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# Sen1-mediated RNAPIII transcription termination controls the positioning of condensin on mitotic chromosomes

Julieta Rivosecchi

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**Julietta RIVOSECCHI**

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**Sen1-mediated RNAPIII transcription  
termination controls the positioning of  
condensin on mitotic chromosomes**

L'hélicase Sen1 contrôle le positionnement de condensine sur les chromosomes  
en régulant la terminaison de la transcription par l'ARN polymérase III

---

Devant le jury composé de :

HERMAND, Damien	Maître de Recherche FNRS-Université de Namur	Rapporteur
LENGRONNE, Armelle	DR2 CNRS-IGH Université de Montpellier	Rapporteuse
MARCAND, Stéphane	DR CEA-Institut de Biologie François Jacob Paris	Examinateur
PADMANABHAN, Kiran	CR1 INSERM-IGFL ENS de Lyon	Examinateur
PALLADINO, Francesca	DR2 CNRS-LBMC ENS de Lyon	Examinatrice
VANOOSTHUYSE, Vincent	CR1 CNRS-LBMC ENS de Lyon	Directeur de thèse



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## Preface

The three-dimensional arrangement of the genome in the nucleus plays a fundamental role in regulating its activities (Fraser and Bickmore, 2007). Disruption of genome architecture is associated with genome instability such as mutations, chromosomal rearrangements and aneuploidy (Fudenberg et al., 2011). These abnormalities can ultimately lead to cancer (Corces and Corces, 2016). The study of genome architecture is a very dynamic and active field of research aiming to understand the relationship between nuclear organization and genome function.

At each cell division, the genome undergoes a dramatic reorganization to form highly compacted and individualized chromosomes, as seen classically as X-shaped chromosomes in metaphase (Figure 1). This process, called chromosome condensation, is essential for the proper transmission of the genome to daughter cells (Hirano, 2016). It was discovered 25 years ago already that the highly conserved condensin complex is a key driver of mitotic chromosome condensation (Hirano and Mitchison, 1994; Saka et al., 1994).

In the last few years, Chromosome Conformation Capture assays in different organisms have revealed that condensin organizes mitotic chromosomes into consecutive loops of chromatin organized around a central axis (Gibcus et al., 2018; Kakui et al., 2017; Naumova et al., 2013; Tanizawa et al., 2017). Very recently, *in vitro* data show how condensin can translocate on naked DNA and extrude DNA to form these loops (Ganji et al., 2018; Kong et al., 2019; Terakawa et al., 2017). However, how condensin behaves *in vivo* on chromatin, in the face of numerous obstacles such as nucleosomes, tightly DNA-bound proteins or the transcription machinery, is still unknown.

The association of condensin with chromatin is a prerequisite for chromosome condensation. How condensin is recruited to chromosomes is still unclear. Studies in various organisms have shown that condensin covers the whole genome but is specifically enriched near highly expressed genes in a transcription-dependent manner (D'Ambrosio et al., 2008; Downen et al., 2013; Gruber and Errington, 2009; Kim et al., 2013, 2016; Kranz et al., 2013; Nakazawa et al., 2015; Sutani et al., 2015). This suggests that transcription can impinge on the distribution of condensin along chromosomes. However, it is not clear whether these high-occupancy condensin sites represent loading sites or sites where condensin accumulates in the face of obstacles. Condensin peaks could represent sites where condensin is initially recruited. Alternatively, they could represent sites where condensin accumulates after sliding from its loading sites. Techniques used to assess genome-wide localization of condensin (based on Chromatin Immunoprecipitation) do not allow to distinguish between these two possibilities.

In vertebrates at least, the link between transcription and condensin is paradoxical, as condensin associates with chromatin mostly in mitosis, when transcription by all three RNA

polymerases is largely repressed (Gottesfeld and Forbes, 1997). How does transcription contribute to establish condensin-positioning sites in mitosis, when transcription is inactive? Recent works have demonstrated that transcription might be maintained at low levels in mitosis (Palozola et al., 2017) and that bursts of transcription at the onset of mitosis precede the full extinction of transcription (Liang et al., 2015), suggesting that transcription and condensin loading on chromosomes could temporally coexist. Some observations show that transcription factors could directly recruit condensin to chromatin at highly transcribed genes (D'Ambrosio et al., 2008; Iwasaki et al., 2010, 2015; Kim et al., 2016). Conversely, it has been suggested that a by-product generated during transcription such as nucleosome free regions at promoters of genes (Toselli-Mollereau et al., 2016), transcription-associated topological stress (Legros et al., 2014), ssDNA or RNA (Nakazawa et al., 2019; Sutani et al., 2015) could impact the loading/positioning of condensin in mitosis. Taken together, these observations suggest that transcription must play a crucial and conserved role in the recruitment and/or the positioning of condensin complexes along chromosomes. However, what transcription-associated features impact the loading/positioning of condensin remains poorly understood (Bernard and Vanoosthuyse, 2015; Robellet et al., 2016).

At the beginning of my PhD project, the literature was quite controversial on this topic. Some publications claimed a positive role for transcription in determining condensin positioning, but other works stated the opposite. During my project I focused on this intriguing interplay between transcription and condensin, using tRNA genes as models.

Prior to my arrival, the lab had used genetic screens in *Schizosaccharomyces pombe* to identify factors associated with the transcription machinery that impact the function of condensin (Vanoosthuyse et al., 2014). When I joined the lab, I focused on one of those transcription-associated regulators of condensin, a conserved DNA/RNA helicase called Sen1. The lab had shown previously that the deletion of Sen1 was able to partially suppress the growth defects of a condensin mutant (Legros et al., 2014).

During my 4 years in the lab (M2 + PhD), I characterised Sen1 as a factor preventing the accumulation of condensin specifically in the vicinity of RNA polymerase III (RNAPIII)-transcribed genes (Chapter 1). I demonstrated that Sen1 is a cofactor of RNAPIII, required for efficient transcription termination (Chapter 2). Finally, I described how the function of Sen1 in RNAPIII transcription termination underlies its role in the positioning of condensin at RNAPIII-transcribed genes (Chapter 3).

