Internship offer
M2 Musculo-Skeletal system, Locomotion, Exercise (MuSkLE)

Title of the Internship: **Deciphering the role of the tubulin code in skeletal muscle regeneration**

Laboratory: NeuroMyoGene institute (INMG) - Pathophysiology and genetics of neuron and muscle (PGNM)
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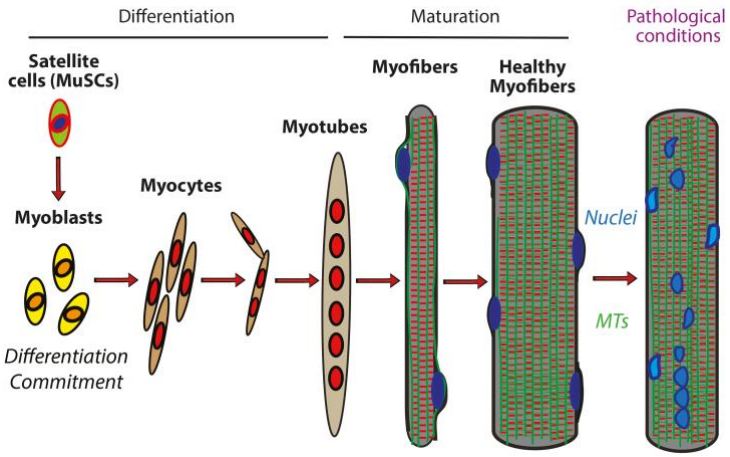
Research team: Muscle Nuclear & Cytoskeleton Architecture (MNCA) Team leader: Vincent Gache

Supervisor to contact: Dr. Vincent Gache (Vincent.gache@inserm.fr)

Research project:



Myonuclei positioning in myofibers: Muscle fibers, or myofibers, are syncytia composed of hundreds of post-mitotic myonuclei that share the same cytoplasm. The precise organization of myonuclei in fibers suggested the existence of MyoNuclear Domains (MNDs), where each myonucleus is responsible for gene expression in its surrounding cytoplasm and guarantees functional muscle integrity[1]. Myonuclear transcriptional flexibility (*i.e.* ability to regulate mRNA expression) is directly determined by the number of myonuclei in myofibers[2]. How each myonucleus reaches its optimal localization and shape along myofibers is a long-term process[3]. We have previously shown that an interplay between molecular motors, Microtubule-Associated Proteins (MAPs), and microtubule network integrity is involved in myonuclei localization along muscle formation and maturation[1,4,5]. Microtubules are built by the association of tubulin dimers and alternative tubulin isotypes and a variety of post-translational modifications control the properties and functions of the microtubule cytoskeleton, a concept known as the "tubulin code"[6]. **The consequences of post-translationnal modification of the tubulin skeletal muscle formation, their specific association with particular proteins and their alterations in myopathies is still pending.**

Preliminary data: We previously found that in the *Macf1* KO mouse muscle, internalization of myonuclei inside muscle fibers is correlated with alteration of the tubulin detyrosination/tyrosination cycle[5]. We then analyzed all the enzymes that control specific microtubule post-translational modification in the time course of myofiber formation. Our investigations now point to a drastic role of the specific post-translational modification of the tubulin (glutamylation/deglutamylation) in the formation and maintenance of muscle fibers. We now aim to decipher its specific involvement in the time course of muscle formation/regeneration.

Proposed project developed during the Master 2

We obtained a specific total knock-out mouse for one particular enzyme that control the "glutamylation/deglutamylation" post-translational modification of the tubulin. The M2 project will consist in (a) the deep analysis of the *in vivo* reformation of muscle after an injury and (b) the *in vitro* description/analysis of Muscle Stem cells (MuSC) behavior, after extraction from the tissue muscle of this knock-out mouse models. Briefly, *Tibialis anterior* of knock-out mouse models will be injured using cardiotoxin injection and muscle reformation will be monitored after 7, 14 and 21 days post injury. A

complete analysis will be realized to appreciate the regeneration in this mouse model as previously described[5]. Additionally, MuSC will be extracted using Facs-sorted/Macs-purification techniques and *in vitro* immunostaining's will be performed to monitor specific transcriptional factor expression during differentiation and maturation. This analysis will be completed with QPCR analysis and time-lapse video microscopy will be performed to access to organelles motility such as myonuclei.

This approach will allow to determine the role of the "glutamylation/deglutamylation" post-translational modification of the tubulin in the skeletal muscle formation

References (red is from the team):

1. Metzger, T. *et al.* MAP and Kinesin dependent nuclear positioning is required for skeletal muscle function. *Nature* **484**, 120–124 (2012).
2. Hansson, K.-A. *et al.* Myonuclear content regulates cell size with similar scaling properties in mice and humans. *Nat Commun* **11**, 6288 (2020).
3. Castellano, L., Caillol, D., Streichenberger, N. & Gache, V. Interplay between microtubule interactome, myonuclei mechanotransduction, and positioning in myopathies. *Nucleus* **16**, 2524909 (2025).
4. Gache, V., Gomes, E. R. & Cadot, B. Microtubule motors involved in nuclear movement during skeletal muscle differentiation. *Mol. Biol. Cell* **28**, 865–874 (2017).
5. Ghasemizadeh, A. *et al.* MACF1 controls skeletal muscle function through the microtubule-dependent localization of extra-synaptic myonuclei and mitochondria biogenesis. *eLife* **10**, e70490 (2021).
6. Janke, C. & Magiera, M. M. The tubulin code and its role in controlling microtubule properties and functions. *Nat. Rev. Mol. Cell Biol.* **21**, 307–326 (2020).

Skills required:

Culture of cells (primary culture cells will be a plus)

Immunofluorescence techniques (fixed cells and/or tissue)

Microscopy and image analysis

Environment

The Gache team (Gache team Website) is an international team, connected to numerous onsite facilities (molecular biology, imaging, cell biology, physiology). It is part of the Institut NeuroMyoGene – Physiopathology and Genetics of the Neuron and Muscle (INMG website), an institute dedicated to basic and translational research in the neuromuscular field that hosts 11 research teams, guaranteeing a high quality and dynamic scientific environment. As the second biggest town in France, Lyon hosts 60 laboratories in the area of Life and Health Sciences. Lyon is ideally located between the Alps and the Mediterranean Sea. Lyon is renowned for its quality of life including outdoor leisure closely the big city, and of course, its gastronomy and wines.