**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:** *The malaria parasite epitranscriptome: Deciphering the role of RNA modifications in malaria parasite virulence and development*

**Keywords:** epitranscriptomics; translation control; non-coding RNA; RNA-protein interactions; CRISPR/Cas9 genome editing

**Department:** Parasites and Insect Vectors

**Name of the lab:** Biology of Host-Parasite Interactions

**Head of the lab:** Prof Artur Scherf

**PhD advisor:** Prof Artur Scherf

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

Presentation of the laboratory and its research topics:

*Plasmodium falciparum* causes the most severe form of malaria, a mosquito-borne infectious disease that killed 400,000 (mostly children) and infected more than 200 million people in 2015. Vaccine development and treatment of the disease has proven challenging due to the complex life cycle of the parasite and the molecular mechanisms behind host immune system evasion. The ‘Biology of Host-Parasite Interactions (BIHP)’ Unit at the Institut Pasteur has spearheaded the elucidation of several of these mechanisms over the past two decades, focusing on the epigenetic control of gene regulation. During the blood stage of infection in the human host, *P. falciparum* parasites invade red blood cells and export antigenic proteins to the host cell membrane, facilitating cytoadherance to the host microvasculature. The proteins involved in this process are encoded by multi-gene families, such as the *var* virulence gene family, in order to facilitate antigenic variation in response to immune pressure. The research in BIHP is generally centered on the transcriptional regulation of these gene families and their encoded proteins, as they are directly involved in pathogenicity and persistent virulence of the parasite.

To study the underlying molecular mechanisms of parasite pathogenesis, we use a wide variety of techniques in all stages of the parasite life cycle, including microscopy, biochemistry, and genome-wide technologies such as chromatin-immunoprecipitation (ChIP-seq) and RNA/DNA sequencing. To characterize individual gene and protein function, we recently adapted the CRISPR/Cas9 system to *P. falciparum*, which has revolutionized genetic engineering in the parasite. The pace ofmolecular and cellular biological research at BIHP is incredibly fast, as our laboratory has its own in-house, state-of-the art equipment including an Illumina next-generation sequencer (NextSeq500), an Agilent BioAnalyzer 2100, a BioRad CFX qPCR machine, and a DeltaVision microscope.

Our laboratory is well connected in the international parasitology community, and we often collaborate within the Institut Pasteur Paris, in France, and throughout the Institut Pasteur global network. BIHP is a truly international team with more than ten nationalities represented. It is a unique research environment, as each team member comes from a different scientific background and offers diverse insight, expertise, and mentoring.

Interested in joining? We are looking forward to meeting you!

Description of the project:

**Malaria is the deadliest disease** in human history. In 2015, nearly half a million died and more than 200 million people were infected with *Plasmodium falciparum -* the causative agent of the most severe form of malaria (1). Malaria pathogenesis results from the asexual reproduction inside erythrocytes where the parasite differentiates and replicates up to 32 daughter cells within 48 hours (2). The developmental stages are characterized by specific profiles of gene expression that are highly coordinated by an as-yet poorly-defined regulatory mechanism (3,4). Interestingly, transcription factors seem to play a small role in transcriptional regulation, and gene activation/repression is believed to be mainly achieved by reversible histone modifications and spatial nuclear organization (5). However, an emerging body of evidence demonstrates that post-transcriptional regulation (i.e. at the RNA level) through specific protein-RNA interactions significantly contributes to coordinated gene expression in the human host and might also play a role in other life cycle stages (i.e. in the mosquito vector) (6). While these processes seem to be key to parasite virulence, many questions remain concerning the extent of protein-RNA interactions, the manner in which proteins recognize specific RNA transcripts, and the consequences of these interactions.

Most intriguingly, recent findings in model organisms point towards a **high level of post-transcriptional regulation** through chemical modifications on mRNA and non-coding RNA transcripts (7,8). We set out to characterize the so-called ‘epitranscriptome’ in *P. falciparum* with mass spectrometry and identified dozens of RNA modifications throughout the parasite life cycle. Of those, we found methylation of **adenosine at N6 (m6A)** to be the most abundant and highly **dynamic RNA modification**. Currently, we are attempting to characterize the m6A methylation machinery, which includes knock down of the putative m6A methyltransferase and genome-wide identification of individual m6A sites.