Two main contributions emerged from my work:

- 1) The identification of Sen1 as the first accessory factor required for RNAPIII transcription termination, challenging the current model that RNAPIII does so autonomously. This work is published in *EMBO Journal*.
- 2) The demonstration that the quality control of RNAPIII transcription contributes to the positioning of condensin in mitosis.



# Introduction

## I. Genome architecture changes throughout the cell cycle

Eukaryotic genomes must be carefully folded and packaged to fit inside the small space of the cell nucleus. The length of total DNA in a single human cell, if stretched out, is nearly 2 meters long, but it is folded inside a nucleus 5-10  $\mu\text{m}$  in diameter. Numerous cellular processes share the same DNA template, including for example transcription and DNA replication, and require the genome to remain accessible. It is therefore crucial that chromosomes are folded in a way that is compatible with these essential cellular processes and also with processes that require a more compact and rigid genome structure, i.e. the segregation of chromosomes towards the two daughter cells in mitosis (Fraser et al., 2015).

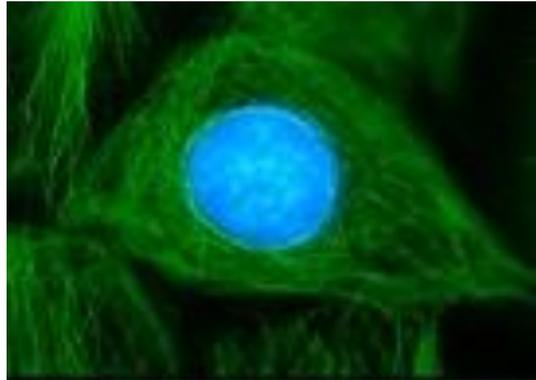
As cells progress through the cell cycle, chromosomes undergo dramatic morphological changes. Decondensed and loose chromosomes in interphase (Figure 1A) are rapidly and efficiently packaged into highly compact mitotic chromosomes (Figure 1B). Thus, chromosome condensation at the onset of mitosis occurs in a short period of time and must therefore be highly efficient. This massive reorganization of genome architecture at each cell division requires the action of dedicated machineries. One of these protein machines, known as condensin, belongs to the Structural Maintenance of Chromosomes (SMC) family of proteins. The conserved SMC complexes, including cohesin and condensin, associate with DNA and influence a large variety of DNA-based processes, including sister chromatid cohesion, chromosome condensation, transcription and replication (reviewed in Hirano, 2016). The role of condensins in shaping mitotic chromosomes is the focus of my research.

In this general introduction, I will first present an overview of genome organization through the cell cycle. I will then introduce the different SMC complexes and their roles in genome organization. Finally, I will focus on the SMC condensin complex and present the currently available data describing the possible links between gene transcription and condensin function.

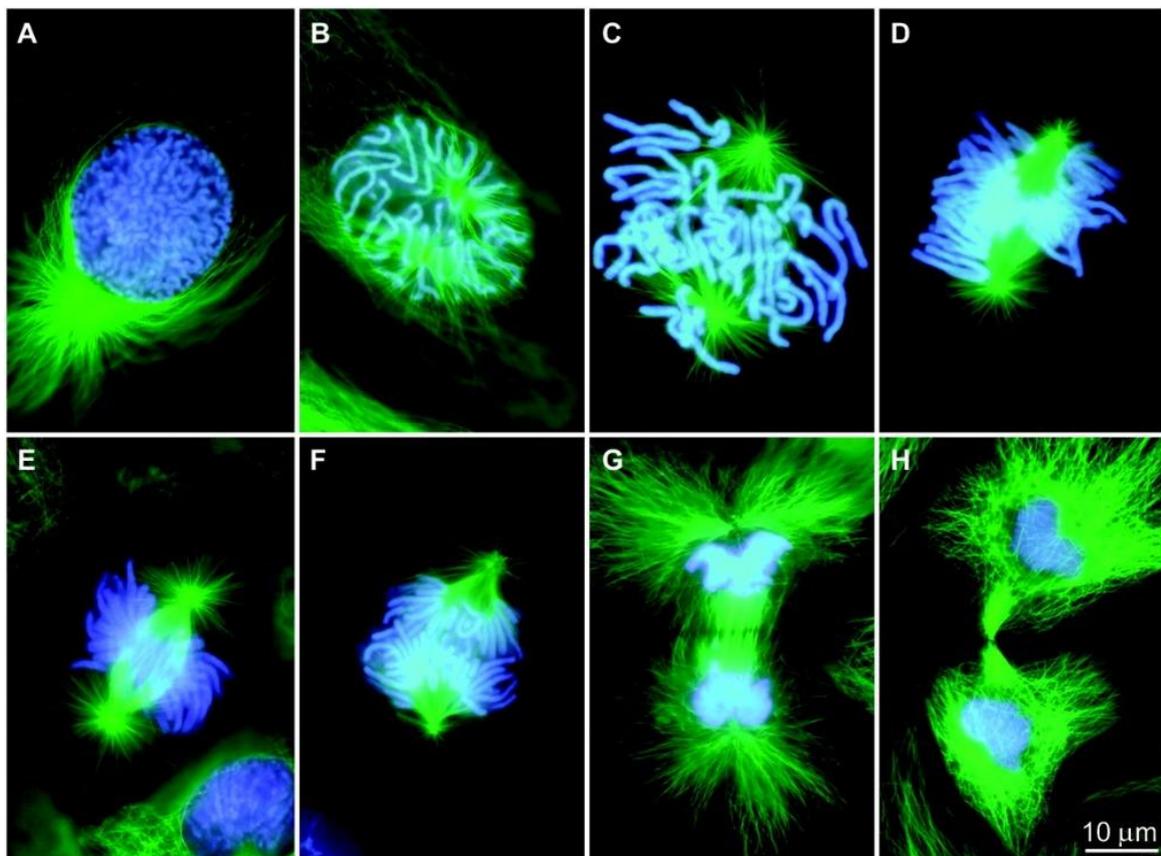
### A) Genome organization in interphase

