ÉCOLE NORMALE SUPÉRIEURE DE LYON

Master 2 internship offer

Chromosome Fragility: understanding how DNA replication in S phase impinges upon condensin-mediated chromosome assembly during mitosis

Key words: 3D genome, mitotic chromosome, condensin, SMC, chromatin dynamics

Where: Laboratoire de Biologie et Modélisation Cellulaire, CNRS/ENS-Lyon. ENS-LYON, 46 allée d'Italie 69007 Lyon. Team P-Bernard: Chromatin Dynamics in Mitotic Chromosome Assembly http://www.ens-lyon.fr/LBMC/equipes/architecture-et-dynamique-fonctionnelle-des-chromosomes.

When: flexible. **Duration:** ~6 months.

Supervisor: Pascal BERNARD (CNRS senior scientist and team leader). **Contact:** first-name.last-name[at]ens-lyon.fr

Current ENS students: Leonard COLIN, Jeremy LEBRETON (contact at first-name.last-name[at]ens-lyon.fr)

Technologies used during the internship

Molecular genetics to control gene expression and protein levels ● Fluorescent microscopy on chromosome spreads ● High resolution ChIP-seq, BrdU-IP-seq and Hi-C genomic approaches ● Bio-informatic analyses.

Candidate profile

- Languages: French or English.

- Good background in basic molecular genetics (DNA replication, chromatin structure and chromosome organization...).

- Curiosity, autonomy and good organization and presentation skills.

- Basic knowledge of the UNIX and *R* programming languages for genomic data analysis is not mandatory, but would be a plus.

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Project description

Pictures of X-shaped metaphase chromosomes abound in school textbooks and yet how such fascinating 3D mega-structures are assembled remains unclear. In essence, mitotic chromosome condensation is the spatial reorganisation of a duplicated chromatin fibre into a new 3D structure, shaped for accurate segregation in anaphase. We know that the protein complex named condensin drives mitotic chromosome assembly[1] (Fig. 1), but the underlying mechanism remains poorly understood. In vivo, condensin binds DNA upon mitotic entry and folds chromatin fibres into arrays of loops, as shown by Hi-C (Fig. 1A). In vitro, condensin extrudes naked DNA into loops of increasing size (Fig. 1A). Such loop-extrusion activity can fully explain the structural properties of mitotic chromosomes. However, inherent to the extrusion reaction is the issue of roadblocks and steric hindrances. Currently, how condensin binds DNA and could conceivably extrude it into loops in the context of a chromatinized genome remain largely unknown.

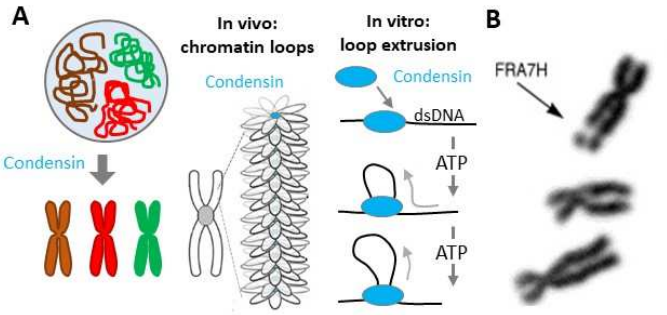

Figure 1. Condensin-mediated mitotic chromosome assembly
A. Condensin shapes mitotic chromosomes by folding chromatin into arrays of loops, possibly via loop extrusion. **B.** An un-condensed lesion in metaphase chromosome at the chromosome fragile site FRA7H.

Our published work and others have shown that histone PTMs, the density of nucleosomes, and arrays of various DNA-binding proteins, play positive or negative roles in the binding of condensin to DNA and mitotic chromosome condensation[2–5]. We aim to understand how chromatin dynamics accommodates condensin in vivo[6]. To investigate this in an integrated manner, we use the fission yeast *S. pombe* as a model system; this single-celled eukaryote being a renowned model for chromatin dynamics and chromosome structure.

Besides the incidence of chromatin, if not related to it, many studies performed in a wide range of organisms suggest the existence of an as-yet enigmatic connection between condensin and DNA replication. A striking manifestation is observed at chromosome fragile sites (CFS) in mammals[7]. CFS are hard-to-replicate regions of the genome whose recurrent instability drives tumorigenesis. Upon replication stress, CFS fail to replicate during S phase and fail to bind condensin in mitosis[7]. They form un-condensed gaps in metaphase chromosomes (Fig. 1B) and cause either chromosome breakage or mitotic segregation errors. Remarkably, hard-to-replicate regions are a conserved feature of eukaryotic genomes, and yet we ignore how a replication stress impinges upon the binding of condensin in the subsequent mitosis. **We collected preliminary results supporting a conservation in fission yeast of a link between replication and condensin. The objective of this internship is to investigate the underlying mechanisms by using (i) state of the art genomic and microscopy approaches and (ii) the exploratory power of the fission yeast system.**

Rational & Preliminary data

We have shown that chromatin micro-environments impinge upon the binding of condensin to DNA and chromosome condensation[2,4,5]. It has also been shown that a replication stress can modify the chromatin landscape. Thus, stalled replication forks might disrupt the establishment or the maintenance of chromatin features conducive for condensin in mitosis (i.e. priming

model). Alternatively, they might propagate chromatin modifications in cis and until mitosis that antagonize condensin binding (antagonism model).

Fission yeast telomeres are hard to replicate. We found that condensin is enriched at telomeres in normal cells arrested in metaphase[5], but strongly reduced when replication is impeded at telomeres, or when their replication timing is switched from late to early S phase, consistent with a conserved coupling with DNA replication. We reasoned that if condensin holds an activity linked to DNA replication, then specific condensin point mutations might impair such activity and confer a hypersensitivity to a replication stress caused by a low (sublethal) dose of the DNA synthesis poison HU. We isolated HU-hypersensitive mutations in condensin. We found that they cause chromosome segregation defects in anaphase that are enhanced upon replication stress, reminiscent of CFS in mammals. **Our results support the existence in fission yeast of a coupling between DNA replication and condensin.**

The aims of the internship

Since hard-to-replicate regions are not restricted to telomeres, the objective is to assess/verify that the link between replication and condensin operates throughout the genome, as it is the case in mammals. To this end, the student will:

(i) modify DNA replication at the genome-wide scale (from totally inhibited to partially impaired) and assess the impact on condensin in metaphase-arrested cells. DNA replication and condensin localisation will be examined by fluorescent microscopy on metaphase chromosome spreads, and at higher resolution by BrdU-IP-seq and calibrated ChIP-seq, respectively. Signals will be compared to assess whether DNA replication per se is needed for condensin binding or whether a replication stress exerts an antagonistic effect.

(ii) assess the formation of mitotic loops upon replication stress. If indeed HU-sensitive condensin mutations impact the link with replication, then the formation of mitotic loops is expected to be altered in mutant cells upon HU treatment. This will be assessed by performing Hi-C on metaphase-arrested cells treated or not with HU.

On a longer-term, these data will be used to further investigate the mechanisms that link mitotic chromosome assembly to DNA replication.

Bibliography

From the hosting team

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