MASTER 2 BMC
PARCOURS GENOPATH
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Deciphering microtubule network re-organization during muscle fiber formation using "Live-Super-Resolution Microscopy" in healthy and pathological conditions

Unit:
Pathophysiology and genetics of neuron and muscle (PGNM)
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Team:
Muscle Nuclear & Cytoskeleton Architecture (MNCA) (Team-website)
Team leader: Vincent Gache

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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

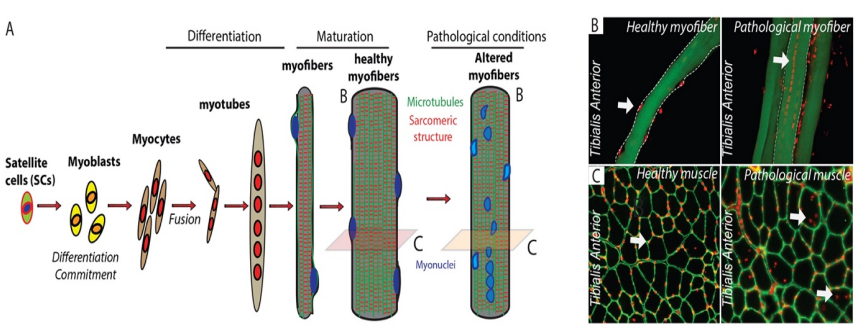

Figure 1. Formation of mature skeletal muscle fibers and myonuclei

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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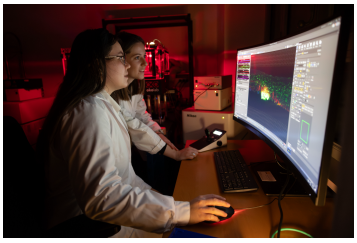
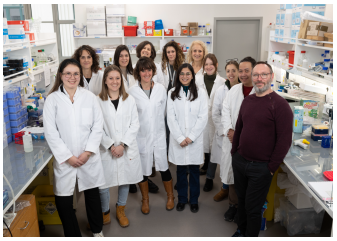

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