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

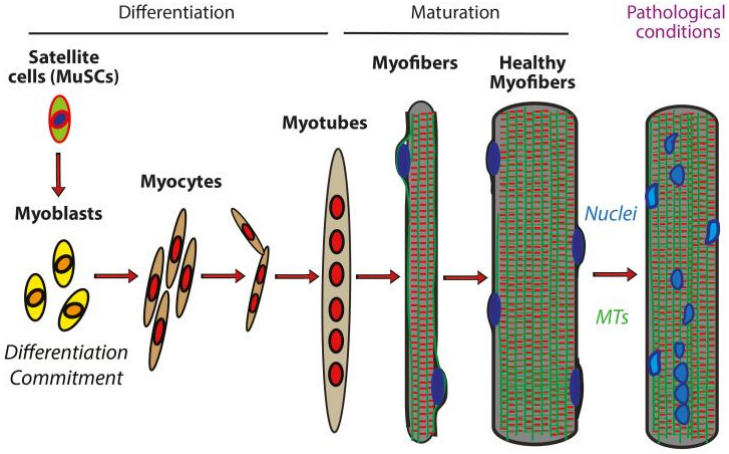
Title of the Internship: **Deciphering the role of tubulin isotypes in the formation & maintenance of skeletal muscle**

Laboratory: NeuroMyoGene institute (INMG) - Pathophysiology and genetics of neuron and muscle (PGNM)
CNRS/UCBL1/UMR5261-INSERM U1315. 8 avenue Rockefeller 69008 LYON

Research team: Muscle Nuclear & Cytoskeleton Architecture (MNCA) Team leader: Vincent Gache

Supervisor to contact: Dr. Muriel Sébastien (muriel.sebastien@inserm.fr) & Dr. Vincent Gache (Vincent.gache@inserm.fr)

Scientific context



Skeletal muscles compose roughly 40% of our body mass and are essential for locomotion, and respiration[1] . They are composed of thousands of multinucleated post-mitotic cells, that are the largest cells in our body. In addition, muscle cells can transform an electrical signal into a mechanical response, and resist the tremendous constraints involved by contraction[2]. These unique dimensions and properties depend on the functional organization of the contractile unit, which is strongly supported by the microtubule network[3]. Microtubules are tubular assemblies of αβ-tubulin heterodimers, that are needed all along a cell's life to support its morphology, motility and intracellular transport[4]. In muscle Stem Cells (MuSCs) the microtubule network is radiating from the centrosome, while in mature muscle cells (myofibers), microtubules form an orthogonal grid at the cell cortex, and a longitudinal mesh at the core. How such versatile functions and organizations arise from the same polymer is still poorly understood, but a growing body of evidence points at the "Tubulin code"[5]. This code is a perfectly balanced regulatory pathway involving a diversity of tubulin isotypes that compose the polymer, but feature various conformations[6], Post-Translational Modifications (PTMs), and capacities to associate with Microtubule-Associated Proteins (MAPs). Interestingly, tubulin isotypes are differently expressed in tissues and developmental stages[7] suggesting specific functions that remain to be explored in cellular contexts. **Taking advantage of *in vitro* and *in vivo* models readily available at the lab, this project aims at a better understanding of the specific roles of tubulin isotypes in organizing the microtubule network during striated muscle development and maturation.**

Proposal

Deciphering the role of a tubulin isotypes in striated muscle cells formation. The M2 candidate will develop new human induced Pluripotent Stem Cell (hiPSCs) lines to analyze the spatial repartition of a specific tubulin isotype and the consequences of its absence and/or mutation in striated muscle formation. Using CRISPR/Cas9 gene-editing, the candidate will generate (1) an endogenous tagging of the tubulin isotype of interest with a fluorophore (GFP), (2) a deletion of the isotype of interest, and (3) a patient mutation involved in a congenital myopathy. These hiPSCs lines will then be differentiated into skeletal or cardiac cells, allowing live-cell imaging and tracking of the target isotype during the differentiation process.

Implication of tubulin isotypes in MuSCs activation. MuSCs are required for skeletal muscle maintenance, growth, and repair. The candidate will extract MuSCs from an already available tubulin isotype knock-out mouse model, using FACs-sorted/MACs-purifications. *In vitro* culture, qPCR and immunostainings will be performed to monitor specific transcriptional factor expression during their differentiation and maturation. This analysis will be completed by live-cell imaging to access to microtubule network organization and usage for intracellular transport (i.e. Tracking of Nuclei, mitochondria, lysosomes…).

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Skills required:

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

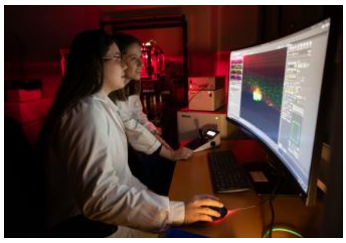
Immunofluorescence techniques (fixed cells)

Biomolecular methods (QPCR, CRISPR/Cas9)

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 – Pathophysiology and Genetics of Neuron and Muscle (INMG website), an institute that hosts 11 research teams, dedicated to basic and translational research in the neuromuscular field. In addition, Lyon hosts many laboratories focused on Life and Health Sciences, guaranteeing a high quality and dynamic scientific environment. As the second biggest city in France, Lyon is also ideally located between the Alps and the Mediterranean Sea, allowing a great quality of life in a large city close to the outdoors, with renowned gastronomy and wines.


Bibliography (In red are publications from team members)

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