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Titre :

Understanding the enigmatic link between DNA replication in S phase and condensin-mediated chromosome assembly during mitosis

keywords: condensin, mitotic chromosome condensation, DNA replication, fission yeast

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Team: Chromatin Dynamics in Mitotic Chromosome Assembly
https://www.ens-lyon.fr/LBMC/equipes/architecture-et-dynamique-fonctionnelle-des-chromosomes
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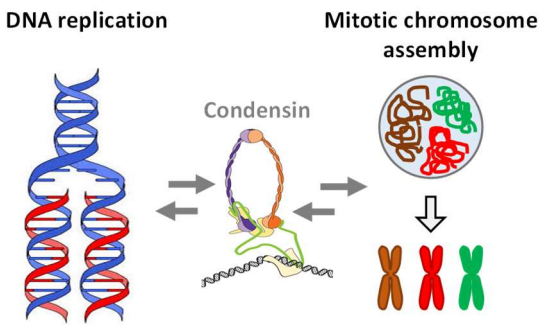
Sujet de stage :



In eukaryotes, DNA replication and the condensation of mitotic chromosomes driven by the condensin complex are two distinct processes, separated in time by the G2 phase. Yet, accumulating evidence indicates that those two essential pathways are functionally linked, that such a link underlies the biogenesis of chromosome fragile sites driving cancer, and that condensin may play a key role in this linkage between DNA replication and mitotic chromosome assembly. **The overall objective of this internship is to decipher the molecular bases of the link between DNA replication, condensin and mitotic chromosome condensation by using a combination of state-of-the-art genomic approaches, microscopy and molecular genetics, and the exploratory power of the fission yeast biological system.**

Condensin is a genome-organizing enzyme that loads onto DNA upon mitotic entry and shapes mitotic chromosomes by extruding DNA into loops[1,2]. How condensin functions in the crowded context of chromatin remains poorly understood. We and others have shown that condensin-mediated chromosome assembly is modulated by the structure of chromatin during mitosis[3–5]. Other studies point towards an unexpected link with DNA replication. It has been observed in human cells[6],

Drosophila[7,8], *C. elegans*[9] and budding yeast[10] that defects in DNA replication (during S phase) impair the association of condensin to DNA in mitosis and/or mitotic chromosome condensation. In human cells, replication stress renders chromosome fragile sites (CFS) refractory for condensin binding during mitosis[6], which creates uncondensed gaps in mitotic chromosomes and drives tumorigenesis. Together these data suggest the existence of an evolutionarily conserved coupling between DNA replication and condensin-mediated chromosome condensation in mitosis. Yet it remains totally unknown how a replication could conceivably impinge "at distance" upon condensin binding in mitosis. Conversely, there is also experimental evidence that condensin itself may take part in DNA replication during S phase[11], but the mechanism remains unknown as well. Thus, current data indicate that DNA replication and mitotic chromosome condensation are functionally linked, despite being separated through time and, though the nature of such a link remains unknown, condensin appears as a prime candidate for "connecting" replication to mitotic chromosome condensation.

Our team studies the integrated functioning of condensin using the fission yeast *S. pombe* as a "simple" and genetically tractable model system. We identified condensin point mutations that confers a hypersensitivity to replication stress and lead to mitotic phenotypes reminiscent of CFS in human cells. By combining functional genomics (Pu-seq), proteomics (ID of condensin binding partners), fluorescent microscopy (to assess mitotic chromosome segregation), we collected further evidence that condensin takes part in DNA replication and, reciprocally, that replication impinges upon mitotic chromosome condensation in fission yeast as in other eukaryotes. Thus, we are in an ideal position to decipher the nature of the interplays between condensin and DNA replication thanks to the exploratory power of the fission yeast system. Notably, using genetics we screened for factors expected to connect condensin to replication. The objectives of this internship will be (1) to further analyse the impact of replication stress on chromatin structure and condensin binding to DNA in mitosis and (2) to further analyse the impact of condensin on DNA replication during S phase and its physical and functional interactions with DNA replication factors.

Modèle et techniques utilisées:

Biological system: the fission yeast *Schizosaccharomyces pombe*. **Techniques:** Cell synchronisation ● Creation and usage of experimentally-controlled mutants using the auxin-induced degradation system. ● Single cell condensation assay using fluorescent microscopy ● Condensin localisation and BrdU-incorporation assay (replication) using chromatin spreading and semi quantitative immunofluorescence. ● Protein co-immunoprecipitation. ● Calibrated ChIP-seq and MNase-seq to assess condensin binding and chromatin structure genome wide. ● Chromosome segregation assay using fluorescent microscopy.

Training outcomes: The student will learn (1) the above-mentioned techniques, (2) to conduct experiments and analyse results autonomously and (3) to present and discuss its results during lab meetings. If successful the internship may lead to a PhD position.

Publications d'intérêt (from the hosting lab)

1. Davidson, I. F. & Peters, J.-M. Genome folding through loop extrusion by SMC complexes. *Nat. Rev. Mol. Cell Biol.* **22**, 445–464 (2021).
2. Hirano, T. Condensin-Based Chromosome Organization from Bacteria to Vertebrates. *Cell* **164**, 847–857 (2016).
3. Toselli-Mollereau, E. *et al.* Nucleosome eviction in mitosis assists condensin loading and chromosome condensation. *EMBO J.* 35, 1565–1581 (2016).
4. Colin, L. *et al.* Condensin positioning at telomeres by shelterin proteins drives sister-telomere disjunction in anaphase. *eLife* 12, RP89812 (2023).
5. Lebreton, J. *et al*. RNAP II antagonizes mitotic chromatin folding and chromosome segregation by condensin. *Cell Rep.* 43, 113901 (2024).
6. Boteva, L. *et al.* Common Fragile Sites Are Characterized by Faulty Condensin Loading after Replication Stress. *Cell Rep.* **32**, 108177 (2020).
7. Loupart, M. L.*et al.* Aberrant replication timing induces defective chromosome condensation in Drosophila ORC2 mutants. *Curr. Biol. CB* **10**, 1547–1556 (2000).
8. Pflumm, M. F. The role of DNA replication in chromosome condensation. *BioEssays News Rev. Mol. Cell. Dev. Biol.* **24**, 411–418 (2002).
9. Sonneville, R. *et al*. Both Chromosome Decondensation and Condensation Are Dependent on DNA Replication in C. elegans Embryos. *Cell Rep.* **12**, 405–417 (2015).
10. Dulev, S., Aragon, L. & Strunnikov, A. Unreplicated DNA in mitosis precludes condensin binding and chromosome condensation in S. cerevisiae. *Front. Biosci.* **13**, 5838–5846 (2008).
11. Aono, N. *et al*, M. Cnd2 has dual roles in mitotic condensation and interphase. *Nature* **417**, 197–202 (2002).