**PhD PROPOSAL FOR THE**

**PASTEUR - PARIS UNIVERSITY INTERNATIONAL DOCTORAL PROGRAM**

Time for applicants to contact host laboratories: September 13 – November 2, 2017

Deadline for full application: November 13, 2017

Interviews: January 30, February 2, 2018

Start of the Ph.D.: October 1, 2018

**Title of the PhD project**: Control of fatty acid degradation in enterobacteria

**Keywords**: stress response, genetic regulation, metabolism, fatty acid, *E. coli, Salmonella*

**Department**: Microbiology

**Name of the lab**: Metabolism and Stress Adaptation

**Head of the lab**: Frédéric Barras

**PhD advisor**: Emmanuelle Bouveret

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**Web site address of the lab**: (unavailable yet) *Doctoral school affiliation and University*: Paris VII

Presentation of the laboratory and its research topics:

UNIT Metabolism and Stress Adaptation (MaSA)

The Unit MaSA will be created in january 2018. It will be directed by Prof. Frédéric Barras and comprises 8 members (E. Bouveret Directrice Recherche CNRS and 3 post doc and 3 engineers to be recruited).

Stress adaptation will be studied in enterobacteria, both pathogens (Salmonella, Shigella) and non pathogens (E. coli), by focusing on several aspects of cell physiology and metabolism, including Fe-S cluster homeostasis, ppGpp control and lipid metabolism, cell envelope integrity and metal biology. These different axes will feed several projects, such as Evolutionary history of Fe-S cluster-based life, Search for new antibiotics and Synthetic biology approach of human Friedreich Ataxia.

Description of the project:

*Background*

Fatty acids (FA) are available in the environment of enterobacteria, either as small chain FAs produced by the microbiote, or through the diet of the host. Enterobacteria use exogenous FAs as carbon source, regulatory molecules in virulence processes or to survive when their own FA synthesis machinery is inhibited by antibiotics (Yao and Rock, 2015; Golubeva *et al*., 2016). Surprisingly, apart from its biochemistry, the FA degradation process is poorly studied, especially its coordination with response to stress. The objective of this thesis is to analyze the way *E. coli* uses FA under different environmental conditions, including anaerobiosis. Understanding how FA get degraded under anaerobic conditions is of great interest in the context of microbiota and pathogen-associated issues as the natural environment of enterobacteria in the mammalian gut is mostly anaerobic.

In the presence of oxygen, FAs are imported in the cell through specific outer membrane receptors and then activated as thioesters of Coenzyme A, the actual substrates of the FA degradation (Fad) enzymes of the beta-oxidation system (DiRusso *et al*., 1999). The *fad* genes are controlled by the FadR repressor, which detects the presence of long chain acyl-CoA, and by the cAMP/CRP complex, which orchestrates the selection of available carbon sources. In the absence of oxygen, *E. coli* uses FA, yet almost nothing is known about how this happens. A second set of Fad enzymes was proposed to be involved in anaerobic conditions but many uncertainties remained as only one study was published (Campbell *et al*., 2003).

*Project*

**The first aim of the project is to identify the anaerobic FA degradation pathways.** A two-genes operon noted *ycfYX* homologs to *fadAB* genes, encoding a acyl-CoA dehydrogenase and a thiolase, respectively was shown to be required identified by several issues remain. In particular, degradation must rely on an electron transfer chain (ETC) to be alimented with reducing power. A dedicated ETC, encoded by the *ydiQRSTD* operon was pointed to as a potential candidate, but much remains to be done. Functional analysis, genetic regulation, and coordination between the Ydi ETC and the YcfXY controlled degradation, will be studied.

**The second aim of the project is to investigate the role and coordination of both aerobic and anaerobic FA metabolism**. One model is that the two systems are functionally redundant but operate under different conditions. Another model is the two systems have overlapping expression pattern but exhibit different substrate specificity, acting on different FA. This part of the project will aim at deciphering the interplay of the different transcriptional factors involved : CRP, FNR, ArcAB, and FadR.

**The third aim of the project is to understand the response of FA degradation pathway(s) in potential mechanism of membrane autophagy.** Transcriptomic studies suggest that *fad* genes are upregulated in stationary phase even in the absence of exogenous FA, possibly via the ppGpp secondary messenger (Traxler *et al.*, 2008; and our unpublished results). We propose that this upregulation to direct an autophagy like process. We will search for the internal source of FAs during growth arrest, and assess the importance of FA metabolism for cell size and shape adaptations upon growth arrest.

This project will address fundamental issues of *E. coli* biology. Conserved pathways between *E. coli* and *Salmonella* will be tested for their role in virulence of Salmonella.This project might fuel in The project will rest on a combination of genetics, cellular biology, biochemistry, single cell analysis and proteomic approaches

References:

Campbell JW, Morgan-Kiss RM, Cronan JE Jr. (2003) A new *Escherichia coli* metabolic competency: growth on fatty acids by a novel anaerobic beta-oxidation pathway. Mol Microbiol. **47**:793-805

DiRusso, CC, PN Black, JD Weimar (1999) Molecular inroads into the regulation and metabolism of fatty acids, lessons from bacteria. *Prog Lipid Res*, **38:** 129-197

Golubeva YA, Ellermeier JR, Cott Chubiz JE, Slauch JM. (2016) Intestinal Long-Chain Fatty Acids Act as a Direct Signal To Modulate Expression of the Salmonella Pathogenicity Island 1 Type III Secretion System. MBio. 7:e02170-15

Pech-Canul Á, Nogales J, Miranda-Molina A, Álvarez L, Geiger O, Soto MJ, López-Lara IM. (2011) FadD is required for utilization of endogenous fatty acids released from membrane lipids. J Bacteriol. **193**:6295-304

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Yao J, Rock CO (2015) How bacterial pathogens eat host lipids: implications for the development of fatty acid synthesis therapeutics. J Biol Chem. **290**:5940-5946

Zhang F, Carothers JM, Keasling JD. (2012) Design of a dynamic sensor-regulator system for production of chemicals and fuels derived from fatty acids. Nat Biotechnol. 30:354-359

Expected profile of the candidate (optional):

Education in fundamental molecular biology, ideallly molecular microbiology, bacterial genetic and/or biochemistry.

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