MASTER 2 BMC
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Establishing cell culture-based experimental setup to track ciliary and extraciliary PKA activity

Unit: NeuroMyoGene Institute (INMG) – Pathophysiology and genetics of neuron and muscle (PGNM), CNRS/UCBL1 UMR 5261 - INSERM U1315, 8 avenue Rockefeller, 69008 Lyon
Unit director: Laurent Schaeffer

Host team: MNCA Team (Muscle Nuclear & Cytoskeleton Architecture)
Team leader: Vincent Gache, PhD, CRCN Inserm
Internship supervisor: Caroline Brun, PhD, CRCN Inserm
caroline.brun@univ-lyon1.fr

Project: Adult skeletal muscle has the outstanding ability to regenerate upon an injury (Brun *et al.*, 2017). Regeneration begins with activation of normally dormant quiescent muscle stem cells (MuSCs) and proceeds with formation of proliferating MuSCs that either differentiate to repair injured myofibers or self-renew and return to quiescence to replenish the stem cell pool.

The primary cilium is a small microtubule-based structure specialized for organizing cellular signaling events (Anvarian *et al.*, 2019). Quiescent MuSCs maintain a primary cilium that is dynamically regulated during activation, almost invariably resorbed during mitosis, reassembled post-cytokinesis and maintained in self-renewing cells (Atmakuru *et al.*, 2023). We and others have recently shown that primary cilia actively control Hedgehog signaling in MuSCs (Brun *et al.*, 2022). Within these novel findings, our work stands out as it shows that cAMP-dependent protein kinase (PKA) in the cilium, but not elsewhere, prevents GLI-dependent transcription and Hedgehog-dependent MuSC activation. PKA has other downstream signaling targets among which the kinases LATS1/2, LKB1 and AMPK, all regulating MuSC quiescence and self-renewal differently. Therefore, we hypothesize that two ciliary and extraciliary pools of PKA regulate downstream signaling effectors according to their subcellular localization in quiescent/self-renewing and activated/proliferating MuSCs.

This project aims to monitor PKA activity in primary cilia of muscle stem cells, as the MuSCs progress through the myogenic lineage. To do so, we will take advantage of the excitation ratiometric A Kinase activity reporter 2 (ExRai-AKAR2, Zhang *et al.*, 2022) that we recently targeted to the primary cilium (referred to as Cilia-AKAR). In brief, muscle cell lines and primary muscle cells will be either transfected or infected with the Cilia-AKAR construct and PKA activity will be measured 24h and 48h post-transfection/infection at the single cell level. Cells will be imaged by time-lapse videomicroscopy to measure the PKA steady-state activity in resting condition and upon several treatments with agonists (Forskolin, IBMX) and inhibitors (H98, PKI[5-24]) to track real-time PKA response.

Methods: Molecular biology (PCR, cloning, sequencing), Cell biology (muscle cell line and primary cell culture, transfection, infection), Microscopy (Immunofluorescence, live single-cell imaging)

References:

- Atmakuru PS, Dhawan J. *The cilium-centrosome axis in coupling cell cycle exit and cell fate*. J Cell Sci, 2023, 136(9):jcs260454.
- Zhang JF, Mehta S and Zhang J. *Protocol for reading and imaging live-cell PKA activity using ExRai-AKAR2*. STAR Protoc, 2022, 3(1):101071.
- Brun CE, Sincennes MC, Lin AYT, Hall D, Jarassier W, Feige P, Le Grand F and Rudnicki MA. *GLI3 regulates muscle stem cell entry into GAlert and self-renewal*. Nat Commun, 2022, 13(1):3961.
- Anvarian Z, Mykytyn K, Mukhopadhyay S, Pedersen LB, Christensen ST. *Cellular signalling by primary cilia in development, organ function and disease*. Nat Rev Nephrol, 2019, 15(4):199-219.
- Brun CE, Chevalier FP, Dumont NA and Rudnicki MA. *Skeletal Muscle: the Satellite Cell Niche*. Biology and Engineering of Stem Cell Niches, 2017, pp. 145-166.3.