UNIVERSITÀ
DI SIENA
1240

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Attività scientifica svolta nel 1° anno di Dottorato in Medicina Molecolare
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Introduction

Contraction in skeletal muscle is started by release of neurotransmitters by motor neurons at the neuromuscular plaques. This initial event induces the depolarization of the sarcolemma (the plasma membrane of the skeletal muscle cells) and of the T-tubules, which are invaginations of the sarcolemma. Depolarization of the sarcolemma/T-tubules is followed by the release of calcium from the sarcoplasmic reticulum (SR) through the activation of the mechanism of Excitation-Contraction coupling (E-C coupling). The site of E-C-coupling is a structure known as triad (or triadic junction) formed by one T-tubule and two terminal cisternae. In the E-C coupling mechanism, depolarization of the sarcolemma induces the activation of voltage gate dihydropyridine receptor (DHPRs) channels, localized on the T-tubule membrane, that trigger the opening of the ryanodine receptors (RYRs), the calcium release channels on the sarcoplasmic reticulum. The functional interaction between DHPRs and RYRs is therefore a key element of the E-C-coupling mechanism.

Junctophilins (JPHs) are a family of proteins that are encoded by four genes: JPH1, JPH2, JPH3 and JPH4. JPH1 and JPH2 are expressed in skeletal muscle, JPH2 is expressed in cardiac muscle, while JPH3 and JPH4 are expressed in neuron. JPH1 and JPH2 are important for maintaining the correct distance between the T-tubule and the terminal cisternae at triads in skeletal muscle.

JPHs contain three specific domains: 1) eight N-terminal MORN motifs (membrane occupation and recognition nexus); 2) an alpha helix domain followed by a divergent region; and a C-terminal transmembrane domain (TMD) that anchors the protein to the membrane of the sarcoplasmic reticulum (SR).

Little is known on the molecular mechanisms that localize JPHs to the triadic junction.

Material and Methods

Previous data from our laboratory showed that the TMD of JPH1 was able to localize at the terminal cisternae in mature muscle fibers and differentiated myocytes. To further estend this finding, we generated different GFP fusion proteins containing the TMD of JPH2, JPH3 and JPH4. These vectors were transfected in muscle fibers and their localization was evaluated by confocal fluorescence microscopy.

Results

The obtained results showed that JPH1 and JPH2 TMDs are able to localize at the triads, while the TMDs of JPH3 and JPH4 are not.

Further work will be performed to increase our understanding of the minimal sequences in TMD that allow the proteins to localize in the triadic junction.

Formative activities

"Corso di formazione sulla tutela della salute e sicurezza nei luoghi di lavoro. Lavoratori di area scientifica-alto rischio (16 ore) " Università di Siena , Siena 26/27 Febbraio 2015.

"Computational systems biology applied to pharmacology and nutrition" (Prof. Corrado Priami, Università di Trento) Siena, 2015.

"Stress, inflammation and reproduction" (IBSA Foundation for scientific research, Università di Siena) Siena, 2015.

<u>Molecular Medicine PhD seminars:</u>

"Regulation of chemokine biology by atypical chemokine receptors" (Prof. Silvano Sozzani, Università di Brescia) Siena, 2014.

"Regolazione del processamento degli mRNA: il ruolo della proteina SAM68 nello sviluppo e nelle patologie umane" (Prof. Claudio Sette, Università di Roma Tor Vergata) Siena, 2014.

"Novel pathway for the targeting and integration of transmembrane proteins" (Prof.ssa Nica Borgese, Università di Milano) Siena, 2015.

"MYCN and the MRN complex: how the replication stress response impacts on neural development and tumorigenesis" (Prof. Giuseppe Giannini, Università di Roma La Sapienza) Siena, 2015.

"Investigation of skeletal muscle Ca2+ homeoastasis in animals models " (Prof. Peter Szentesi, University of Debrecen, Hungary) Siena, 2015.