**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:** Study of RNA molecules contained in extracellular vesicles in *Cryptococcus neoformans*

**Keywords:** RNA, Pathogenic fungi, vesicles, Cryptococcus neoformans

**Department:** Mycology

**Name of the lab:** RNA Biology of Fungal Pathogens

**Head of the lab:** Guilhem Janbon

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***Doctoral school affiliation and University*:** BioSPC

Presentation of the laboratory and its research topics:

The Unit RNA Biology of Fungal Pathogens is part of the Mycology department. It focusses on the study of different aspects of the RNA metabolism of pathogenic fungi. These themes of research include the study of alternative splicing, alternative polyadenylation, alternative transcription start and lncRNA expression in these fungi. The team of Guilhem Janbon is proficient in NGS data analysis and in the molecular biology of the main fungal pathogens. Most of the research is done using the basidiomycete yeast *Cryptococcus neoformans*,

Description of the project:

*Cryptococcus neoformans* is an encapsulated basidiomycete yeast responsible for more than 300,000 deaths per year and principally infects immunocompromised patients [1]. This fungus lives in the environment and can be isolated from the soil, decaying wood or bird’s droppings. As such it has to cope with different stresses and predators including worms and amebae and has for that developed specific features, the most prominent ones being its polysaccharide capsule and its ability to synthesize melanin. Associated with its ability to grow at 37°C, these features, now recognized as virulence factors, make this fungus a deadly opportunistic pathogen [2]. More recently, we, and others have shown that *C. neoformans* possessed a very elaborated and plastic transcriptome structure. It has been hypothesized that this complex RNA metabolism might provide a mechanism for this yeast to respond to different environmental cues and to be an efficient pathogen.

*C. neoformans* as well as other pathogenic fungi has been shown to release extracellular vesicles [3]. These extracellular vesicles have recently shown to contain RNAs and proteins [4] although the origin or the function of these molecules remain unclear. The regulation of their expression is completely unknown. Nevertheless, it is tempting to hypothesise that they could be implicated in cell-to-cell communication or interact with the host and thus be important for virulence [5]. The present project aims to identify and characterize the RNA molecules present in these extracellular vesicles, and to study their regulation and their potential roles during the infection.

The first part of this project will use RNA-Seq analyses to characterize the RNA molecules that could be specific or enriched in these vesicles. Due to the spectacular diversity of RNA molecules produced by *C. neoformans*, the challenge will be here more the analysis of the data than in their production. Indeed, the unit recently performed a large set of RNA-Seq experiments in *C. neoformans* that were used to re-annotate its genome [6, 7]. The analysis of data revealed a fascinating and complex pattern of RNA molecules. Thus, both coding and non-coding genes are colonized by thousands of introns which are alternatively spliced [7]. Moreover, thousands of coding or non-coding transcripts resulting from alternative 3’ or/and 5’ ends usages further increase the complexity of the transcriptome of this pathogen (unpublished data).

The second objective of this project is to use some of the molecules (RNA or proteins) present in these extracellular vesicles to specifically tag them. These tagged vesicles producing strains will be then used to study the mechanism regulating vesicles production in *C. neoformans*. For instance, the influence environmental cues will be studied. A large collection of mutant strains (more than 4000) is available and will be also used to identify genes and mechanisms regulating vesicles production and composition. Here classical genetics and molecular biology based experiments will be performed.

The last part of the project will be to study the production of the molecules in vivo and their influences on the pathophysiology of the infection. First, strains expressing tagged vesicles will be used to study the production of these vesicles in vitro and in vivo by microscopy approaches. Molecular beacons could be used also to study the expression of some of these RNA molecules within the extracellular vesicles. Finally, using collaborations within the Institut Pasteur and outside, in vitro cellular models or/and animal models of infection will be used to study the potential modular roles of these molecules on the host response.

References:

*1. Kwon-Chung, K.J., et al., Cryptococcus neoformans and Cryptococcus gattii, the etiologic agents of cryptococcosis. Cold Spring Harb Perspect Med, 2015.* ***4****: p. a019760.*

*2. May, R.C., et al., Cryptococcus: from environmental saprophyte to global pathogen. Nat Rev Micro, 2016.* ***14****(2): p. 106-117.*

*3. Rodrigues, M.L., et al., Traveling into Outer Space: Unanswered Questions about Fungal Extracellular Vesicles. PLOS Pathogens, 2015.* ***11****(12): p. e1005240.*

*4. da Silva, R.P., et al., Extracellular vesicle-mediated export of fungal RNA. Scientific Reports, 2015.* ***5****: p. 7763.*

5. Buck, A.H., et al., *Exosomes secreted by nematode parasites transfer small RNAs to mammalian cells and modulate innate immunity.* Nature Communications, 2014. **5488**: p. doi:10.1038/ncomms6488.

6. Janbon, G., et al., *Analysis of the genome and transcriptome of Cryptococcus neoformans var. grubii reveals complex RNA expression and microevolution leading to virulence attenuation.* PLoS Genetics, 2014. **10**: p. e1004261.

7. Gonzalez-Hilarion, S., et al., *Intron retention-dependent gene regulation in Cryptococcus neoformans.* Scientific Reports, 2016. **6**: p. 32252.

Expected profile of the candidate (optional):

The candidate should be interested in genetics and molecular biology. An interest in bioinformatics would be a plus.

Contact:

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