The folding of interphase chromosomes follows a hierarchical organization. In recent years, microscopic techniques such as Fluorescence *In Situ* Hybridization (FISH) and molecular approaches including Chromosome Conformation Capture (3C) technologies allowed scientists to interrogate chromatin organization at multiple resolutions (reviewed in Fraser et al., 2015). In particular, the high-throughput version of the 3C method, called Hi-C, enables the detection of chromatin contacts

A



B



**Figure 1: Chromosome compaction changes during the cell cycle.** Fluorescent micrographs of mitotic newt lung cells. Microtubules are stained with Anti- $\beta$ -Tubulin. Chromosomes are counterstained with Hoechst 33342. **(A)** In interphase, chromatin is in its least condensed state and appears loosely distributed throughout the nucleus. **(B)** Reorganization of chromosomes in mitosis. Stages of mitosis: A-B: prophase. Chromosome compaction begins during prophase. C: prometaphase. Chromosomes are highly compacted. D-E: Metaphase. Alignment of chromosomes at the metaphase plate. F: anaphase. Chromosomes are segregated into daughter nuclei. G: telophase. In late anaphase and telophase the mitotic chromatin decondenses to re-establish its interphase structure. H: cytokinesis. From Rieder & Khodjakov, 2003.

at a whole-genome scale (Lieberman-Aiden et al., 2009). The progressive improvement in the resolution of Hi-C techniques in the last decade uncovered general principles of chromosome folding (Figure 2) (Szalaj and Plewczynski, 2018).

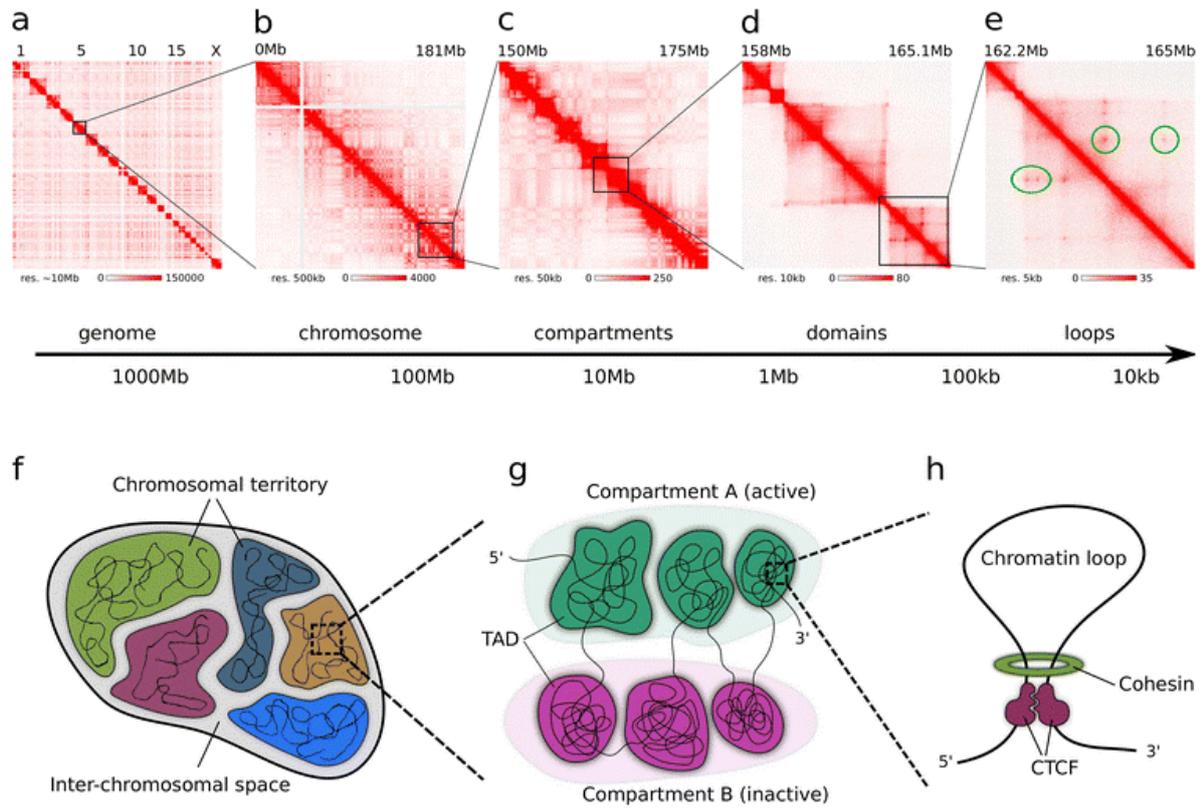
At the level of the whole nucleus, individual chromosomes occupy separated territories that are irregular in shape but typically about 1–2  $\mu\text{m}$  in diameter (Figure 2A, B and F). In higher eukaryotes, every interphase chromosome has its preferred location and preferred neighbouring chromosomes within the nucleus. These chromosome territories constitute the largest feature of nuclear architecture (Cremer and Cremer, 2010).

At the megabase level, chromosomal arms segregate into regions of preferential long-range interactions that form two mutually excluded types of chromatin, referred to as “A” and “B” compartments ( $\sim 5$  Mb in size) (Figure 2C and G). “A” compartments correlate with gene density, transcriptional activity, chromatin accessibility, and activating chromatin marks. “B” compartments are mostly enriched in repressive chromatin. Each compartment is characterized by extensive contacts with multiple domains of the same type (A or B) (Nagano et al., 2017; Rao et al., 2014).

