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

Title of the Internship: Defining the quiescent ID of muscle stem cells

Laboratory (name, n°, website): Pathophysiology and Genetics of Neuron and Muscle, CNRS UMR 5261 - INSERM U1315, Université de Lyon - Université Claude Bernard Lyon 1
https://pgnm.inmg.fr/en/

Research team (name, website): *Muscle Nuclear & Cytoskeleton Architecture* – MNCA team
https://pgnm.inmg.fr/en/gache/

Supervisor to contact (name, email address): Caroline E. BRUN, caroline.brun@inserm.fr

Project description including a short introduction, aim/objectives and methods/approach to be used

Adult muscle stem cells (MuSCs) reside in quiescence, a reversible G0 cell-cycle arrest essential for long-term regenerative capacity, but can rapidly re-enter the cell cycle upon injury to generate myogenic progenitors and self-renew [1]. Long viewed as a uniform dormant state, quiescence now encompasses distinct functional phases, such as a GAlert state in which G0 stem cells reversibly transition in response to systemic signals released from a distant injury [2,3]. Recent efforts focus on elucidating the molecular, epigenetic, and functional signatures of these quiescence depths to enable targeted modulation in regenerative contexts [4-6]. However, a major challenge is obtaining truly quiescent MuSCs, as any disruption of their microenvironment inevitably modifies their molecular signature. To overcome this technical issue, we developed a mouse model, the MuSC-CIBOP mouse, that allows capturing proteome snapshots of MuSC in their physiological microenvironment, unlike current strategies requiring mechanical or enzymatic dissociations, which inevitably alter MuSC profiles. This model relies on the tamoxifen (TMX)-inducible expression of the engineered biotin ligase TurboID, specifically in the MuSC population [7,8]. Upon biotin supplementation, the TurboID biotinylates MuSC proteins that can be further purified and identified by mass spectrometry. Using MuSC-CIBOP mice, we aim to determine the molecular signature of quiescent MuSCs in homeostasis, through genetic loss-of-function approaches or in different pathological contexts.

The student will confirm that the MuSC-CIBOP mice are reliable for identifying quiescent muscle stem cells in all skeletal muscles by i) immunofluorescence using Fluorophore-coupled Streptavidin and ii) western blotting. She/He will be in charge of setting up the protocols for the streptavidin-based affinity purification of biotinylated proteins from different muscles of MuSC-CIBOP mice. Following identification of the purified proteins by mass spectrometry, she/he will perform *in silico* analysis to define the molecular signature of quiescent muscle stem cells.

References:

1. **Brun, C.E.**, Chevalier, F.P., Dumont, N.A., and Rudnicki, M.A. (2017). *Chapter 10 - The Satellite Cell Niche in Skeletal Muscle*. In Biology and Engineering of Stem Cell Niches, A. Vishwakarma, and J.M. Karp, eds. (Academic Press), pp. 145-166. https://doi.org/10.1016/B978-0-12-802734-9.00010-X
2. **Brun, C.E.**, Sincennes, M.C., Lin, A.Y.T., Hall, D., Jarassier, W., Feige, P., Le Grand, F., and Rudnicki, M.A. (2022). *GLI3 regulates muscle stem cell entry into G(Alert) and self-renewal*. Nature communications 13, 3961. 10.1038/s41467-022-31695-5.
3. Rodgers, J.T., King, K.Y., Brett, J.O., Cromie, M.J., Charville, G.W., Maguire, K.K., Brunson, C., Mastey, N., Liu, L., Tsai, C.R., et al. (2014). *mTORC1 controls the adaptive transition of quiescent stem cells from G0 to G(Alert)*. Nature 510, 393-396. 10.1038/nature13255.
4. Krauss, R.S., and Kann, A.P. (2023). *Muscle stem cells get a new look: Dynamic cellular projections as sensors of the stem cell niche*. BioEssays: news and reviews in molecular, cellular and developmental biology 45, e2200249. 10.1002/bies.202200249.

5. Urban, N., and Cheung, T.H. (2021). *Stem cell quiescence: the challenging path to activation*. Development 148. 10.1242/dev.165084.
6. Esper, M.E., **Brun, C.E.**, Lin, A.Y.T., Feige, P., Catenacci, M.J., Sincennes, M.C., Ritso, M., and Rudnicki, M.A. (2025). *Intrinsic Muscle Stem Cell Dysfunction Contributes to Impaired Regeneration in the mdx Mouse*. Journal of cachexia, sarcopenia and muscle 16, e13682. 10.1002/jcsm.13682.
7. Murphy, M.M., Lawson, J.A., Mathew, S.J., Hutcheson, D.A., and Kardon, G. (2011). *Satellite cells, connective tissue fibroblasts and their interactions are crucial for muscle regeneration*. Development 138, 3625-3637. 10.1242/dev.064162.
8. Rayaprolu, S., Bitarafan, S., Santiago, J.V., Betarbet, R., Sunna, S., Cheng, L., Xiao, H., Nelson, R.S., Kumar, P., Bagchi, P., et al. (2022). *Cell type-specific biotin labeling in vivo resolves regional neuronal and astrocyte proteomic differences in mouse brain*. Nature communications 13, 2927. 10.1038/s41467-022-30623-x.

Skills required: The internship requires hands-on experience with core lab technics, including histology (e.g., tissue sectioning, immunostaining), molecular assays (PCR, qPCR for gene expression, Western blot for protein analysis), and proficiency in standard software (Word, Excel). Knowledge of mouse genetics is an asset. Finally, the candidate should demonstrate precision and strong organizational skills, and must be able to work effectively in a collaborative team environment.