**M2 Internship description**

**Chromatin Dynamics Team**

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 <https://www.i2bc.paris-saclay.fr/equipe-chromatin-dynamics/>

*Functional dissection of a chromatin domain boundary in mouse embryonic stem cells*

The three-dimensional (3D) organization of chromosomes is an essential component of gene regulation in mammalian cells. The promoters of many genes form DNA loops with enhancer elements that are located far away on the chromosome, thereby regulating their activity. This process is aided by the presence of TADs, which are insulated 3D chromatin domains that promote the formation of contacts within the same domain and prevent contacts between neighboring domains. Perturbation of TADs leads to a range of diseases and embryonic defects, confirming the importance of these domains for gene regulation (see e.g. Lupianez et al, 2015).

TADs are formed by the binding of a specific protein, the CTCF insulator protein, at their boundaries. The Noordermeer team at the I2BC (Gif-sur-Yvette) has recently shown that most TAD boundaries contain multiple binding sites for this protein (Chang et al, 2021). This clustered binding of CTCF adds a new level of gene regulation, through its capacity to influence the formation of enhancer-promoter loops. The ultimate aim of the team is to decipher and functionally characterize this regulatory grammar of CTCF binding.

The aim of this M2 project is to functionally dissect the contribution of individual CTCF binding sites to the formation of TADs. We will focus on a TAD boundary where 4 CTCF binding sites are clustered (see Chang et al, 2021). Genome-editing will be used to remove individual or combinations of CTCF binding sites at this boundary in mouse embryonic stem cells. The functional impact of these deletions will then be determined using genomics approaches, including the effect on CTCF binding elsewhere at the boundary (ChIP-seq), the integrity of neighboring TAD structure (tiled Capture Hi-C, see Franke et al., 2016) and the effect on gene activity. Depending on the progress of the project, similar genome-editing experiments will be performed in embryonic stem cells where other factors involved in TAD formation can be removed.

This M2 project will incorporate:

1. Removal of CTCF binding sites in mouse embryonic stem cells using CRISPR-Cas9 genome editing.

2. Analysis of genome-wide CTCF binding using ChIP-seq in WT and genome-edited mouse embryonic stem cells.

3. Analysis of 3D genome reorganization upon CTCF binding site deletions using Capture Hi-C.

The student will be supervised by the team leader, with support from a bioinformatician and an engineer in the team. The focus of the project will be on experimental (wetlab) studies, but will incorporate a limited amount of bioinformatics as well.

**Techniques used during the internship**

Embryonic stem cell culture

Genome editing

CTCF

ChIP-seq

Capture Hi-C

Bioinformatics analysis of Hi-C data (under direct supervision of a bioinformatician in the team)

**Bibliography**

* D. G. Lupianez, K. Kraft, V. Heinrich, P. Krawitz, F. Brancati, E. Klopocki, et al. **Disruptions of topological chromatin domains cause pathogenic rewiring of gene-enhancer interactions (2015)** Cell 161, Pages 1012-1025.
* L. H. Chang, S. Ghosh, A. Papale, M. Miranda, V. Piras, J. Degrouard, et al. **A complex CTCF binding code defines TAD boundary structure and function (2021)** bioRxiv. <http://biorxiv.org/content/early/2021/04/15/2021.04.15.440007.abstract>
* M. Franke, D.M. Ibrahim, G. Andrey, W. Schwarzer, V. Heinrich, R. Schöpflin, et al. **Formation of new chromatin domains determines pathogenicity of genomic duplications (2016)** Nature 538, pages 265-269.

**The team**

The Chromatin Dynamics team studies the function of 3D genome organization in biological processes, with a focus on gene regulation. They primarily focus on mammalian cells, including models for human disease (cancer and imprinting disorders). The team consists of 3 permanent scientists, 2 PhD students a postdoc and a research engineer.

See website for more detail and recent publications:

<https://www.i2bc.paris-saclay.fr/equipe-chromatin-dynamics/>

The team is part of the I2BC and will move to new rooms in September 2023.

**How to apply**

Send an email, including your CV and your reasons to apply for the project, directly to the main supervisor: daan.noordermeer@i2bc.paris-saclay