Master Structural & Functional Biochemistry

INTERNSHIP PROJECT 2021-2022

Towards the discovery and characterization of protein partners from Fatty Acid Synthase-II complex, an antituberculous therapeutic target.

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Each year, *Mycobacterium tuberculosis* is responsible for 1.4 million deaths and 8.6 million new cases of tuberculosis (TB). The emergence of drug resistant strains is one the main factors behind this phenomenon and the WHO called for the development of new molecules efficient against resistant TB. Recent phenotypic screening surveys revealed the relevance of the envelope metabolic pathways from *M. tuberculosis* as therapeutic targets. The major and atypical cell wall lipids, mycolic acids, play a central role in the architecture and the permeability of the *M. tuberculosis* envelope as well as in the bacterial pathogenicity. Their biosynthesis pathway is essential to the mycobacterial survival. Furthermore, currently used TB drugs, such as isoniazid, as well as several new promising molecules, including the recently approved drug delamanid, inhibit the mycolic acid metabolism. Notably, the Fatty Acid Synthase type II (FAS-II) involved in mycolic acid biosynthesis constitutes a highly relevant and validated pharmaceutical target (1,2).

The proposed project is part of a larger study aimed to characterize the mycobacterial FAS-II multienzyme system at the scale of the entire complex. Thus, an integrative approach is used to decipher the protein composition, the 3D structure and the enzymatic function of the whole purified FAS-II complex. In this context, we recently identified two new dehydratase partners of FAS-II (3-5) and several other candidate protein partners using an approach of affinity purification coupled to proteomic analysis from mycobacteria expressing various tagged FAS-II enzymes.

The Master project itself will be divided into two main axes:

1) The involvement of a recently identified candidate protein of the FAS-II complex and its function will be investigated by performing phenotypic analyses on an available mycobacterial mutant strain for which the corresponding gene has been deleted. Notably, the impact of the gene inactivation on FAS-II function and consequently on the mycolic acid biosynthesis will be examined after extraction, separation by chromatography and fine structural analyses of the lipids of interest by diverse technics such as mass spectrometry (MALDI-TOF MS, LC-MS) and NMR.

2) To go deeper in the study of protein-protein interactions within FAS-II complex itself and discovering potential new partner proteins, further experiments of affinity purification coupled to proteomic analysis (6) will be performed. FAS-II sub-complexes will be purified by an optimized affinity chromatography method from mycobacteria that produce different tagged bait proteins at the physiological level, thanks to an innovative strategy. The composition and stoichiometry of the purified sub-complexes will be analyzed both by bottom up and top down mass spectrometry, with or without prior complex stabilisation by already set up cross-linking strategies.

The deep knowledge of the FAS- II system will bring crucial and physiologically relevant data for the development of TB drug candidates. Screening strategies based on results obtained by such approaches are currently developed in the lab.

Methods:

Bacterial cultures, lipid extraction, thin layer and liquid chromatographies, MALDI-TOF MS, LC-MS, NMR, protein production in mycobacteria, preparation of bacterial extracts, SDS-PAGE, Western blotting, purification of protein complexes by affinity chromatography and size exclusion using FPLC, sample preparation for mass spectrometry, proteomics, native MS, cross-linking, bioinformatic analyses.

References:

- 1. Quémard A. (2016) New Insights into the Mycolate-Containing Compound Biosynthesis and Transport in Mycobacteria. *Trends Microbiol.* 24, 725-738.
- Daffé M., Quémard A., Marrakchi H. (2017) Mycolic acids: from Chemistry to Biology. In Biogenesis of Fatty Acids, Lipids and Membranes - Handbook of Hydrocarbon and Lipid Microbiology Series, ed Geiger O. (Springer, Cham)
- 3. Bardou F., Ducoux M., Quémard A. *et al.* (2017) HadD, a novel protein of the mycobacterial fatty acid synthase type II. Europe patent EP17306041.9.
- 4. Lefebvre C., Boulon R. *et al.* (2018) HadD, a novel protein of the mycobacterial fatty acid synthase type II, is essential for alpha- and epoxy-mycolic acid biosynthesis, cell envelope integrity and bacterial fitness. *Sci. Rep.* 8: 6084.
- 5. Lefebvre, C., W. Frigui, *et al.* (2020). Discovery of a novel dehydratase of the fatty acid synthase type II critical for ketomycolic acid biosynthesis and virulence of *Mycobacterium tuberculosis. Sci. Rep.* 10: 2112.
- 6. Jonik-Nowak B. *et al.* (2018). PIP30/FAM192A is a novel regulator of the nuclear proteasome activator PA28gamma. *Proc. Natl. Acad. Sci. USA.* 115: E6477-E6486.