Deciphering microtubule network re-organization during muscle fiber formation using “Live-Super-Resolution Microscopy” in healthy and pathological conditions

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Research project:
The building block of skeletal muscle is the post-mitotic muscle fiber (myofibers). Myofiber is formed by the fusion of hundreds of specialized mononucleated cells (myoblasts/myocytes), which shape syncytial cells (myotubes). Myotubes are immature myofibers in which positioning of nuclei (i.e. myonuclei), referred as myonuclei localization and shape, is finely regulated (Roman & Gomes, 2017). During muscle development, myonuclei actively spread within myofibers. Myonuclei finally adopt a specific localization in the mature myofiber, regularly positioned at its periphery (Fig.1 B-C Healthy muscle – white arrows). Myonuclei are located between the plasma membrane of myofibers and myofibril structures (Sanger et al, 2010). This peripheral localization of myonuclei induces drastic changes in their shape, mainly due to forces applied on their nuclear envelope. This myonuclei organization is set by an interplay between the various cytoskeletons in which the microtubule network is key in the contribution of myofiber functional integrity. In accordance, we previously showed that myonuclear positioning within myofibers is required for proper muscle function (Metzger et al, 2012; Ghasemizadeh et al, 2021; Couturier & Gache, 2017; Cadot et al, 2012; Guiraud et al, 2020; Gache et al, 2017).

The proposed project aims to monitor during the precocious steps of myoblast fusion and maturation the reorganization/dynamics of (a) microtubule cytoskeleton, (b) actin cytoskeleton and (c) mitochondria. We will use different tools such as dyes (actin-Chromobody®, SiR-Actin® and SiR-tubulin® PKmito-Orange-FX®) and various constructs that will label some Microtubule associated proteins (MACF1/MAP7/EB3). This project benefit of the acquisition of a wide-field confocal microscope equipped with a motorized stage, an incubation chamber and Super-resolution module (Nikon-AX-NSPARC).

Models and techniques:
Culture of primary cells (rat/mouse)
transfection
Immunofluorescence
Real-time imaging (using confocal microscopy and Super-resolution)

How to apply?
Please send to the PI: 1) a motivation letter and 2) a CV listing education and any other skills of interest; 3) reference contacts.

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References:

Cadot B, Gache V, Vasyutina E, Falcone S, Birchmeier C & Gomes ER (2012) Nuclear movement during myotube formation is microtubule and dynein dependent and is regulated by Cdc42, Par6 and Par3. EMBO reports 13: 741–749


Roman W & Gomes ER (2017) Nuclear positioning in skeletal muscle. Seminars in Cell and Developmental Biology