M2 Master internship
2024-2025
Team Delattre

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Deciphering the mechanisms of chromosome breakage and repair during Programmed-DNA Elimination

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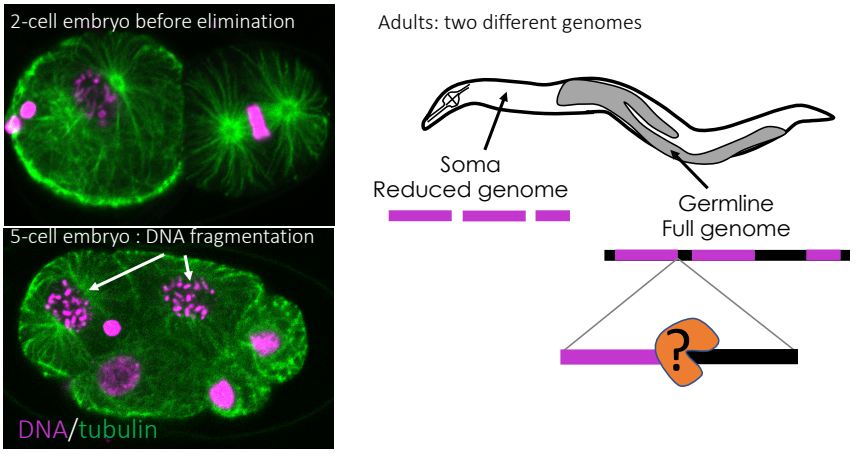
https://www.ens-lyon.fr/LBMC/equipes/NematodeCell

Scientific background

Some species systematically undergo excision and elimination of portions of their genome in somatic cells, in a process called programmed-DNA elimination (PDE) (while the germline cells maintain an intact genome). PDE has emerged multiple times throughout evolution. It has been extensively studied in unicellular Ciliates, which led to breakthrough discoveries on the role of smallRNAs in genome stability (1). In animals, although spotted for the first time in 1887, we still don't know how the genome is excised at specific locations and what is the ultimate function and PDE (2).

We fortuitously discovered PDE in the free-living nematodes *Mesorhabitis* (*3*). In contrast to the other animal species so far described, *Mesorhabditis* are lab tractable (*4–6*). This offers a unique opportunity to, at last, understand the mechanism, the role and the evolution of PDE. We expect this work to shed light on new mechanisms of genome regulation and, in the longer term, provide new tools for genome engineering.



Mesorhabditis nematodes eliminate portions of their genome during development in somatic cells only (in 5-cell stage embryos). The germline-restricted (*i.e.* eliminated) genome (shown in black) contains mainly repeated elements and few genes. The molecular machinery responsible for chromosome breakage is still unknown but the breakpoints are very precise, suggesting a specific nuclease (shown in orange) is involved.

Project description

The project aims to identify the molecular machinery responsible for chromosome breakage and fragmentation in *Mesorhabditis*. We have recently found that chromosome break sites are sharp, at the base, and have identified a specific motif near break sites. We will be looking for the protein(s) that are recruited to this motif. To do this, we will test candidate genes

(previously identified using an RNAseq approach) by generating CRISPR-Cas9 mutants. We will look for mutants in which genome elimination is abolished. Candidates will also be tested in a yeast one-hybrid assay to determine which protein is able to bind and/or cut the motif. To this end, a yeast strain with a reporter assay has already been established. In parallel, we will develop a forward approach: using cell extracts from embryonic nuclei, we will identify which proteins bind directly to the DNA motif.

What you will learn

During this project, you will become proficient in techniques related to nematode rearing, genome engineering (CRISPR/Cas), microscopy, cytology (immuno-fluorescence, DNA FISH and single molecule FISH) and biochemistry (DNA pull-down and co-IP). You will learn how to independently develop ideas, pursue a research project and communicate your results in oral and written form.

Your qualification

We are seeking a student with interest in genomics and evolutionary biology. The ideal candidate is keen to combine wet lab work, imaging and bioinformatics (previous training in these areas is not necessary).

Contact: send a CV and a cover letter to Marie Delattre marie.delattre@ens-lyon.fr

References (*from the lab)

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6. *C. Blanc, N. Saclier, E. Le Faou, L. Marie-Orleach, E. Wenger, C. Diblasi, S. Glemin, N. Galtier, M. Delattre, Cosegregation of recombinant chromatids maintains genome-wide heterozygosity in an asexual nematode. *Sci. Adv.* **9**, eadi2804 (2023).