2023-12-19

International PhD Program IC-PhD

PhD thesis project

2024 Call for application

Role of architectural proteins in the spatial organization of the Bombyx mori genome and Mitotic chromosome architecture in holocentric insects

General information

Call	2024
Reference	2024-09-MULLER
Keyword(s)	Genome 3D architecture; comparative genomics; holocentromeres; Hi-C; mitosis.

Director(s) and team

Thesis director(s)	Héloïse Muller
Research team	Evolution of centromeres and chromosome segregation
Research department	UMR3664 - Nuclear dynamics

Description of the PhD thesis project

Abstract

Chromosome number abnormalities are common in cancer and often result from centromere defects. To understand how centromere dysregulation is associated with malignancies, it is essential to explore the range of natural behaviors through the study of biological diversity. We focus on the fundamental role of centromeres in 3D chromosome organization in interphase and mitosis by analyzing holocentric organisms exhibiting a drastic evolution in centromere layout, where centromeres distribute over the entire length of chromosomes. We initiated our study on the silkworm and described its unconventional 3D genome organization in interphase. This reveals a unique third type of compartment, in addition to the conventional active A and inactive B. We labeled it "S" for "secluded," as it frequently makes short-range contacts and very rarely engages in long-range contacts. The project investigates the role of architectural proteins in organizing this peculiar genome's spatial architecture. We will systematically investigate the roles of cohesin, condensin, and insulators through Hi-C and ChIP-seq experiments in both normal and perturbed conditions. To understand the interplay between centromere organization and genome conformation, we need to characterize functional centromeric regions during mitosis. The analysis of this specific cell cycle stage will also shed light on how architectural proteins contribute to shaping centromeric chromatin when its function is most crucial.

Background

The group of Ines Drinnenberg in Institut Curie focuses on the evolution of centromeric regions, using the silkworm *Bombyx mori* as a model organism. *B. mori* has an unconventional centromere organization, called holocentric, where chromosomes have no primary constriction point for kinetochore formation and spindle

attachment during cell division, but instead have multiple spindle attachment points all along their length. As centromeres have been shown to be majors players in chromosome architecture, we are using this model to understand the relationship between the linear and spatial organization of genomes. To evaluate the interplay between centromere organization and genome architecture we first need to describe them in interphase and mitotic cells of *B. mori*. We already described conserved and specific features in interphase. At the genome-wide scale, chromosome form very strong territories with very sparse signal in the interchromosomal space, a specific signature of the absence of regional monocentromere. At the chromosomal scale, chromosomes do not only segregate between two compartments (A: active, B: inactive); instead, we identified a third type of compartment. We labeled it "S" for "secluded," as it frequently makes short-range contacts and very rarely engages in long-range contacts. This 3rd compartment segregates away from both A and B, seems to host a specific combination of genetic and epigenetic features of both and shows strong loop extrusion signatures.

Objectives

In other organisms, chromosome architecture has been shown to results from the interplay between phenomena dictated by the epigenetic status of loci (compartments), and others that result from the intervention of so-called architectural proteins (TADs; loops, …). Among these, we find SMCs, and in particular cohesin and condensin, but also anchor proteins or complexes, like CTCF, Beaf-32, Mod(mdg4), CP190, etc…, that are positioned at architectural borders. To complement our analysis, we propose to evaluate the role of the homologs of architectural proteins that are found in *B. mori* in mediating the described genome architecture.

In mitosis, centromeres are the cornerstone of chromosome architecture. For mitotic segregation to occur, chromosomal DNA must be highly compacted and centromere regions with associated kinetochore proteins appear pointing outward from sister chromatids. First, we propose to isolate mitotic cells from *B. mori* embryos and analyze the localization of kinetochore at this stage to identify regions of metaphasic chromosomes that function in segregating *B. mori* chromosomes. In parallel, we will describe the 3D architecture of chromosomes as well as the localization of SMC complexes, believed to play a critical role in metaphasic chromosomes organization. This study will allow us to explore how the organization described in monocentric organism can be extrapolated to holocentrics as well as exploring the role of the kinetochore in metaphase chromosome folding.