At the scale of several hundreds of kilobases (kb), chromosomes fold into smaller domains. Chromatin interactions are much favoured within a domain compared to inter-domain interactions with neighbouring chromatin domains on the same chromosome. These contacts ( $\sim 1$  Mb in size) are referred to as “topologically-associating domains” (TADs) and have been described in many species, indicating that they may represent a conserved feature of genome organization (Figure 2D and G) (Dekker and Heard, 2015; Lieberman-Aiden et al., 2009).

At lower scales (10-100 kb), chromatin is able to form long-range interactions in which two distant DNA segments are brought close to each other forming a loop (Figure 2E and H). These loops exhibit a great variability in their length and stability. Different types of loops may be involved in various cellular mechanisms. One of the best-characterized function of loops is to bring together distant enhancers and promoters (Szalaj and Plewczynski, 2018).

Disruption of this hierarchical organization of the genome in interphase has been linked to changes in gene transcription (Fraser and Bickmore, 2007). Illustrating this functional link, tethering of chromosomes to the nuclear periphery has been shown to influence gene expression (Finlan et al., 2008) and deletion of the sequence at a TAD boundary has been shown to produce transcription misregulation (Nora et al., 2012). Numerous studies have pointed out that chromatin looping plays a role in gene regulation by promoting or preventing contacts between gene promoters and regulatory elements. Studies investigating the impact of DNA looping on gene expression have looked at several loci such as the prolactin gene (Cullen et al., 1993), the  $\beta$ -globin locus (Tolhuis et al., 2002), the H19/Igf2 locus (Murrell et al., 2004) and the Shh locus (Amano et al., 2009).



**Figure 2: Hierarchical genome organization in interphase.** Hi-C heatmaps at different scales: **(A)** Whole genome (chromosomes occupy separate chromosomal territories and rarely interact with each other). **(B)** Whole chromosome. **(C)** Megabase (checked pattern corresponding to compartments A and B) **(D)** Megabase (clear square formations along the diagonal are indicative of topological domains). **(E)** Hundred kilobases (individual peaks corresponding to chromatin loops). **(F-H)** Model of genome folding at these scales. Adapted from Szalaj and Plewczynski, 2018.

In conclusion, chromatin loops in interphase constitute the structural unit of the hierarchical organization of the genome. The loops are subjected to a complex and tight regulation, aiming at controlling cellular processes such as transcription.

## B) Genome reorganization in mitosis

The fidelity of mitosis is essential for life, and successful completion of this process relies on drastic and rapid changes to the organization of chromosomes (Hirano, 2016).

### i. Chromosomes are individualized and compacted in mitosis

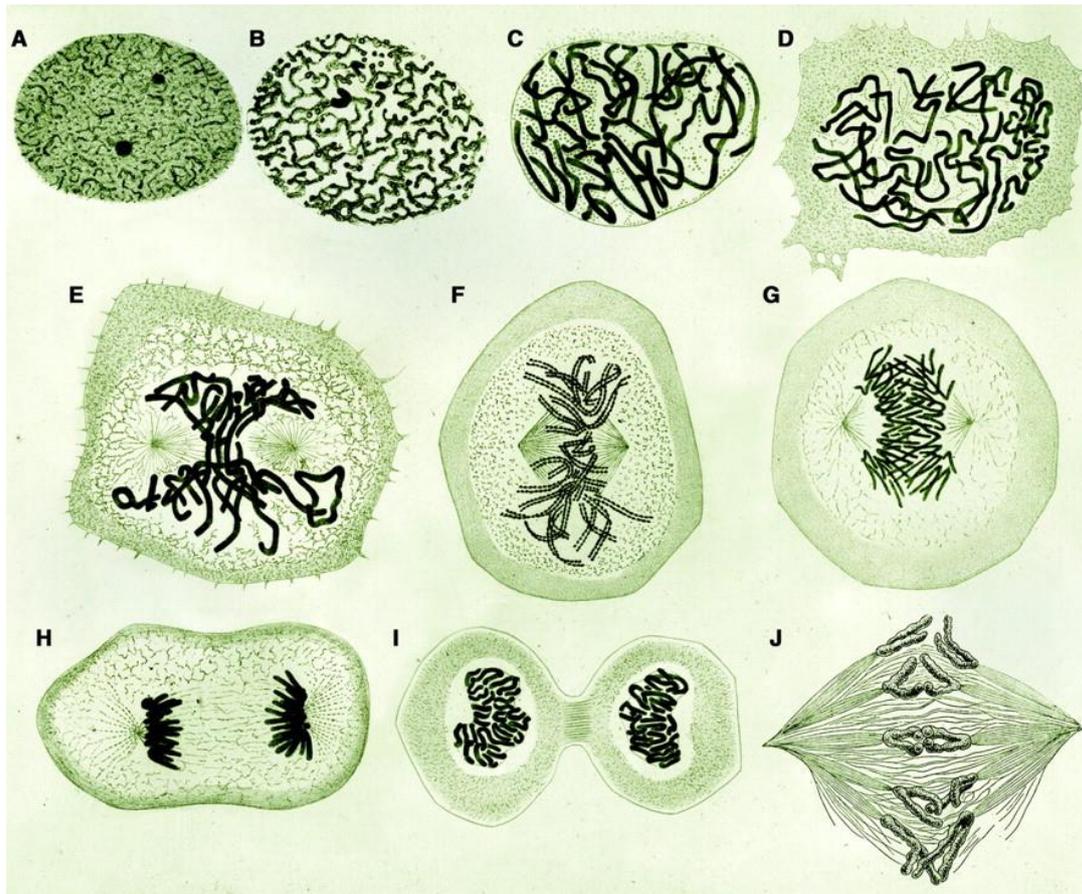
The dramatic reorganization of the nucleus during cell division has fascinated microscopists for more than a century. The German anatomist Walther Flemming described cell division in 1882 as we know it today. The substance in the nucleus was termed “chromatin” because of its affinity for dyes. Flemming proposed the term “mitosis” to characterize the formation of paired threads (greek = mitos) during division of the cell nucleus. At the time, Flemming proposed that chromatin network in resting nucleus (interphase) transforms into threads (mitotic chromosomes), thereby representing continuity of the nuclear material (Figure 3, compare to Figure 1B) (Flemming, 1882).

