Al collegio docenti del Dottorato in Medicina Molecolare

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Introduction

Muscle contraction is based on the Excitation Contraction Coupling (ECC) mechanism that occurs at specialized structures called triads. These are composed by the juxtaposition of two terminal cisternae of the sarcoplasmic reticulum with one T-tubule, an invagination of the sarcolemma. At triads many proteins interact to regulate the ECC process, including junctophillins (JPHs), which tether the sarcolemma and the sarcoplasmic reticulum cisternae. By using the Proximity-dependent labelling with BioID2 method, we identified the microtubule associated protein CLIMP63 as a novel interactor of JPHs. During the second year of my PhD, I performed experiments aimed at confirming and characterizing the interaction between JPHs and CLIMP63 in muscle cells.

Methods

Plasmids encoding JPH1/2 conjugated with GFP were transfected in mouse Flexor Digitorum Brevis (FDB) muscles or in HEK293T cells. Total protein lysates were used for Co-Immunoprecipitation (Co-IP) experiments using antibodies against GFP. Expression of CLIMP63 was assessed by Western Blot analysis and Immunofluorescence (IF) in skeletal muscles of mice at different ages.

Results

Co-IP analysis performed on FDB muscle or HEK293T expressing GFP tagged JPH1/2 showed that CLIMP63 can interact with JPHs in both experimental systems confirming previous results obtained by Proximity-dependent labelling with BioID2 method and suggesting that it may have a role in triad assembly and/or maintenance. Quantitative western blot analysis revealed that CLIMP63 is highly expressed in the embryonic and early post-natal life, while it is drastically reduced during adult and old age. CLIMP63 appears to localize at the I Band, with a minor staining colocalizing with JPH1/2 at triads.