Our current efforts and the main focus of the proposed PhD project will be on characterizing the m6A methylation-dependent processes and phenotypes in *P. falciparum*. Specific questions include:

1. Which proteins are involved in ‘writing’ m6A methylation on RNA and which proteins specifically recognize this modification?
2. How do m6A modifications affect mRNA translation and stability?
3. Does it control parasite virulence?
4. Does m6A occur on types of RNA transcripts other than mRNA?
5. On a broader ‘organismal’ level, how does m6A affect the parasite in its development and progression through the life cycle?

The PhD student will learn and apply several targeted and **genome-wide approaches** to answer these questions. Protein immunoprecipitations will be used to identify new members of the m6A methylation complex and RNA pull-downs will help to identify specific m6A ‘reading’ proteins. Genome-wide approaches such as ribosome profiling will be used to measure translation efficiencies across the parasite life cycle in methylation-deficient versus wild-type parasites. In follow-up experiments, the PhD student will have the opportunity to characterize individual proteins involved in RNA methylation by creating inducible knock out or knock down cell lines with CRISPR/Cas9. Importantly, the PhD student will have the flexibility to investigate unforeseen and interesting developments in the proposed project.

The proposed project takes the **emerging hot topic of post-transcriptional regulation** and applies it to a pathogen that has a huge **impact on global public health**. The student will receive training in a wide variety of techniques in parasitology, cell and molecular biology, and genetics, ranging from parasite cell culture to generation and bioinformatic analysis of NGS data. To facilitate scientific interactions, BIHP has weekly lab meetings and participates in an annual lab and departmental retreat. Finally, each student in BIHP is encouraged to attend international workshops and conferences where she/he will have the opportunity to present her/his data and interact with experts from all over the world. The project will be supervised on the day-to-day basis by an experienced postdoctoral fellow (Dr. S. Baumgarten).

References:

*1. World Health Organization. World Malaria Report 2015. (2015). 2. Boddey, J. A. & Cowman, A. F. (2013) Plasmodium Nesting: Remaking the Erythrocyte from the Inside Out. Annu. Rev. Microbiol.* ***67,*** *243–269.*

*3. Bozdech, Z., Llinas, M., Pulliam, B. L., Wong, E. D., Zhu, J. & DeRisi, J. L. (2003) The transcriptome of the intraerythrocytic developmental cycle of Plasmodium falciparum. PLoS Biol.* ***1,*** *85–100.*

*4. Foth, B. J., Zhang, N., Chaal, B. K., Sze, S. K., Preiser, P. R. & Bozdech, Z. (2011) Quantitative time-course profiling of parasite and host cell proteins in the human malaria parasite Plasmodium falciparum. Mol. Cell. Proteomics* ***10,*** *M110.006411.*

*5. Llinás, M., Deitsch, K. W. & Voss, T. S. (2008) Plasmodium gene regulation: far more to factor in. Trends Parasitol.* ***24,*** *551–556.*

*6.*  *Vembar, S. S., Droll, D. & Scherf, A (2016). Translational regulation in blood stages of the malaria parasite Plasmodium spp.: systems-wide studies pave the way. Wiley Interdiscip. Rev. RNA* ***7,*** *772–792.*

*7. Zhao, B. S., Roundtree, I. A. & He, C (2016). Post-transcriptional gene regulation by mRNA modifications. Nat. Rev. Mol. Cell Biol.* ***18,*** *31–42*

*8. Meyer, K. D. & Jaffrey, S. R. (2014) The dynamic epitranscriptome: N6-methyladenosine and gene expression control. Nat. Rev. Mol. Cell Biol.* ***15,*** *313–26.*

Expected profile of the candidate (optional):

* General interest in infectious disease biology and parasitology
* High proficiency in English
* Previous experience in a molecular biology lab very desirable
* Team player

Contact:

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