Structural investigation of components of the muscle excitation-contraction coupling machinery and their associated disease mutations

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Muscle excitation-contraction (EC) coupling requires communication between voltage-gated calcium channels (CaVs), located on the plasma membrane, and Ryanodine Receptors (RyRs), situated in the Sarcoplasmic reticulum. Whereas this process requires calcium signals in cardiac muscle, it relies on mechanical coupling in skeletal muscle. We investigate the role of additional proteins involved in the process in both tissues, including STAC proteins, Calmodulin (CaM), and kinases such as PKA. STACs and CaM can associate with CaVs and also directly affect their function. Whereas CaM can mediate calcium-dependent inactivation in L-type calcium channels, STAC proteins can abolish this process. Mutations in CaM have been associated with cardiac arrhythmia, whereas various mutations in STAC3 have been linked to Native American Myopathy. We analyzed the binding of both proteins to their target sequences in L-type calcium channels using X-ray crystallography and quantitative protein-protein interaction methods. Disease-associated mutations fall in different categories, either interfering with protein folding, directly affecting binding interfaces, or interfering with the ability to bind calcium. Finally, we analyzed the ability of PKA to bind and phosphorylate both Cavs and RyRs. We find that PKA uses extensive interfaces that reach far beyond the consensus sequence, using up to 33 residues in the case of RyRs. Phosphorylation induces changes in the structure, including the formation of additional secondary structure elements. These changes underlie the ability of kinases to promote channel opening.