**INTERNSHIP PROPOSAL M2 RESEARCH 2023-2024**

**TITLE:**

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Number of PhD currently supervised: 2 (1 defense in fall 2023)

Possibilty to give rise to a PhD proposal : x YES ☐ NO

Title: How does an organism recognize and regulate Transposable Elements?: Crosstalks between two epigenetic silencing systems in Arabidopsis

INTRODUCTION, SCIENTIFIC CONTEXT :

Transposable Elements (TE) are repetitive sequences present in all living organisms that are mobile and can multiply in the genome. As such, they are potential mutagens that can be deleterious, but can also be adaptive by creating genetic or expression variants that can be selected. They are now considered as a driving force of evolution and the importance of TE in gene regulation well established. The mechanisms that mediate TE repression as well as the mechanisms by which TE can escape repression are thus of critical importance in these processes. **How does an organism recognize and regulate Transposable Elements**? While DNA methylation (associated with Histone 3 Lysine 9 dimethylation) has long been recognized as the primary epigenetic mechanism that mediates the stable transcriptional silencing of TE, it has been recently revealed in various organisms including in plants (demonstrated by our team) that they could also be, in certain genetic/genomic contexts, also targeted by Histone 3 Lysine 27 trimethylation (H3K27me3)—another **epigenetic mark** associated with Polycomb-group proteins (PcG). This is unexpected, given that H3K27me3 is a hallmark of plastic transcriptional repression associated with protein-coding genes in particular developmental and stress-responsive genes. Our team explores this **alternative mode of TE epigenetic regulation** which could favor TE propagation by allowing discrete windows of opportunities (during development or environmental stresses) for them to be expressed and/or confer nearby genes with a dynamic epigenetic regulation. We thus investigate the **the role** as well as the **molecular bases of recruitment of H3K27me3 at TEs**.

RESEARCH PROPOSAL :

To investigate the roleas well as the **molecular bases of recruitment of H3K27me3 at TEs**, we aim in this project to answer the following questions:

1. **What are the intrinsic features of TEs that trigger recruitment of Polycomb and can sometimes favour it over DNA methylation? Can TE act as recruitment platforms that impact nearby gene regulation?**
2. **What are the specific epigenetic factors involved in this recruitment and that can favour it over DNA methylation? Are there crosstalks between DNA methylation and Polycomb machineries at transposable elements?**

Project

1. We have made hypotheses about sequences motives involved in PcG recruitment that could arise during TE evolution, based on what is known for Polycomb recruitment at genes; in addition, we also use natural genetic and epigenetic variation at TEs across Arabidopsis ecotypes (sub-populations of Arabidopsis collected throughout the word) as a tool to predict the nature of these determinants in a unbiased manner. During the project, the student will validate these predictions, by **implementing CRISPR-Cas9 genome-editing to delete or mutate specific TE sequences** and then **analyze the resulting epigenetic profile at the modified TE** by **ChIP-qPCR** (H3K27me3 patterns) and **Bisulfite-sequencing** (DNA methylation patterns).

In CRISPR-edited lines, the H3K27me3 profile of nearby genes will also be analyzed to test whether H3K27me3-marked TEs play a role as recruitment platform and cis-regulatory module that could impact nearby gene epigenetic profile and expression.

1. The student will also **characterize mutants of Arabidopsis** that we predicted- based on previous work- to be involved in the differential recruitment of H3K27me3 versus DNA methylation. This will be done by analyzing H3K27me3 (ChIP) and DNA methylation (Bisulfite-sequencing) at endogenous TEs and transgenic TEs which model TE neo-insertions after transposition- a system which is well established in the lab to study the crosstalk between DNA methylation and Polycomb when a TE arrives “free” of epigenetic marks in the genome.

METHODOLOGIES :

**Genome-editing (CRISPR)** (some constructs and edited lines already available in the line, some to be generated), **Chromatin immunoprecipitation/ bisulfite-sequencing** (ChIP\_qPCR/seq, bisulfite-sequencing), **introduction to NGS analyses** (IGV Browser/ introduction to ChIP-seq); general molecular biology (cloning, nucleic acid analysis), genetics and Arabidopsis culture and transformation.

REFERENCES

* **Angélique Déléris**, Sandra Duharcourt and Fred Berger.

A Role of Polycomb in the control of transposable elements, **Trends in Genetics**, 2021 June Volume 37, Issue 10, Pages 882-889, https://doi.org/10.1016/j.tig.2021.06.003.

* Jérôme Zervudacki, A.Yu, D. Amesefe, J.Wang, J.Drouaud, L.Navarro and **Angélique Déléris** (2018)

Transcriptional control and exploitation of an immune-responsive family of plant retrotransposons, **The EMBO journal**, 2018 Jul 13;37(14):e98482

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Polycomb mutant partially suppresses DNA hypomethylation–associated phenotypes in Arabidopsis, **Life Science Alliance** . 2020 Dec 21;4(2): e202000848