**Sujet de stage M2 2023-24**

**Mammalian epigenomics team**

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**Title: Cryo-EM analysis of subnucleosomal particles generated in open chromatin regions**

The binding of transcription factors (TFs) to cis-regulatory elements (CREs) is a major step in the mechanism that controls gene transcription. TFs interact with specific DNA motifs (6-25 bp), enriched at promoters and enhancers. The mammalian genome is packaged into nucleosomes, and the tight interaction of DNA with histones makes the DNA sequence inaccessible to DNA-binding proteins, thus generating a barrier to the binding of TFs. This barrier can be relieved by members of a large family of enzymes called ATP-dependent chromatin remodelers, which interact with nucleosomes to alter their positioning and occupancy. The Gérard team showed that chromatin remodelers are enriched at CREs, where they play an essential role in generating "open" chromatin regions (REF 1). Open chromatin has been identified as regions of increased DNA accessibility using nuclease and transposase-based methods. However, the nature of the open chromatin at the molecular level is not well defined.

Recently, the Gérard team performed an in-depth analysis of open chromatin regions at CREs in mouse embryonic stem (ES) cells, using chromatin fragmented by micrococcal nuclease (MNase) as input for ChIP-seq with antibodies against histones and high-coverage deep-sequencing (REF 2). This analysis revealed that the open chromatin of promoters and enhancers contains a discrete combination of interspersed fragile nucleosomes, subnucleosomal particles, and histone-free DNA regions. In particular, we identified a new class of subnucleosomal particles containing the four core histones H2A, H2B, H3 and H4 associated with 50-80 bp of DNA.

We showed that the SWI/SNF chromatin remodeler generates these particles in mouse embryonic stem cells (mESCs). We found that these subnucleosomal particles interact with TFs independently of DNA motifs, revealing a new mechanism for the recruitment of TFs at CREs in mammalian cells (REF 2).

The M2 student will be involved in a team project dedicated to exploring the structure of this new class of subnucleosomal particles. First, she/he will purify the subnucleosomal particles from mESC. Second, the student will contribute to the analysis of their structure by cryo-EM in collaboration with the team of Pierre Legrand (Soleil synchrotron).

**References:**

1. de Dieuleveult, M., Yen, K., Hmitou, I., Depaux, A., Boussouar, F., Dargham, D.B., Jounier, S., Humbertclaude, H., Ribierre, F., Baulard, C., et al. (2016). Genome-wide nucleosome specificity and function of chromatin remodellers in ES cells. Nature *530*, 113–116. 10.1038/nature16505.

2. Nocente, M.C., Karamitsos, A.M., Drouineau, E., Albawardi, W., Dulary, C., Ribierre, F., Picaud, H., Alibert, O., Acker, J., Aude, J.-C., et al. (2022). BRG1 generates subnucleosomes that expand OCT4 binding and function beyond DNA motifs at enhancers (Genomics) 10.1101/2022.09.15.507958.

**Mots Clefs**

Nucleosome, subnucleosome, chromatin remodeler, cis-regulatory element, cryo-EM