Master Biologie Moléculaire et Cellulaire 'BMC',
Université de Paris - *UFR Sciences du Vivant*

Parcours : **Biologie et Développement Cellulaires 'BDC'**
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Fiche de Projet de Stage M2, Année 2021-2022

Unité INSERM ou CNRS ou Université : INSERM U1016. CNRS UMR 8104. U.Paris **Intitulé Equipe :** Développement neuromusculaire, Génétique et Physiopathologie **ED d'appartenance :BioSPC** **Responsable de l'Equipe :Pascal Maire**	**Responsable du Stage :Pascal Maire** **Contacts** Adresse : 24 rue du Fg St Jacques. 75014 Paris Email :pascal.maire@inserm.fr Tel :01 44 41 24 13

Titre du projet : Regulation of the super enhancer at the fast Myosin heavy chain locus

Résumé du Projet de Stage

The overall goal of this project is to define the regulatory mechanisms determining the myofiber type of adult skeletal muscle and more specifically the mechanisms controlling the expression of a single myosin heavy chain (*Myh*) gene in the hundreds of nuclei present in a given fiber. The major myofiber types are generally defined by the *Myh* genes that they express. However, we lack a comprehension of the regulatory mechanisms that determine which *Myh* gene will be expressed within a myofiber. The fast *Myh* (f*Myh*) locus in mammals is composed of *Myh2*, *Myh1*, *Myh4*, *Myh8* and *Myh13* genes arranged in 350Kb. Previous work from our group has identified by 4C experiments a 42kb cis-regulatory module (CRM) upstream of *Myh2* that can interact with the promoters of *Myh2* in the slow soleus and with the promoter of *Myh4* in the fast quadriceps. This CRM is composed of discrete DNA elements as revealed by single nucleus ATAC-seq (**snATAC-seq**) experiments performed with adult skeletal muscles and we have shown that this CRM acts as a **super-enhancer** (SE) controlling **fast *Myh* genes expression**. We demonstrated that **innervation is required to coordinate** f*Myh* gene expression in the hundreds of myonuclei present in an adult myofiber.
The research proposed will allow to fully characterize how the fast subtype myofiber gene expression program is established. In particular, we will test how the SE at the *Myh* locus is responsible for organizing alternate DNA conformations to ensure that only a single *Myh* gene is expressed in the muscle fiber. **SnRNA-seq** and snATAC-seq experiments will be performed with adult muscles of wt and PMA (peroneal muscular atrophy animals whose distal hindlimb muscles have never been innervated). These experiments will identify the **transcription factors and signaling pathways** linking fast motoneuron firing, SE activity and myonuclei coordination. Identified DNA regions relaying fast motoneuron subtypes will be validated by transient transfection experiments in adult muscles.

Publications de l'équipe relatives au projet de stage (max 5)

Dos Santos,M.,et al. 2020. Single-nucleus RNA-seq and FISH identify coordinated transcriptional activity in mammalian myofibers. *Nat Commun* 11, 5102. https://doi.org/10.1038/s41467-020-18789-8

Wurmser,M.,et al. 2020. SIX1 and SIX4 homeoproteins regulate PAX7+ progenitor cell properties during fetal epaxial myogenesis. Development. 147(19):dev185975. doi: 10.1242/dev.185975.

Randrianarison-Huetz, V. et al. 2018. Srf controls satellite cell fusion through the maintenance of actin architecture. J.Cell.Biol. 217(2):685-700.

Santolini, M.*,et al. 2016. MyoD reprogramming requires Six1 and Six4 homeoproteins: genome-wide cis-regulatory module analysis. Nucleic Acids Res. 44(18):8621-8640.

Sakakibara,I., et al 2014. Six homeoproteins and a linc-RNA cooperate at the fast MYH locus to lock terminal fast myofibre phenotype. PLoS Genet. 10 (5) : e1004386