MASTER 2 BMC
PARCOURS GENOPATH
ANNÉE 2024-2025

Titre du sujet de stage :

Sperm chromatin organization and establishment of the paternal epigenetic identity in the zygote

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Epigénétique et Formation du Zygote dirigée par Benjamin Loppin

https://www.ens-lyon.fr/LBMC/equipes/epigenetique-et-formation-du-zygote

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Sujet de stage :

Background:

Our group study sperm chromatin organization, function and evolution using genetics and imaging in model insects. Many animals, including mammals and insects, package sperm DNA with specialized, non-histone proteins called protamines. During the differentiation of haploid spermatids into mature spermatozoa, the histone-to-protamine transition contribute to sperm chromatin compaction, a complex process associated with the shutdown of basic nuclear activities. At fertilization, protamines are rapidly eliminated and replaced with maternally-provided histones to reconstitute a nucleosomal organization of paternal chromosomes.

We use two main model insects: the fruitfly *Drosophila melanogaster* and the cricket *Gryllus bimaculatus* and develop a variety of projects.

Project: Investigating the mechanism and function of the histone-to-protamine transition in *Drosophila*

In *Drosophila*, histones are almost completely replaced with protamines during spermiogenesis but the functional significance of this ultraspecialized chromatin organization is poorly understood. We have recently discovered, through the functional analysis of a paternal effect mutant named *paternal loss* (*pal*), that the elimination of histones in sperm is critical to protect paternal chromosomes in the egg at fertilization (*Dubruille et al.*, 2023, Science). *pal* encodes a transition protein required for the eviction of histone H3 and H4 during the histone-to-protamine transition. In

pal mutant males, histones H2A and H2B are eliminated, but H3 and H4 are aberrantly retained, without affecting sperm function. At fertilization, the presence of H3 and H4 in *pal* sperm leads to the misrecognition of paternal chromosomes as maternal chromosomes by the egg cytoplasm and the fragmentation of the male pronucleus (Figure 1). We are currently characterizing another paternal effect mutant, *vrs* in which whole nucleosomes, i.e H2A-H2B H3-H4, are retained in the sperm chromatin.

We are looking for a highly motivated student to decipher the molecular mechanisms of histone eviction by Pal and Vrs during spermiogenesis. The project aims also at characterizing the sperm chromatin organization in these mutants as well the consequences of these organizations on early embryonic development.

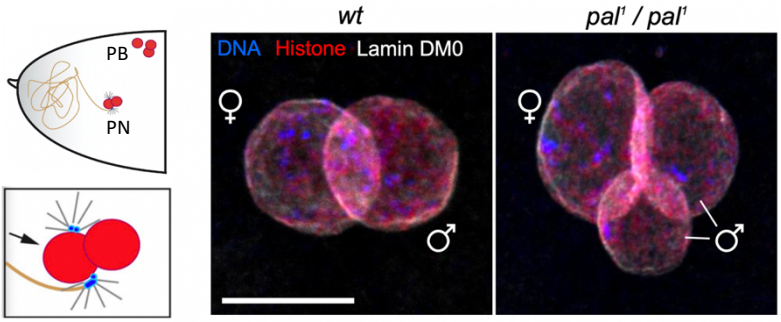

Figure 1 : Left panels : Schemes of a *Drosophila* egg at the apposition stage (PB: polar bodies; PN: pronuclei). The arrow shows the male pronucleus. Right panels : Confocal images of a control egg and an egg fertilized by a *pal* sperm at the apposition stage. Note the fragmented male pronucleus in *pal* eggs

Technologies utilisées :
Drosophila genetics (crosses, phenotypic analyses, CRISPR/Cas9, ...), cytology/microscopy (dissections, embryo collections, immunofluorescence, confocal microscopy …), molecular biology and biochemistry

Mots clés : *Drosophila melanogaster*, chromatin, histones, protamines, histone modifications, zygote, spermiogenesis, spermatozoa.

Publications du laboratoire (5 max):

Dubruille R, Herbette M, Revel M, Horard B, Chang CH, Loppin B. (2023) Histone removal in sperm protects paternal chromosomes from premature division at fertilization. ***Science.*** 382(6671):725-731.

Orsi GA, Tortora MMC, Horard B, Baas D, Kleman JP, Bucevičius J, Lukinavičius G, Jost D, Loppin B. (2023) Biophysical ordering transitions underlie genome 3D re-organization during cricket spermiogenesis. ***Nat Commun.*** 14(1):4187.

Horard B, Terretaz K, Gosselin-Grenet AS, Sobry H, Sicard M, Landmann F, Loppin B. (2022) Paternal transmission of the Wolbachia CidB toxin underlies cytoplasmic incompatibility. ***Curr Biol.*** 32(6):1319-1331.e5.

Herbette M, Wei X, Chang CH, Larracuente AM, Loppin B, Dubruille R. (2021) Distinct spermiogenic phenotypes underlie sperm elimination in the Segregation Distorter meiotic drive system. ***PLoS Genet.*** 17(7):e1009662

Loppin B, Dubruille R, Horard B (2015) The intimate genetics of Drosophila fertilization. ***Open Biol.*** 5(8). pii: 150076.