C-6

Identification of binding motifs for skeletal ryanodine receptor and triadin

Jae Man Lee and Do Han Kim

Kwangju Institute of Science and Technology (K-JIST), Kwangju, Republic of Korea

In skeletal muscle cells, depolarization of the transverse tubules (T-tubules) results in Ca^{2+} release from the sarcoplasmic reticulum (SR), leading to elevated cytoplasmic Ca^{2+} and muscle contraction. This process has been known as excitation-contraction coupling (E-C coupling). Several proteins, such as the ryanodine receptor (RyR), triadin, junctin, and calsequestrin (CSQ), have been identified to be involved in the Ca^{2+} release process. However, the molecular interactions between the SR proteins have not been resolved. In the present study, the mechanisms of interaction between RyR1 and triadin have been studied by in vitro protein binding and $^{45}Ca^{2+}$ overlay assays. Our data demonstrate that the intraluminal loop II of RyR1 binds to triadin in Ca^{2+}-independent manner. Moreover, we could not find any Ca^{2+} binding sites in the loop II region. GST-pull down assay revealed that a KEKE motif of triadin, which was previously identified as a CSQ binding site (Kobayasi et al., 2000 JBC) was also a binding site for RyR1. Our results suggest that the intraluminal loop II of RyR could participate in the RyR-mediated Ca^{2+} release process by offering a direct binding site to luminal triadin.