

Dr.ssa Sara Roccabianca

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Tutor: prof.ssa Daniela Rossi

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Introduction. The structural integrity of the triadic junction is crucial for the excitation-contraction coupling (ECC). The formation and maintenance of the triad, formed by two terminal cisternae of the junctional sarcoplasmic reticulum (j-SR) and the invagination of the plasmatic membrane known as the transverse tubule (T-tubule), is due to the concerted interaction of structural proteins. In particular, junctophilin 1 (JPH1) forms a molecular bridge between the membranes of the T-tubule and the j-SR, allowing the mechanic interaction of DHPR and RyR1 receptors and thus the release of Ca^{2+} necessary for muscle contraction. Previous studies conducted in our laboratory identified cytoskeleton-linking membrane protein 63 (CLIMP-63), a well-known spacer of endoplasmic reticulum (ER) cisternae and microtubular cytoskeleton linker, as one of JPH1 and JPH2 interactors, leading us to hypothesize a crucial function for this protein in triad biogenesis and maintenance. During my first year of PhD, I focused my research on characterizing the interaction between CLIMP-63 and JPH1.

Methods. To characterize the domain of CLIMP-63 responsible for the interaction with JPH1, I performed co-immunoprecipitation (co-IP) studies using recombinant proteins. To identify the region of CLIMP-63 responsible for interaction with JPH1, plasmids coding for either full length mCherry-tagged CLIMP-63 or CLIMP-63 proteins deleted in their cytosolic or luminal domains were designed and synthesized by an external company. Proteins were expressed in HEK293T cells and co-IPs were performed by selectively precipitating recombinant proteins with magnetic beads conjugated with antibodies directed against mCherry. The obtained samples were analyzed by SDS-Page/Western Blot analysis.

Results. The results obtained from the co-IP studies confirmed that CLIMP63 interacts with JPH and that the region of CLIMP63 responsible for the interaction with JPH1 is contained within the cytosolic domain of the protein.

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Congressi:

19th IIM Meeting - Assisi (Italy), 20-23 Ottobre 2022

Pubblicazioni

Feliciano Protasi, Barbara Girolami, Sara Roccabianca and Daniela Rossi. 2022. StoreOperated Calcium Entry: from physiology to tubular aggregate myopathy. Current Opinion in Pharmacology. Under review.