

Deciphering the structural environment of transcription *in situ*: interplay between RNA polymerase II and chromatin structure

Project aim & background

Eukaryotic genomes are compacted in the nucleus as chromatin, an array of nucleosomes (~147 bp of DNA wrapped around a histone octamer) connected by short linker DNA segments. This *bead-on-string* structure restricts access to DNA sequences, modulating all nuclear processes, including transcription, a key step of gene expression. In eukaryotes, transcription is catalysed by RNA polymerase II (Pol II). Nucleosomes stand a major energy barrier to Pol II progression, which is associated to partial disassembly and displacements of the particle. Despite many structural studies *in vitro*, the interplay between chromatin organization and transcription remains poorly understood, especially *in situ* where the local chromatin architecture during transcription is unknown. Our project explores how transcription couples to local chromatin organisation, assembly and disassembly *in situ*. Using cryo electron tomography (cryo-ET) combined with image analysis and simulations, we aim at characterizing the variety of nucleosome, Pol II and chromatin fold conformations within transcriptionally active regions of interphase polytene chromosomes.

Methods and internship program.

Cryo-ET provides visualization of macromolecular complexes at work within flash-frozen tissue and cells and at nm to sub-nm resolution. We have recently demonstrated the potential of this approach to resolve [nucleosome and chromatin folding *in situ*](#), and explore their structural heterogeneity in relationship with their functional compartmentalization. Transcription hotspots will be targeted by cryo correlative light and electron microscopy (cryo-CLEM) in native *Drosophila* polytene chromosomes with GFP and m-Cherry labelled Pol II subunits. We will rely on our recent technical developments of specimen preparation and a deep learning-based method dedicated to automation of polymorphic particle picking in complex and crowded environments. The experimental internship includes complete cryo-ET workflow from sample preparation (sample vitrification by high pressure freezing, cryo-sectioning, cryo-CLEM) to cryo-tomogram acquisition on high end instruments. According to the advancement, it will also include first data analyses: tomogram reconstruction & denoising, particle picking & segmentation, sub-tomogram averaging. We dispose of a large set of preliminary data for training and test of data analysis tools.

Supervision

We are seeking candidates with a background in Cell Biology, Biophysics and/or Physics. The student will be supervised by Amélie Leforestier (LPS, Orsay) & M. Eltsov (IGBMC, Strasbourg), biophysicists specialist of cryo-EM/ET of DNA, nucleosomes and chromatin. He/she will be hosted by the Laboratoire de Physique des Solides (Université Paris-Saclay), with travels to IGBMC. This internship is part of a global interdisciplinary collaborative work gathering biophysicists (A. Leforestier & M. Eltsov), physicists (J.M. Victor & M. Barbi, LPTMC, Sorbonne Université: multiscale chromatin modelling), and data analyst (M. Harastani, Institut Pasteur: deep learning, macromolecular conformational variability).

