Dott.ssa Caterina Amato

Attività scientifica svolta nel II° anno di Dottorato, a.a. 2017/1018

Introduction

In skeletal muscle, several proteins are organized at typical membrane contact sites between the plasma membrane and the sarcoplasmic reticulum, known as triads. Interactions between proteins at triads are essential to organize the macromolecular complex that mediates the mechanism of excitation-contraction coupling. Not every single contact is already known between muscle proteins; therefore it is necessary to gain insight into protein-protein interactions (PPIs). A novel method to investigate PPIs in living cells is the Proximity-dependent labelling with BioID2. This technique takes advantage of the ability of an enzyme that covalently label, with biotin, the interacting proteins. During the second year of my PhD I focused on this approach with the purpose of studying Junctophilin 1 and its protein interactors in muscle cells. JPH1 is a protein that stabilizes the interaction between plasmalemma/sarcolemma and ER/SR, working like a molecular bridge. The already known JPH1 interactors are RyR1, DHPR, Triadin, CASQ1, Cav3, STIM1 and Orai1.
The aim of my work is to set the experimental conditions to apply the Proximity-dependent biotin identification approach in order to find out and characterize direct and unknown interactions between proteins in the triadic junction, with particular attention to JPH1 interactors.

Methods

3xmycBioID2JPH1 plasmic vector was transfected in C2C12 cells with Lipofectamine PlusTM Reagent, the day after 3.2 μM of biotin were added and after 20 hours BioID pulldown (Roux et al., 2013) and Western Blot analysis were carried out.

Preliminary results

3xmycBioID2JPH1 transfection let to several biotinylated proteins identification in Hek293T cells, among which we identified CLIMP63. Since this cellular system was useful during the first year of my PhD, to set the system, we are currently working to verify the interaction between JPH1 and CLIMP63 in skeletal muscle cell line (C2C12) to extend our knowledge on the assembly and maintenance of triadic junctions.

Partecipazione a Seminari

- 11th – 14th October 2018 – Assisi (PG) - "Pathogenesis and Therapies of Neuromuscular disease" iiM – Myology Meeting

Oral presentation: "Binding of JPH1, a protein of the triadic membrane contact site of skeletal muscle cells, to CLIMP63, a microtubule-binding protein"

- 21th - 25th October 2018 – Lucca (LU) – "Endoplasmic reticulum function in health and disease" – EMBO Workshop

Poster: **Caterina Amato**, Maria Rosaria Catallo, Stefania Lorenzini, Daniela Rossi and Vincenzo Sorrentino "Binding of JPH1, a protein of the triadic membrane contact site of skeletal muscle cells, to CLIMP63, a microtubule-binding protein"

Il Tutor **La Dottoranda**

Prof.ssa Daniela Rossi **Dott.ssa Caterina Amato**