1 are known. We have investigated the NMR-derived structure of the α_{1s} II-III loop and its binding to a region of the skeletal RyR1. We find that the II-III loop is highly flexible, with a strong N-terminal helix followed by several nascent helical/turn elements and unstructured segments, but it possesses no stable tertiary fold. The II-III loop thus belongs to a burgeoning class of functionally important, intrinsically unstructured, proteins. We have mapped the area of II-III loop interaction with RyR1 as a SPRY domain (1085-1208) and have identified which regions of II-III loop that are directly involved in binding by NMR methods. The principle site of interaction is through the N-terminal helix (A region, 671-690) but the B region (691-723) is also found to participate in binding. Confirmation that the A region of the II-III loop is indeed involved in SPRY2 binding is demonstrated through a series of mutations that clearly implicate a stretch of basic residues (R^{681}-K^{685}) as an important structural determinant. Evidence was found suggesting that there are weaker interactions between the C region of the loop and SPRY2. We propose that the flexible nature of the II-III loop is required for segments of the loop to associate and disassociate with RyR1 when the surface membrane potential changes, so that the loop may act as a conformational switch in EC coupling.