

Al collegio docenti del Dottorato in Medicina Molecolare

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Ciclo XXXVII Tutor: prof.ssa Daniela Rossi

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Introduction. Triads, formed by two terminal cisternae of the junctional sarcoplasmic reticulum (j-SR) and an invagination of the plasma membrane known as transverse tubule (T-tubule), represent the skeletal muscle-specific structure where excitation-contraction coupling occurs. These structures are formed and maintained by Junctophilins (JPHs), a family of membrane-tethering proteins, which participate in the formation of junctional membrane complexes in all excitable cell types. Previous studies performed in our laboratory identified the cytoskeleton-linking membrane protein 63 (CLIMP-63) as one of JPHs interactors. During the second year of my PhD, I extended the characterization of CLIMP-63/JPHs interaction and I started to set up the experimental protocol for CLIMP63 overexpression and silencing.

Methods. Co-immunoprecipitation experiments using recombinant proteins were performed in non-muscle cells and *Flexor Digitorum Brevis* (FDB) muscles to study the interaction between CLIMP63, JPHs and other triadic proteins.

To study the effect of overexpression and/or silencing of CLIMP63 in skeletal muscle we designed AAV9 vectors containing the murine sequence of CLIMP63 under the control of a CMV promoter and a siRNA sequence targeting CLIMP63 under the control of a U6 promoter, respectively.

Results. Co-immunoprecipitation experiments in non-muscle cells showed that both the cytosolic and luminal regions of CLIMP63 and JPHs are engaged in protein-protein interactions. A significant interaction was also observed between the transmembrane regions of the two proteins. To further characterize this point we will repeat co-immunoprecipitation experiments in a CLIMP63 knockout cell line, that will allow us to exclude the role played by the endogenous protein in this interaction. Expression of recombinant JPHs in FDB confirmed interaction with CLIMP63. Studies concerning the interaction of CLIMP63 with other triadic proteins, such as triadin, calsequestrin and junctin, are in progress.

Protocols of AAV9 infection of mouse skeletal muscles are currently under evaluation.



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DIPARTIMENTO DI MEDICINA MOLECOLARE
E DELLO SVILUPPO

Corsi:

- *Corso di formazione in materia di protezione degli animali utilizzati a fini scientifici – Prima Edizione* (Accreditato dal Ministero della Salute secondo D.M. 5 agosto 2021 e D.D. 18 Marzo 2022 Autorizzazione del 17/04/2023), dal 05/06/2023 al 05/07/2023

Soft skills per i dottorandi:

- *Comunicare in ricerca*, dr.ssa Elena Meli (21/06/2023)
- *How to exploit your research idea: a quick introduction to EU project design and management*, dr.ssa Donata Franzi (27/09/2023)

Lezioni per i dottorandi:

- *How to study genetics in complex diseases I*, prof. Stefano Landi – Pisa (Italy), 19/01/2023 dalle 14:30 alle 17:30
- *How to study genetics in complex diseases II*, prof. Stefano Landi – Pisa (Italy), 26/01/2023 dalle 14:30 alle 17:30
- *Microvesicles in cell-to-cell communication*, dr.ssa Laura Governini – Siena (Italy), 27/02/2023 dalle 9:00 alle 13:00
- *Biomarkers discovery in precision medicine*, dr.ssa Alice Luddi – Siena (Italy), 27/02/2023 dalle 14:00 alle 18:00
- *Through thick and thin... and titin: defining titin's role to muscle function*, dr. Anthony Hessel – Florence (Italy), 04/04/2023 dalle 9:30 alle 11:30
- *Advanced optical methods to monitor and control the cardiac electrical activity*, prof. Leonardo Sacconi – Florence (Italy), 04/04/2023 dalle 11:30 alle 13:30
- *Cellular mechanisms of heart failure*, prof. Pieter de Tombe – Florence (Italy), dalle 14:30 alle 16:30
- *Drug development, sex-dependent difference, pharmacovigilance*, prof. Elisabetta Cerbai e dr.ssa Elisabetta Bigagli – Florence (Italy), 04/04/2023 dalle 16:30 alle 18:30

Congressi:

- *50th European Muscle Conference (EMC)* – Florence (Italy), 2 Settembre 2023
- *20th IIM Meeting* – Assisi (Italy), 12 Ottobre 2023

Pubblicazioni

- Feliciano Protasi, Barbara Girolami, Sara Roccabianca and Daniela Rossi. 2022. Store-Operated Calcium Entry: from physiology to tubular aggregate myopathy. *Current Opinion in Pharmacology*.