Mitotic chromosome condensation at the onset of mitosis is responsible for the individualization and compaction of chromosomes (Hirano, 2016). As DNA replication results in the entanglement of the two DNA molecules (Sundin and Varshavsky, 1980), the resolution of such intertwinings (catenanes) upon mitotic entry is crucial for efficient and faithful chromosome segregation. Therefore, the formation of mitotic chromosomes requires both the resolution of replicated sister chromatids and the compaction of chromatin. Additionally, condensation of chromatin into sturdy chromosomes is also necessary to confer the physical properties required for their segregation. Chromosomes must be stiff, resilient, and elastic enough to withstand forces coming from pulling microtubules during anaphase to prevent damage and breaks caused by external tensions (Piskadlo and Oliveira, 2016).

How are chromatin fibres organized within mitotic chromosomes?

### ii. Mitotic chromosomes are organized into arrays of consecutive loops

In the late 1970s, Laemmli and colleagues proposed that mitotic chromosome structure arises from a set of non-histone proteins that fold the chromatin fibres into loops (Figure 4). To test their hypothesis, Laemmli and colleagues isolated histone-depleted chromosomes after treating mitotic chromosomes from HeLa cells with an excess of polyanions dextran sulphate and



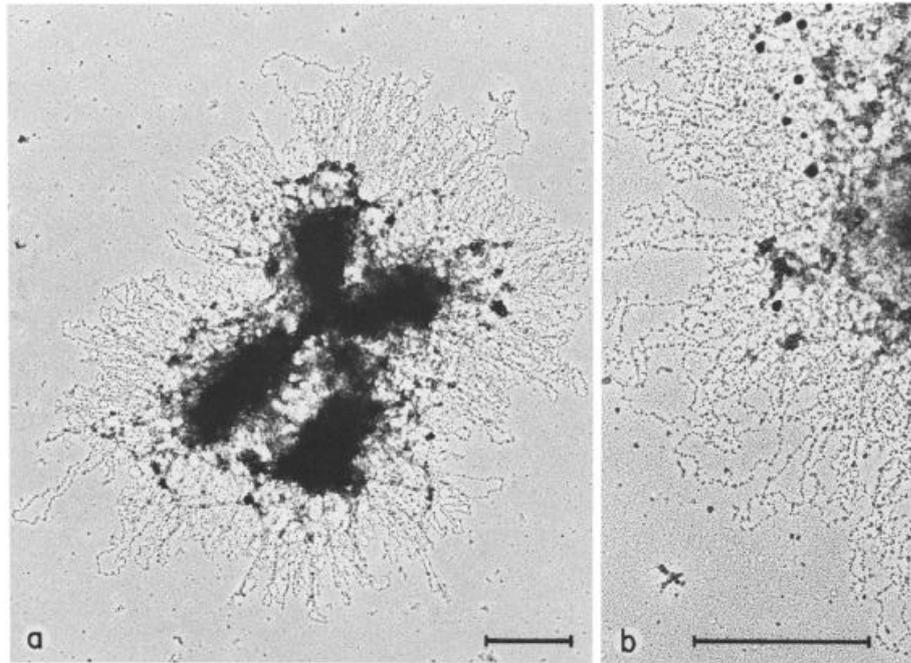
**Figure 3: Drawing of mitosis in newt cells by Walther Flemming.** Sequence of chromosome movements during mitosis. **(A to C)** During prophase the chromosomes form within the nucleus from a substance termed “chromatin” because of its affinity for dyes. **(D)** After nuclear envelope breakdown, the chromosomes interact with the two separating “centrosomes” to form a spindle-shaped structure. **(E)** Prometaphase. **(F)** Metaphase. After the chromosomes attach to the spindle, they become positioned on its equator, halfway between the two poles. **(G)** Anaphase. The two chromatids move toward the opposing poles. **(H)** Telophase. During the final stages of mitosis, neighbouring chromosomes within the two groups fuse to form the daughter nuclei. **(I)** Cytokinesis. **(J)** Microtubules pulling apart sister chromatids to opposite poles in anaphase. Compare to Figure 1B. Adapted from Flemming, 1882.

heparin (Paulson and Laemmli, 1977). They found that DNA remained highly organized by a core of non-histone proteins whose structure retained the size and shape of the original chromosomes. The authors referred to these proteins as the “chromosome scaffold” and observed that loops of DNA extended outward from the scaffold (Paulson and Laemmli, 1977). Laemmli and colleagues demonstrated later that in intact mitotic chromosomes swollen in a low-salt buffer, chromatin loops emanate from the chromosome scaffold (Figure 4) (Earnshaw and Laemmli, 1983; Lewis and Laemmli, 1982; Maeshima et al., 2005).