Experimental approaches

During his training, the student will have the opportunity to learn a large number of technics, from basic molecular biology to high-throughput and NGS production and analysis. He will also be familiarized with the difficulties of studying a non-model organism through very trendy and state-of-the-art problematics such as the 3D organization of chromosomes inside the nucleus.

Aim I. Role of architectural proteins in the spatial organization of the *B. mori* genome

To obtain the chromosomal distribution of proteins of interests, the student will perform ChIP-seq experiments using cloned and tagged proteins transfected to cell lines or using some custom antibodies currently being produced. Then, the student will carry out perturbation experiments on architectural proteins in *B. mori* by RNAi depletion and analyze the consequences on the 3D organization using Hi-C. Finally, he will consolidate both sets of experiments to conclude about the implication of the architectural proteins in the 3D genome architecture, and in particular in forming topological frontiers.

Aim II. Mitotic chromosome architecture in holocentric insects

After FACS cell sorting of M-phase cells, the student will perform ChIP-seq experiments to systematically investigate the localization of cohesin, condensin I and II and kinetochore along the compacted chromatin and analyze these results in light of the 3D architecture of metaphasic chromosome (determined by Hi-C).

International, interdisciplinary & intersectoral aspects of the project

The preliminary part of this project, which will be published soon, already involved collaborations which will be continued with Leah Rosin (NIH) for microscopy and Leonid Mirny (MIT) for biophysical modeling. In addition, we also collaborate with Susumu Katsuma (University of Tokyo) for the expertise in genome modification and RNAi techniques in B. *mori* embryos.

Recent publications

1. Gil Jr., J., Rosin, L., Navarrete, E., Chowdhury, N., Abraham, S., Cornilleau, G., Lei, E., Mozziconacci, J, Mirny, L., **Muller, H**.#, Drinnenberg, I.# (2023). Holocentric Bombyx mori chromosomes are highly territorial and segregate into three genome-wide compartments. BioRxiv

2. Senaratne, A. P., **Muller, H.**, Fryer, K. A., Kawamoto, M., Katsuma, S., and Drinnenberg, I. A. (2021) Formation of the CenH3-Deficient Holocentromere in Lepidoptera Avoids Active Chromatin. Curr. Biol. CB. 31, 173-181.e7

3. **Muller, H.**, Gil Jr., J., and Drinnenberg, I. A. (2019) The Impact of Centromeres on Spatial Genome Architecture. Trends Genet. TIG. 35, 565–578

4. **Muller, H.***, Scolari, V. F.*, Agier, N., Piazza, A., Thierry, A., Mercy, G., Descorps-Declere, S., Lazar-Stefanita, L., Espeli, O., Llorente, B., Fischer, G., Mozziconacci, J., and Koszul, R. (2018) Characterizing meiotic chromosomes' structure and pairing using a designer sequence optimized for Hi-C. Mol. Syst. Biol. 14, e8293

5. Mercy, G., Mozziconacci, J., Scolari, V. F., Yang, K., Zhao, G., Thierry, A., Luo, Y., Mitchell, L. A., Shen, M., Shen, Y., Walker, R., Zhang, W., Wu, Y., Xie, Z.-X., Luo, Z., Cai, Y., Dai, J., Yang, H., Yuan, Y.-J., Boeke, J. D., Bader, J. S., **Muller, H.**#, and Koszul, R.# (2017) 3D organization of synthetic and scrambled chromosomes. Science. 355(6329):eaaf4597.

Expected profile of the candidate

We are looking for a highly motivated candidate with excellent academic results and a good theoretical knowledge of genetics and genomics. Laboratory experience in basic molecular biology techniques (PCR, agarose gel electrophoresis, cloning, etc.) is required. Dry-lab experience in bioinformatics would be a plus. A basic knowledge of a computer language such as python is required, and at least a very good willingness to develop bioinformatics skills will be necessary, as the student will be expected to analyze the NGS data produced.