Ecole Doctorale COMPLEXITE DU VIVANT – Fiche Projet CONCOURS

Nom et prénom du directeur de thèse (et si besoin du co-directeur) : HUYNH Jean-René (DR et HDR)

Le directeur de thèse et le co-directeur doivent impérativement avoir l'HDR ou équivalent

Coordonnées
Tel : +33 1 44 27 17 01
e-mail : jean-rene.huynh@college-de-france.fr

Nom et prénom du co-encadrant (non Hdr) (s’il y a lieu) : MOLLA HERMAN Anahi (CR)

Coordonnées
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Y-a-t-il un candidat déjà identifié pour le projet: OUI

Nom et prénom du responsable de l’équipe : Jean-René HUYNH

Intitulé de l'équipe : Evolution and Development of Germ Cells

Nombre de chercheurs et enseignants-chercheurs statutaires de l’équipe titulaires d’une HDR (ou équivalent) : 1

Nom et prénom du responsable d'UMR ou de département : Marie-Hélène VERLHAC

Intitulé et N° d’UMR ou de département : CNRS/UMR 7241 - INSERM U1050

Titre du projet de thèse : tRNAs and tRFs roles in the maintenance of genomic integrity

Signature du directeur d'UMR ou de département (vaut avis favorable pour le dépôt du projet):

Spécialité : Génétique et Développement

Résumé du projet de thèse (1 page maximum, en anglais)
Pour les thèses avec 2 co-directeurs, ou en partenariat entre 2 laboratoires ou structures, indiquer la participation de chaque co-directeur et structure dans la gestion du projet.
Transposable Elements (TEs) are mobile genetic elements that have colonized all genomes: they represent 20% of the fruit fly genome and 50% of the human genome. They are of great functional importance since they endanger the genome by creating mutations upon insertion. This effect is especially crucial in the germline, the cell lineage that will give rise to the gametes. If sperms and oocytes undergo mutations, they will be passed on to the next generation.

In recent years, an adaptive “genome immune system” against these threats has been uncovered. It is based on small non-coding RNAs called piRNAs (PIWI-interacting RNAs of 23-29 nt) that target other RNAs by sequence complementarity, similarly to microRNAs and small interfering RNAs [1]. piRNAs are produced by piRNAs clusters, organized as discrete loci in the genome. They act at two levels. At the RNA level, they can induce the cleavage of RNAs by nuclease from the AGO family (Ago3, Piwi, Aubergine for instance) to which they are bound, blocking then TEs expression. At the DNA level, they can induce changes in chromatin structure, thus silencing TEs transcription.

A genetic screen to find genes involved in the development of the germline in Drosophila melanogaster was performed in the lab. Among others, Rpp30 mutation leads to oogenesis arrest and sterility in females: ovariess remain very small and don’t form any eggs. In Rpp30 mutant ovaries, piRNAs collapse, TEs are upregulated and replication checkpoints are activated [3]. Surprisingly, Rpp30 is a subunit of RNase P, a highly conserved ribozyme required for tRNAs precursor maturation [2]. tRNAs are notably known for their role in aminoacyl transfer during translation but translation is not altered in Rpp30 mutants. Epigenetic marks on the chromatin close to tRNAs clusters are disturbed, showing an effect of the mutation at the DNA level [3]. Yet, the mutation could also have an effect at the RNA level. Indeed, a new class of small non-coding RNAs derived from different regions of precursor or mature tRNAs was identified recently: they are called tRFs (tRNA fragments) [5]. tRFs might make the link between tRNAs biology and piRNAs biology. In the lab, A. Mollá-Herman, in a work in which I was involved, gave evidence that tRFs population is completely deregulated in Rpp30 mutant ovaries compared to control ovaries [4]. Therefore, the mutation of RNase P could also have an effect at the RNA level by changing tRFs-dependent RNAs regulation.

My PhD project lies at the cross-road of developmental biology, germline biology, genetics and genomics. It will be tackled with tools already mastered in the lab: Drosophila genetics, confocal microscopy, small-RNA sequencing and bioinformatics analysis.

The objective of my PhD project is to address two important complementary though independent aspects of tRNAs biology, crucial for germline integrity and transposon control (see graphical abstract):

1 – At the RNA level, demonstrate the in vivo function of tRFs in TEs repression. I will use tRFs-GFP sensors made in the lab to check whether tRFs can recognize and downregulate TEs expression. I will also decipher which proteins are involved in this silencing process with a small-scale genetic RNAi screen involving piRNAs pathway proteins, among others.

2 – At the DNA level, look for a role of tRNAs in the establishment of a genomic immune system. I will insert ectopic tRNAs clusters in the genome of flies, studying new TEs landing sites and prospective new piRNAs clusters formation and piRNAs production. This will be done in wild type and sensitized conditions that lead to higher amounts of TEs expression or replication stress, such as piRNAs and tRNAs pathways.

Graphical abstract: left panel. Oogenesis is arrested (no eggs formation) in Rpp30 homozygous mutants. Right panel: In WT conditions, RNase P is required for tRFs biogenesis. piRNAs silence TEs. TEs are in violet. TEs and new piRNAs cluster are in yellow. PhD tasks 1 and 2 are depicted.