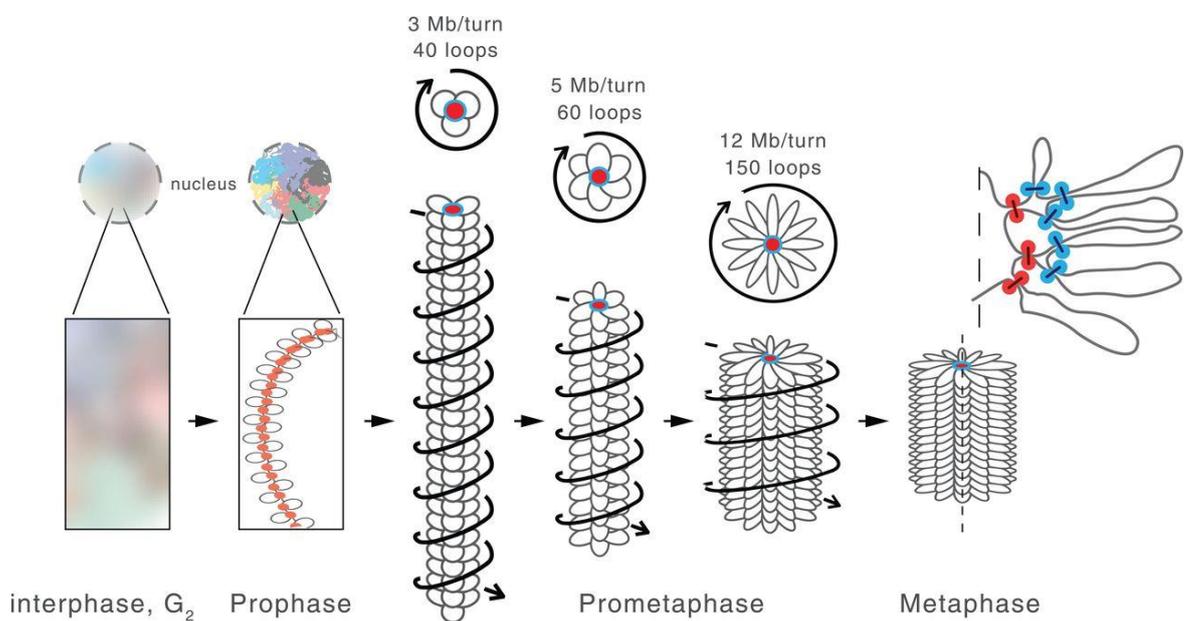
Recent studies, using chromosome conformation capture techniques confirmed such organisation in chromatin loops observed in electron micrographs (Gibcus et al., 2018; Kakui et al., 2017; Naumova et al., 2013; Tanizawa et al., 2017). *Naumova et al.* performed chromosome conformation capture experiments in different human cell types in interphase and in mitosis. These experiments revealed that mitotic genome organization strongly differs from the compartmentalized and cell type-specific organization of the genome in interphase (Naumova et al., 2013). In metaphase, chromosomes are made of consecutive and homogenous arrays of chromatin loops. This folding of chromatin within mitotic chromosomes is consistent among cell types and mitotic chromatin loops are formed by loci located 2-12 Mb apart (Naumova et al., 2013). Consistent with this work, in fission yeast, Hi-C studies have shown that short range (<100 kb) interactions in interphase are replaced by long range (~1 Mb) interactions in mitosis (Kakui et al., 2017; Tanizawa et al., 2017).

*Gibcus et al.* have described a pathway for mitotic chromosome formation using DT40 chicken cells. The authors collected synchronous cells at different time points during mitotic progression and analysed chromosome organization by microscopy and Hi-C. Their study revealed that upon mitotic entry, interphase organization is lost and chromatin fibres are converted into arrays of consecutive loops (Gibcus et al., 2018), consistent with previous studies (Naumova et al., 2013). The authors proposed a model in which, in prophase an inner chromosome scaffold forms with a radial arrangement of loops from 30 to 40 kb up to 60 kb in size around the scaffold (Figure 5). As cells progress through mitosis, the distance between interacting loci increases. During prometaphase, the central scaffold acquires a helical arrangement with loops rotating around the scaffold as steps in a spiral staircase. As prometaphase progresses, outer loops grow, and they are subdivided into smaller loops producing a nested arrangement of loops. The number of kilobases per turn continues to grow and results in the shortening of chromosomes to form the mature mitotic chromosome (Figure 5) (Gibcus et al., 2018).

In conclusion, mitotic chromosomes show a highly uniform organization in which large chromatin loops constitute the structural unit and are consecutively arranged.



**Figure 4: Architecture of metaphase chromosomes.** (A) Electron micrographs of a metaphase chromosome swollen in a low-salt buffer showing radial loops emanating from points all along the chromatid arms. (B) Higher magnification view showing the nucleosomal arrangement of the chromatin in the loops. Adapted from Earnshaw & Laemmli, 1983.



**Figure 5: Model for mitotic chromosome formation.** In prophase, chromosomes are compacted into arrays of consecutive loops around a chromosome scaffold. During prometaphase, the central scaffold acquires a helical arrangement with loops rotating around the scaffold as steps in a spiral staircase. As prometaphase progresses, outer loops grow, they split into smaller loops, the number of loops per turn increases, resulting in the shortening of chromosomes to form the mature mitotic chromosome. Adapted from Gibcus et al, 2018.

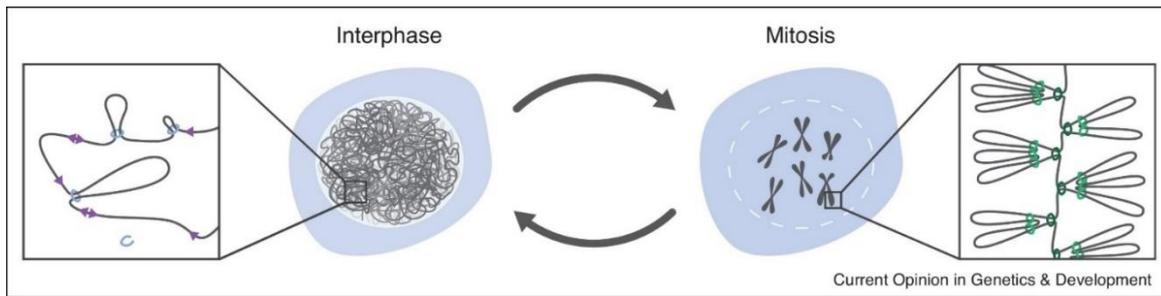
### C) Chromatin loops are different between interphase and mitosis

As described above, the genome is extremely structured in the cell nucleus and its organization undergoes drastic changes throughout the cell cycle. Interphase chromatin is hierarchically organized but remains, however, accessible to transcription and DNA replication machineries. In addition, the loose conformation of chromatin allows the control of interactions between genomic loci, through chromatin loops. On the other hand, mitotic chromosomes are organized in helically arranged loop arrays and chromatin acquires a compact structure. Chromatin loops represent, therefore, the structural units of chromosomes during all the cell cycle (Figure 6).

