Al collegio docenti del Dottorato in Medicina Molecolare

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Introduction

Activation of the excitation-contraction (e-c) coupling mechanism requires a multiprotein complex organized at typical membrane contact sites defined triads or diads. Assembly of triads and diads is mediated by members of the junctophilin (JPH) family, namely JPH1 and JPH2, which tether the sarcolemma and the sarcoplasmic reticulum cisternae. Different studies showed that JPHs are also required for recruitment and clustering of e-c coupling proteins, as well as for stabilization of membrane contact sites in physiological and pathological conditions, suggesting that they may be involved in interactions with additional cellular components. In order to investigate novel potential interactors of JPHs, we used the Proximity-dependent labelling with BioID2 method. This technique takes advantage of the ability of a biotin ligase enzyme to covalently label with biotin neighboring proteins in the range of 10 nm.

Methods

Plasmids coding for either 3xMYCBioID2JPH1 or 3xMYCBioID2JPH2 were validated by transfection into HEK293T using the Lipofectamine Plus Reagent. Cell growth medium was supplemented with 50 μM biotin and cells were lysed using a specific cell lysis buffer. Biotinilated proteins were purified using the BioId pulldown protocol (Roux et al. 2013) and analyzed by Western Blot

Results

Western Blot analysis revealed that the 3xmycBioID2JPH1/2 fusion proteins were able to tag various neighboring proteins, as showed by streptavidin analysis. Purification via the BioId pulldown method, identified one of the interacting proteins as the microtubule associated proteins CLIMP-63. Experiments to confirm interaction between JPHs and CLIMP63 in muscle cells are currently in progress.