

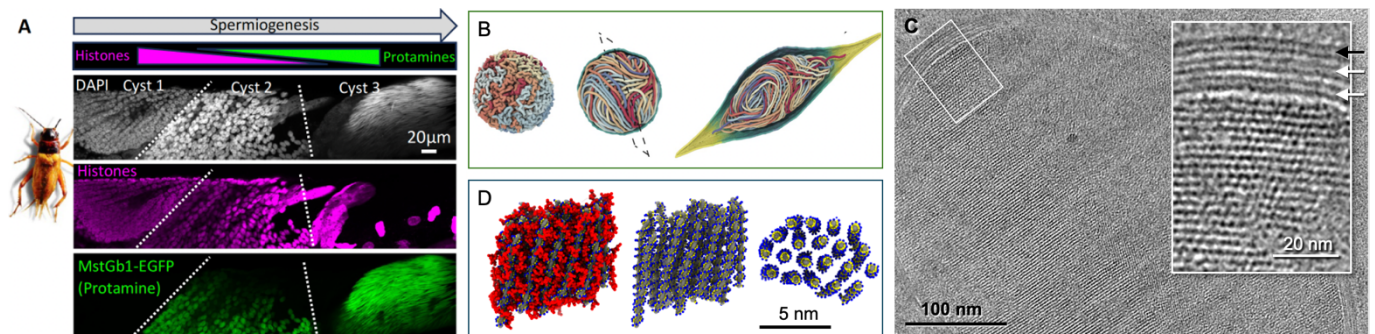
Chromatin remodelling into a crystal upon spermiogenesis

Project aim and background

Spermiogenesis is a radical process of cell differentiation whereby sperm cells acquire a compact and specialized morphology. In many animals, this entails dramatic chromatin remodelling: most histones are replaced by sperm-specific nuclear basic proteins (SNBPs), including protamines. *In vitro*, protamines, acting as multivalent cations, can organize long DNA molecules (>10kbp) into ~150 nm-wide toroids, and short DNA molecules (<200bp) into crystalline rods with hexagonal order. Yet, the *in situ* configuration of the “protaminized” genome in mature sperm is largely unknown, as well as the structural transitions occurring from the initial histone-containing “bead-on-string” chromatin.

In this internship, we propose to explore the structural transitions in cricket genome folding along spermiogenesis, focusing on how protamines organize the paternal genome in mature sperm in the two-spotted cricket *Gryllus bimaculatus*. A precise spatio-temporal dynamics of histone acetylation and the histone-to-protamine transition are critical to achieve this process. In *G. bimaculatus*, it occurs within cysts of synchronous spermatids, with a succession of (i) early round spermatids (i.e nucleosome-based chromatin) with isotropic DNA orientation resembling typical somatic cell nuclei; (ii) elongating spermatids (protamine-based) with spooled ~25nm thick chromatin fibers with nematic-like order along the elongation axis of the nucleus; and (iii) a compact uniform DNA orientation and in mature sperm cells (from left to right in Figure A). Our previous studies and preliminary results (Fig. A, C) suggest the entire genome folds as a nucleus-scale (liquid) crystal in mature sperm. Polymer biophysical modelling reveals that this spatio-temporal pattern is compatible with a nucleated increase in chromatin rigidity, which would critically determine the 3D genome organization (Fig. B).

G. bimaculatus is an emerging model organism offering a paradigmatic case of folding principles and mechanisms conserved in other animals, including mammals.



(A) Histone-to-protamine transition revealed by immunofluorescence in a testicular follicle of an MstGb1-GFP transgenic cricket. (B) Polymer modeling of DNA folding upon spermiogenesis. (C) CryoEM on a mature sperm nucleus reveals DNA organization as a hexagonal lattice. (D) Polyelectrolyte simulation of DNA (grey) and protamine (red) self-assembly recapitulates a hexagonal lattice.

Methods and internship program.

We will use cryo electron microscopy and tomography (cryo-EM/ET) of vitreous sections, combined with biophysical modelling. Cryo-EM/ET provides visualization of macromolecular complexes in flash-frozen tissue and cells and at nm to sub-nm resolution. We have recently demonstrated its potential to resolve [nucleosome and chromatin folding *in situ*](#). Preliminary observations reveal that the genome of mature sperm nuclei adopts a crystal-like structure with compact hexagonal order (Fig. C), as in [rods](#) and [toroids](#) *in vitro*. We will follow structural transitions during spermiogenesis by cryoEM/ET of whole testes to access the successive chromatin structural states during spermiogenesis and follow the transformation from the nucleosome bead-on-string chromatin expected in early cysts to the unidirectional hexagonal dense packing in mature sperm cells. In parallel, we will use polarization microscopy, a simple tool to monitor DNA ordering *in vitro* and *in situ*.

According to the candidate background and interests, cryo-EM/ET will be coupled to computational modelling. We will simulate DNA local bundling via protamines at the local scale. We will model DNA and proteins as polyelectrolytes with a coarse-grained bead-spring model, using the numerical tools of statistical mechanics. Preliminary simulations recapitulate DNA organization as a hexagonal lattice (Fig. D), compatible with cryo-EM *in vitro* and preliminary results *in situ* (Fig. C). In the long term, we will seek to model the histone-to-protamine exchange dynamics leading to a genome-scale hexagonal lattice, to predict which quantitative and dynamic parameters are critical.

Supervision

Amélie Leforestier (DR2 CNRS) leads the “Self-assembled biological objects” team at Laboratoire de Physique des Solides (LPS, Paris-Saclay) and a fully equipped cryo-EM facility. She is a specialist of condensed states of DNA and chromatin *in situ* and *in vitro*.

Guillermo Orsi (CRCN INSERM) leads the “Epigenetics and Chromatin” team at the Institute for Advanced Biosciences (IAB), Grenoble, and the Epinomics platform for epigenomics. Over the years, the team has developed multidisciplinary approaches to study mechanisms of gene regulation and gametogenesis.

Yves Lansac (Associate Professor), is an expert in molecular modelling applied to soft matter and biophysical systems, in particular DNA and nucleosomes.