A variety of microscopy-based and sequencing-based techniques have shown, however, that the nature of interphase and mitotic chromatin loops is different (Figure 6). Interphase loops are smaller (~10-100 kb), heterogeneous, they are not arranged around a central axis and they control gene expression. On the contrary, mitotic loops are larger (~1-10 Mb), they form all over mitotic chromosomes, they are arranged in homogenous arrays of loops, they are formed at the onset of mitosis in a very short period of time, and their purpose is to compact chromosomes (Figure 6). These differences between interphase and mitotic loops could be explained either by a unique machinery responsible for the formation of the loops that is differently regulated between interphase and mitosis, or alternatively, two different machines that form different loops.

Biochemical and genetic experiments led to the discovery of Structural Maintenance of Chromosomes (SMC) complexes as major components of chromosomes. Numerous studies demonstrated that the SMC complex condensin is central for chromatin looping during mitosis while the SMC complex cohesin underlies the formation of chromatin loops in interphase (Gibcus et al., 2018; Naumova et al., 2013; Rao et al., 2014, 2017).

In the next sections, I will describe the discovery, structure and function of SMC complexes, with particular focus on cohesin and condensin. I will then describe the *in vitro* data supporting the model in which cohesin and condensin fold the genome by extruding loops of DNA. I will discuss whether structural differences between cohesin and condensin could underlie the formation of different loops. Finally, I will focus on condensin and its function in the context of chromatin.



**Figure 6 : Chromatin loops in interphase and mitosis.** The interphase genome is organized at a low scale by irregular loops. Mitotic chromosomes by contrast are compact rod-like structures containing arrays of homogenous nested loops. Adapted from Sedeño Cacciatore and Rowland, 2019.

## II. The SMC complexes organize the chromosomes in the nuclear space

SMC complexes are key players in the spatiotemporal organization and maintenance of chromosomes from bacteria to humans.

### A) Discovery of SMC

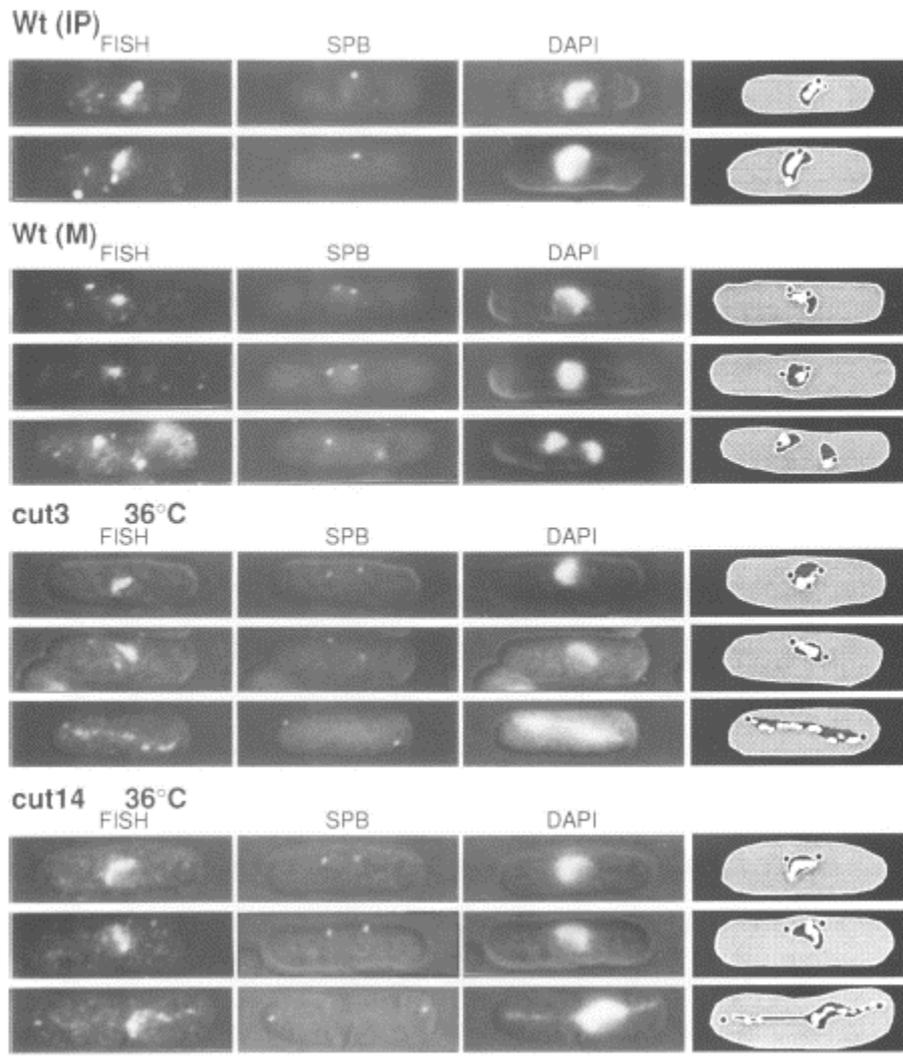
SMC proteins were discovered independently by both biochemical and genetic approaches.

As mentioned previously, Laemmli and colleagues used electron microscopy to analyse the structure of mitotic chromosomes. The authors observed that a chromosome scaffold forms the backbone of mitotic chromosomes with chromatin loops attached to this central axis (Figure 4) (Earnshaw and Laemmli, 1983; Lewis and Laemmli, 1982; Paulson and Laemmli, 1977). It was shown that the scaffold consists of a subset of non-histone proteins that includes two major proteins, Scl and SclI (Lewis and Laemmli, 1982). Scl was identified as topoisomerase II (Earnshaw et al., 1985). Topoisomerase II is an evolutionary conserved protein that can untangle DNA and relax the intertwined supercoils in a DNA molecule by passing one DNA molecule through a transient double-stranded break in another (Wang, 1996). The requirement of Topoisomerase II for chromosome condensation was also shown in fission yeast using genetic approaches, as Topoisomerase II mutants fail to segregate chromosomes (Uemura et al., 1987).