References:
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Faisabilité du projet de thèse (1/2 page maximum, en anglais)

Expliciter la faisabilité du projet en terme d'expertise de l'équipe d'accueil, des collaborations potentielles qui pourront être mises en place pour certains aspects du projet, de la disponibilité des appareils nécessaires au bon déroulement du projet...

J.R. Huynh is a Senior group leader at the Collège de France. His team consists of 5 permanent researchers (CNRS/INSERM). He has successfully supervised 3 PhD students, several M2-M1 students as well as 7 post-docs. I am the only student in the laboratory in this moment. J. R. Huynh is able to coordinate several innovative and ambitious projects in the laboratory in the same time (http://germcells.fr). He has been awarded for several grants and distinctions (ANR Plastisipi 2013-20 on small non coding RNAs and TEs repression, FRM labellisation, Fondation Bettencourt Schueller, ARC, Médaille de Bronze du CNRS...). He has already coordinated several consortiums: ANR AbCyStem (2015-19) with A. Echard (I. Pasteur) and O. Gavet (I. G. Roussy) on cytokinesis in the Pilp region; “Stem Cells” Labex DEEP (2012-17) at the I. Curie... He was a partner in several ANR grants and international grants such as the Japanese JSPS (M. Tanaka, Nagoya University).

A. Molla-Herman obtained a permanent CNRS position in J. R. Huynh’s lab in 2016. She has supervised several M1 and M2 students during her PhD. During her post-doc she supervised an engineer and a researcher who participated in her project together with J. R. Huynh. She was awarded with L’Oréal Prize in 2015 for her discoveries on tRNAs-piRNAs new crosstalk in genome integrity defense. This project is ambitious, original and highly appreciated in the field [3, 4]. During the last 3 years, she has supervised one M2 student and myself. She included me to study tRFs populations in WT and Rpp30 mutant conditions, recently published in Frontiers in Genetics [2]. For bioinformatics, A. Molla-Herman and J. R. Huynh have been successfully collaborating with C. Antoniewski (ART BIO platform, Jussieu) since 2013 [1, 2]. A. Molla-Herman is a highly dynamic supervisor and would like to obtain the HDR in the following years, and she has the support of J. R. Huynh. She has followed 2 EMBO courses on “lab management” and “female leadership and negotiation” in Heidelberg. She is also involved in PSL teaching activities in which I will have the opportunity to participate during my PhD. She is also involved in the scientific life of the CIRB, organizing seminars and scientific events, which would help for eventual future collaborations. The CIRB is an interdisciplinary research center for microbiology, developmental biology, neuroscience, oncology, biophysics, cancer biology or cardio-vascular physiology. tRFs are present in all branches of life, and we already have some teams interested in studying tRFs in their systems.

My skills in cell biology, biochemistry, genomics, bioinformatics and molecular biology obtained during my different internships and my academic studies will be very helpful for my PhD. Preliminary data are promising. My project is feasible in time and combines tasks with low and medium risk. The Huynh lab in the CIRB Collège de France is thus a highly suitable place to do my PhD project. I will have good supervision and resources to perform experiments already mastered in the laboratory : techniques in molecular biology, microscopy, genetics and sequencing, protocols, kits, machines, pipelines= … I will also have access to their networks and collaborations to make this project a success.

[1] Molla-Herman et al., EMBO J 2015, tRNA processing defects induce replication stress and Chk2-dependent disruption of piRNA transcription
[3] Yamanaka and Siomi, EMBO J 2015, Misprocessed tRNA response targets piRNA clusters

Thèses actuellement en cours dans l’équipe

Tous les encadrements doivent être indiqués (y compris les co-directions avec un autre HDR pour des doctorants d'une autre ED, et les encadrements dans le cadre de programmes doctoraux tels qu’IPV, FDV...)

<table>
<thead>
<tr>
<th>Nom et Prénom du doctorant</th>
<th>Directeur(s) de thèse</th>
<th>Année de 1ère inscription</th>
<th>ED</th>
<th>Financement</th>
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3
Ecole Doctorale COMPLEXITE DU VIVANT – Fiche Projet CONCOURS

Trois publications récentes du directeur de thèse (du co-directeur ou du co-encadrant s’il y a lieu). Mettre en gras le nom du directeur de thèse.

**tRNA Fragments Populations Analysis in Mutants Affecting tRNAs Processing and tRNA Methylation.**  
*Molla-Herman A, Angelova MT, Ginestet M, Carré C, Antoniewski C, Huynh JR.*  

**Collective Cell Sorting Requires Contractile Cortical Waves in Germline Cells.**  

**The replicative histone chaperone CAF1 is essential for the maintenance of identity and genome integrity in adult stem cells.**  
*Clémot M, Molla-Herman A, Mathieu J, Huynh JR, Dostatni N.*  

Docteurs encadrés par le directeur de thèse ayant soutenu entre la date de dépôt de ce dossier et il y a 5 ans et publications relatives à leur sujet de thèse. Mettre en gras le nom du directeur de thèse et celui du docteur.

**Abscission is regulated by the ESCRT-III protein shrub in Drosophila germline stem cells.**  
*Matias NR, Mathieu J, Huynh JR.*  

**Chromatin modifications regulate germ cell development and transgenerational information relay.**  
*Molla-Herman A, Matias NR, Huynh JR.*  