PhD offer : Physiopathological roles of a poorly understood myelin lipid

Dynamics of lipid membranes and protein coats Lab
(https://www.ipmc.cnrs.fr/cgi-bin/site.cgi)

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Myelin, which plays a crucial role in nerve conduction, is essential for neuronal function. It is composed of several layers of membrane tightly stacked around the axons. Among the characteristic lipids that make up myelin, we have identified a polyunsaturated plasmalogen with unknown functions whose levels decrease markedly in patients with Alzheimer's disease (AD) (1). Unlike DHA and other polyunsaturated lipids (PUFAs) studied in AD, this plasmalogen has never been studied despite its abundance in myelin and the discovery of its decrease in AD patients already thirty years ago (2). PUFAs esterified in membrane lipids affect the way proteins with amphipathic helices, e.g. synuclein, interact with membranes (3). The main protein that shapes myelin, MBP (myelin basic protein), contains amphipathic helices that fold upon membrane binding (4). Thus, the identified plasmalogen could regulate myelin stacking and structure by regulating MBP-membrane interaction. Myelin deficiencies are increasingly considered as early events in AD (5,6). The involvement of this plasmalogen in myelin and the enzymes responsible for its synthesis in AD may pave the way for new therapies.
The proposed PhD project focuses on the specific lipid metabolism that ensures the synthesis of this specific plasmalogen in oligodendrocytes and involves a lysophospholipid acyltransferase named AGPAT4. AGPAT4 KO mice show a memory deficit (7). In preliminary experiments using lipidomics, RNAseq and quantitative immunostaining on human frontal white matter sections, we observed in AD patients that the decrease of this plasmalogen correlates with the decrease of AGPAT4 and MBP suggesting that this plasmalogen might be involved in the interaction of MBP with the plasma membrane.
The objective of the project is to demonstrate that AGPAT4 promotes the synthesis of the identified plasmalogen and that its decrease may impair the proper compaction of myelin and the excitability of neurons, thus increasing the vulnerability of the brain to Alzheimer's disease.

To test the hypothesis that AGPAT4 in oligodendrocytes regulates this specific plasmalogen synthesis and that its depletion leads to a defect in myelin organization, two axes will be considered:

- **A fundamental axis in biochemistry and cell biology to investigate the interaction of MBP with the identified plasmalogen:**

1/ study of the interaction of MBP through binding experiments of the purified protein with artificial liposomes whose plasmalogen composition can be perfectly controlled.
2/ study of the interaction of MBP-GFP with the plasma membrane in a cell model deficient in endogenous AGPAT4 whose membrane lipid profile will be modulated by increasing the levels of the candidate plasmalogen by overexpression of AGPAT4 associated with lipid diets.
3/ study of the interaction of MBP in human iPS cells differentiated into oligodendrocytes in which AGPAT4 will be invalidated or not by genome editing (Crispr/Cas9).*

- **A morphological axis using spectral microscopy and electron microscopy to quantify the alteration of the myelin sheath on brain sections in mice and humans.**

1/ Comparative study of the morphological characteristics of myelin on brain sections from AGPAT4 KO mice vs. control mice (Hideo Shindou collaboration, Japan). A similar study will be carried out on APP/PS1 AD-mouse model which show a significant loss of myelin (8) to be linked to altered levels of AGPAT4 and MBP. A functional readout of myelin integrity by electrophysiology in

both mouse models is envisageable in a collaborative framework with a laboratory having this expertise.
2/ In humans, a comparative study of AD patients vs. controls on brain sections (male and female subjects) in the white matter region bordering the hippocampus will be conducted to explore the levels of AGPAT4 and MBP in correlation with altered myelin morphology in AD patients.

1. Horrocks et al. 1990 In Phospholipids. pp.51-58. DOI: 10.1007/978-1-4757-1364-0_4
2. Skinner et al. 1993 Brain 116, 717-725. DOI: 10.1093/brain/116.3.717
3. Pinot et al. 2014 Science 345, 693-697. DOI: 10.1126/science.1255288
4. Raasakka et al. 2017 Sci Rep 7, 4974. DOI: 10.1038/s41598-017-05364-3
5. Papuć et al. 2020 A Med Sci 16, 345-341. DOI: 10.5114/aoms.2018.76863
6. Mathys et al. 2019 Nature 570,332-337. DOI: 10.1038/s41586-019-1195-2
7. Bradley RM et al. (2017) Mol Cell Biol 37, e00245-00217. DOI.org/10.1128/MCB.00245-17.
8. Chen et al., 2021, Neuron 109, 2292–2307. July 21, 2021 DOI.org/10.1016/j.neuron.2021.05.012

All the articles below demonstrate how polyunsaturated lipids are important for membrane dynamics in cells:

- Pinot M, S. Vanni, S. Pagnotta, S. Lacas-Gervais, S. Payet, T. Ferreira, R. Gautier, B. Goud, B. Antonny, H. Barelli. 2014 Lipid Cell Biology: Polyunsaturated phospholipids facilitate membrane deformation and fission by endocytic proteins. **Science**. 345, 693-697. DOI: 10.1126/science.1255288.
- Barelli H, Antonny B. 2016 Lipid unsaturation and organelle dynamics. **Curr Opin Cell Biol**. 7;41:25-32.
- Manni et al. 2018 Acyl chain asymmetry and polyunsaturation of brain phospholipids facilitate membrane vesiculation without leakage. **eLife** 7. pii: e34394
- Tsai M-C, Fleuriot L, Janel S, Gonzalez-Rodriguez D, Morel C, Mettouchi A, Debayle D, Dallongeville S, Olivo-Marin J-C, Antonny B, Lafont F, Lemichez E, Barelli H. DHA-containing phospholipids control membrane fusion and transcellular tunnel dynamics. **J Cell Sci**. 8 Fev 2022.135 (5): jcs259119. doi:10.1242/jcs.259119

Scientific skills required by the candidate: Scientific and technical skills in neuroscience, neurobiology, cell biology, histology, microscopy and image analysis will be appreciated.

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