**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**: Aberrant RNA detection and degradation through nonsense-mediated mRNA decay

**Keywords**: RNA degradation, RNA translation, yeast, nonsense-mediated mRNA decay

**Department:** Genomes & Genetics

**Name of the lab:** Genetics of Macromolecular Interactions

**Head of the lab:** Alain Jacquier

**PhD advisor:** Cosmin SAVEANU

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***Doctoral school affiliation and University*:** Complexité du Vivant (CDV) - University Pierre et Marie Curie (UPMC)

Presentation of the laboratory and its research topics:

Our laboratory has a long-term interest in mechanisms of RNA synthesis, ribosome assembly and RNA degradation in the yeast *S. cerevisiae*, a model organism for fundamental cellular processes in eukaryotes. Three inter-related topics are currently studied in the laboratory - one centered on DNA transcription and the transcriptome landscape, another focused on co-translational protein degradation and a third, on RNA degradation through nonsense-mediated mRNA degradation (NMD). The strength of the laboratory is the development and use of advanced techniques of RNA sequencing, mass-spectrometry based protein quantitation and large-scale phenotyping.

The PhD project will be done in the NMD group led by Cosmin Saveanu, in tight collaboration with Laurence Decourty, an exceptional research technician, working full time. Additionally, Varun Khanna (staff bioinformatics engineer) will be working part-time for the project on data analysis. The team closely collaborates with Abdelkader Namane, research engineer and mass-spectrometry expert, for proteomics. The project will benefit from the ANR CLEANMD funding (2015-2019).

Description of the project:

Context: The proposed PhD project focuses on NMD, non-sense mediated mRNA decay, a major degradation pathway linked with translation of mRNAs that contain premature termination codons. The pathway affects many essential processes in eukaryotes, from telomere maintenance to embryo development. It affects a large variety of transcripts, characterized by short coding sequences (Decourty et al, 2014) and long 3' untranslated regions, as shown in our laboratory (Malabat, Feuerbach et al., 2015).

Independent of their origin, NMD substrates are recognized through translation and rapidly degraded in a process that is dependent on ribosomes and three highly conserved factors, Upf1, Upf2 and Upf3. Upf1, an ATP dependent RNA helicase, is one of the main factors in recognizing NMD substrates and triggering their degradation. The prevalent molecular model for NMD proposes the intervention of the exon-exon junction complex (EJC) in the recognition of aberrant stop codons, together with phosphorylation and dephosphorylation cycles of Upf1, the most abundant and highly conserved NMD factor. The current model, however, does not explain how NMD works in the absence of mRNA splicing, in organisms devoid of the kinase that phosphorylates Upf1, or in organisms in which the phosphorylated residues are absent. **Preliminary results:** The results of the current PhD student working on NMD, Marine Dehecq, suggest that a *EJC-independent* and *phosphorylation-independent* model could be a widely conserved molecular mechanism in eukaryotes. These observations, based on more than 100 affinity-purification and mass-spectrometry experiments represent the basis for the current PhD project.

**Aim 1: Obtain a binary interaction map for the two newly described NMD complexes.** To this end, the PhD candidate will dissect the Upf1 helicase and search for point mutations that change the composition of the complexes, their binding to RNA and the shift from one complex to the next. The effect of these mutations on the stabilization of NMD substrates will be tested genome-wide by RNA sequencing. In a parallel approach, affinity-purified complexes will be cross-linked *in vitro* and the cross-linked peptides will be identified by mass-spectrometry.

**Aim 2: Investigate alternative degradation mechanisms for NMD substrates.** Our data indicate that one of the NMD complexes contains a potential RNA endonuclease that is directly bound to the helicase domain of Upf1. The PhD candidate will investigate the potential for an active role of this endonuclease on specific NMD targets by working in mutant strains in which the major 5' to 3' RNA degradation pathway is inhibited. *In vitro* studies will estimate the potential catalytic activity of the putative endonuclease and its activation, or not, by Upf1.

**Aim 3:** **Understand how RNA degradation is triggered in NMD.** Current biochemical models of mammalian NMD invoke the important role of a protein kinase in the activation of Upf1. However, equivalents of this kinase either absent or not required for NMD in many species. Moreover, the Upf1 phosphorylated residues are not universally conserved. In this context, it is possible that not phosphorylation, but another molecular event allows the activation of RNA degradation during NMD. Such an event is the switch from the newly identified detector and effector complexes. This switch could involve the dimerization of Upf1 and the hypothesis will be investigated by co-purification of Upf1 domains with tagged Upf1 in the presence or absence of the other essential co-factors, Upf2 and Upf3. The dimerization and its dynamics will also be studied in the presence of translation inhibitors.

**Significance:** The main aim of this project is to provide insights into NMD mechanisms conserved from yeast to humans. The study of NMD is intimately linked with the study of translation, mRNA stability and the diversity and “fuzziness” of DNA transcription. Knowledge of these processes is essential to understand gene expression.

References:

1. *Decourty L, Doyen A, Malabat C, Frachon E, Rispal D, Séraphin B, Feuerbach F, Jacquier A & Saveanu C (****2014****) Long open reading frame transcripts escape nonsense-mediated mRNA decay in yeast. Cell Rep.* ***6:*** *593–598*
2. *Malabat C, Feuerbach F, Ma L,* ***Saveanu C*** *& Jacquier A (****2015****) Quality control of transcription start site selection by Nonsense-Mediated-mRNA Decay. Elife* ***4*** *10.7554/eLife.06722*

Expected profile of the candidate (optional):

A candidate who is not afraid of generating and analyzing big data - dozens of results on thousands of genes, transcripts and proteins - both experimentally and through results analysis. The project will provide a solid formation in yeast genetics, molecular biology, functional genomics and biochemistry, with the added bonus of competence in statistics for data analysis.

Contact:

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