Master de Sciences et Technologies
Mention Biologie Intégrative et Physiologie
Parcours : Neurosciences
Responsable : Professeur Régis Lambert

Internship Proposal
Academic Year 2019-2020

1. Host team :

Research Unit (e.g. Department or Institute) : ICM UPMC UMRS 1127 INSERM 7225 CNRS
Research Unit Director : Pr Alexis Brice
Research Team Director : Pr Bruno Stankoff and Pr Catherine Lubetzki
Team name : Mechanims of myelination and remyelination in the CNS

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Supervisor of the Research Intern for this project : Dr Anne Desmazieres
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2. Internship project title:

Live-imaging study of nodal protein targeting and of oligodendrocyte-nodal structure interaction

3. Internship Description :

In myelinated nerve fibers, fast saltatory conduction relies on the alternance of myelinated axonal segments, the internodes, and of small unmyelinated domains enriched in voltage-gated sodium channels (Na_v), the nodes of Ranvier. Nodes of Ranvier allow the regeneration and fast propagation of action potentials along the axon. In demyelinating diseases such as Multiple sclerosis (MS), nodes of Ranvier are deeply altered in demyelinated area and can also present abnormalities in normal appearing white matter (NAWM) surrounding the lesions, which participates in the functional deficits. During remyelination, the nodes reassemble, allowing the restoration of electrical conduction along the axon. Previous works from Catherine Lubetzki team show that the reclustering of the nodes of Ranvier is an early event of the remyelination process in MS (Coman et al, 2006) and could thus influence repair. Further studies *in vitro* confirmed the clustering of nodal structures along axons prior to myelination in specific neuronal subpopulations, as well as a potential functional impact of these structures on axonal conduction velocities and on myelin initiation guidance (Kaplan et al, 1997 and 2011; Freeman, Desmazieres et al, 2015; Thetiot et al, in review). Better understanding how these nodal structures are formed and how they can participate in myelination process guidance is of major interest as it could allow to better understand (re)myelination regulation.

The present project aims at addressing by live-imaging approaches how nodal proteins are targeted at early nodal structures and mature nodes of Ranvier, as well as how myelinating glial cells, the oligodendrocytes, interact with the early nodal structures at myelination onset.

We will take advantage of the *in vitro* live-imaging techniques of mixed hippocampal cultures and unique molecular tools we recently developed (Freeman, Desmazieres et al, 2015; Thetiot et al, in review and unpublished data) in order to address these questions.

Regarding the targeting of nodal proteins, we generated and validated constructs allowing to follow the membrane targeting of nodal markers by coupling them to a GFP variant sensitive to pH, which only fluoresces when inserted at the axonal membrane and thus will allow to localize membrane delivery events. We will transfect mixed hippocampal cultures to induce the expression of these markers in neurons (using the pan-neuronal Synapsin promoter). We will then follow by videomicroscopy how these proteins are targeted at the membrane, and in particular whether they are directly inserted at the nodal structures or are randomly inserted and cluster later on. By coupling tagged nodal markers expression and live nodal staining prior and during myelination, we will compare the characteristics of the targeting in early and mature nodal structures.

The second axis of this study is to better characterize the physical interaction that we recently observed between premyelinating oligodendrocytes (OLs) and early nodal clusters in mixed hippocampal cultures at myelination onset (Thetiot et al, in review). Briefly, we repeatedly observed in our live-imaging studies that some OLs processes (visualized by GFP expression) contact early nodal clusters, prior to relocalizing at their direct vicinity, where they initiate myelination. To gain insight in the mechanism underlying this interaction and how it impacts myelination, we will first complement our previous study by further spinning-disk confocal live-imaging studies and immunocytological stainings. We will in particular address the timing of enrichment, at the interacting site, of axonal markers implicated in the axo-glial junction (Caspr-mCherry, expressed under the control of the Synapsin promoter). We will further address the exact stage of differentiation of the contacting glial cells, and whether the glial processes contacting nodal clusters are enriched in “post-synaptic” receptors, as described in the literature for oligodendrocytes progenitors interacting with electrically active axons. In case we characterize some of these receptors at the site of OLs-nodal clusters interaction, treating the cultures with some of their specific agonists or antagonists would allow us to see whether we can perturb this OLs-node interaction, and the consequence of this alteration. In the longer-term, this study will be transferred to ex vivo de/remyelinating models.

During his/her internship, the student will participate in mixed hippocampal cultures generation and maintenance, as well as transfection and live-imaging by videomicroscopy and spinning disk confocal imaging. He/she will further perform immunocytochemistry on fixed cells and associated characterization and quantifications of glial-node contacts.