Functional interactions within the skeletal ryanodine receptor calcium release complex

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The ryanodine receptor Ca^{2+} release channel (RyR1), embedded in the internal sarcoplasmic reticulum (SR) Ca^{2+} store membrane in skeletal muscle, forms a stable multiprotein complex that stretches from the SR lumen to the quasi-extracellular environment in transverse (t-) tubule invaginations of the surface membrane. This complex senses environmental changes in 3 separate compartments of the muscle fibre. The extracellular space is sensed by the a_{1S} subunit of the dihydropyridine receptor (DHPR, CaV1.1). The cytoplasm is sensed by the cytoplasmic domains of the DHPR a_{1S} subunit and by the cytoplasmic domains of RyR1, triadin and junctin (embedded in the SR membrane), and by associated soluble cytoplasmic proteins including the DHPR b_{1a} subunit, the 12 kDa FK506 binding protein (FKBP12) and CLIC2 (type 2 Cl^- intracellular channel). The SR lumen is sensed by luminal domains of RyR1, triadin and junctin, and through calsequestrin (CSQ) - the Ca^{2+} binding protein in the SR linked to the RyR via triadin and junctin. Excitation-contraction (EC) coupling is initiated when t-tubule membrane depolarisation is sensed by the DHPR a_{1S} subunit. Environmental and membrane potential factors are relayed to the RyR pore to modify its resting activity and Ca^{2+} release during EC coupling.

Our aim is to understand how proteins in the complex respond to environmental changes to regulate RyR1 activity, Ca^{2+} release and contraction. We correlate Ca^{2+} release from SR vesicles and single channel activity in lipid bilayers with protein/protein binding to define specific molecular interactions that cannot be studied in the same detail in intact cells.

Our most recent findings provide further evidence for functional interactions between the DHPR b_{1a} subunit and RyR1, supporting a role of DHPR b_{1a} subunit in transmitting surface membrane depolarisation signals from the DHPR a_{1S} to the RyR1 during EC coupling (Rebbeck *et al.*, 2015) with additional evidence for a communication between b_{1a} and the EC coupling-critical region of the a_{1S} II-III loop. STAC3 is a newly identified protein that maintains the RyR1 protein complex and functional EC coupling (Polster *et al.*, 2015). STAC3 (1-10 μM) increases the single RyR1 channel activity 10±2-fold. This occurs with an increase in sub-conductance activity in single channels and is associated with dissociation of ~50% of the FKBP12 from the RyR1 channel complex. FKBP12 is a major factor in stabilizing RyR1 channel activity and in preventing myopathy-associated resting Ca^{2+} leak through the channel. The association with FKBP12 is central to RyR1 channel gating and is destabilized by many endogenous factors including CLIC2, and by CSQ dissociation.

It is intriguing that triadin and junctin, inserted in the SR membrane, have short cytoplasmic domains and longer luminal domains that independently alter channel activity (Wium *et al.*, 2012; Li *et al.*, 2015) in a manner that can depend on their binding to CSQ. Triadin binding to RyR1 in the lumen of the SR determines the efficacy of EC coupling (Goonasekera *et al.*, 2007; Wang *et al.*, 2009). Triadin residues K^{218}, K^{220} and K^{224} are required for RyR1 regulation, and this regulation site may be occluded by CSQ in resting muscle. Junctin on the other hand, conveys inhibitory signals from CSQ to RyR1 under resting conditions, helping to prevent the damaging resting Ca^{2+} 'leak' from the SR (Wei *et al.*, 2009). Cytoplasmic interactions between junctin and RyR1 are dominant in determining the overall effect of the protein on channel activity and we have identified the cytoplasmic and luminal domains of RyR1 that bind to the corresponding domains of junctin (Li *et al.*, 2015). These residues can now be located in the atomic resolution structure of RyR1 (Yan *et al.*, 2015) and intramolecular pathways identified that allow information flow from the binding sites on external surfaces of the protein to the RyR pore and gating machinery.

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