Another biochemical approach used mitotic chromosomes assembled *in vitro*. When incubated in *Xenopus* egg extracts, sperm chromatin is converted into condensed mitotic chromosomes (Hirano and Mitchison, 1994). Hirano and Mitchison identified two chromosome-associated proteins (CAPs) present in *Xenopus* egg extracts, XCAP-C and XCAP-E (Hirano and Mitchison, 1994).

Independent genetic studies led to the discovery of two gene products required for chromosome segregation in yeast. Cloning of a budding yeast gene responsible for the 'Stability of Mini Chromosomes' led to the identification of Smc1 (Strunnikov et al., 1993). Yanagida's group demonstrated that the proteins mutated in the *cut3* and *cut14* fission yeast mutants showing the "cell untimely torn" (cut) phenotype, were homologues of the SMCs involved in chromosome condensation and segregation (Figure 7) (Saka et al., 1994). Almost simultaneously, Saitoh et al. showed that SclI, one of the major components of the chromosome scaffold, is a chicken homologue of an SMC protein (Saitoh et al., 1994).

Hirano and his colleagues later characterised the CAP proteins and discovered a pentameric complex, that they named condensin, including a heterodimer of XCAP-C (SMC4) and XCAP-E (SMC2) and three non-SMC subunits XCAP-D2, XCAP-G, and XCAP-H (Hirano et al., 1997). The authors showed that immunodepletion of condensin results in chromatin decompaction, suggesting that the



**Figure 7 : Thermosensitive mutants of fission yeast condensin lead to chromosome condensation and sister chromatid separation defects .** Painting of the chromosome arm in *cut3-477* and *cut14-208* by FISH. Wild type, *cut3-477* and *cut14-208* cells were cultured at 36°C and fixed. The probes for FISH consisted of mixed DNAs derived from 11 cosmids in the left arm of chromosome 2. The spindle pole bodies (SPB) (functionally equivalent to the centrosome) were visualized using anti-sad1 antibodies. DAPI-staining of nuclear chromatin is shown in the third column. Superimposed images of FISH, SPB and DAPI are depicted on the right. Note in *cut3-477* and *cut14-208* cells the formation of chromosome bridges in anaphase. Wt IP: wild type interphase. Wt M: wild type mitosis. The bar indicates 10  $\mu$ m. Adapted from Saka et al, 1994.

complex has a key role in chromosome condensation (Hirano et al., 1997). In 1997 also the laboratory of Kim Nasmyth discovered the cohesin complex in budding yeast (Michaelis et al., 1997).

In 1995, Smc2 was identified in budding yeast and it was shown to be required for chromosome segregation (Strunnikov et al., 1995).

The identification of a second condensin complex in vertebrate cells was later reported (Ono et al., 2003). The condensin complex initially discovered was named condensin I, while the newly identified condensin complex was named condensin II. Whereas the two SMC subunits are identical in both complexes, the non-SMC subunits are different (Ono et al., 2003). Both condensins I and II are almost ubiquitous in eukaryotes, and only a limited number of organisms have condensin I only (such as *S. cerevisiae* and *S. pombe*).

The results from many diverse technical approaches have converged in the discovery of a large superfamily of SMC. SMC have been found in all eukaryotes examined, and numerous prokaryotes as well, and play crucial roles in chromatid cohesion, chromosome condensation, and DNA repair. Although SMC complexes have diverse functions, they share a conserved architecture.

## B) Structure of SMC complexes

SMC complexes are characterized by their ring-like shape structure (Figure 8). The ring is made up of three proteins along its circumference: two SMC subunits (homo or heterodimers) complemented by a kleisin subunit (Figure 8B). The SMC family of proteins regroups large ATPases with an unusual domain organization. Each SMC subunit is 1,000–1,500 amino acids in length and has a central hinge domain flanked by two long coiled-coils. The N- and C-terminal domains of the coiled coils contain Walker A and Walker B motifs, crucial for ATP binding (Figure 8A). The SMC folds back on itself through antiparallel coiled-coil interactions, creating an ATP-binding head domain.

Within a complex, two SMC proteins interact at the hinge region, forming long-armed V-shaped dimers (Figure 8B). The length of each arm is ~50 nm, equivalent to the length of 150 bp of dsDNA.

The ATPase domain is structurally similar to an ATP-binding cassette (ABC) domain and their ATP-binding and hydrolysis cycle modulates engagement and disengagement of the two head domains (Hirano, 2016). In the presence of ATP, the two ATPase heads engage and create a compartment between the hinge and the engaged heads (Chapard et al., 2019; Vazquez Nunez et al., 2019).

The activities of the SMC ring are regulated by peripheral subunits, many of which are either composed of  $\alpha$ -helical HEAT (Huntingtin, Elongation factor 3, protein phosphatase 2A, TOR1 domain) repeats or Tandem Winged-Helix Domains (WHD) and associate via the kleisin subunit (Haering and Gruber, 2016a; Uhlmann, 2016). Non-SMC subunits are responsible for differences in localization,



dynamic and function of SMC complexes (Andrews et al., 2005; Fujimoto et al., 2005; Green et al., 2012; Ono et al., 2003).

The ring-shaped structure and the ATPase activity of SMC lead to the proposal that they can act as machines that manipulate chromosomal DNA within their compartments, in order to shape chromosomes. SMC could embrace DNA inside the ring or inside the compartment created when SMC heads engaged through ATP. It has been shown that SMC can encircle DNA (Cuylen et al., 2011; Haering et al., 2008; Ivanov and Nasmyth, 2005; Kanno et al., 2015; Wilhelm et al., 2015) and more recently, a better description of DNA entrapment inside the SMC compartments has been reported (Chapard et al., 2019; Kong et al., 2019; Vazquez Nunez et al., 2019). Interestingly, disrupting the integrity of the condensin ring, results in segregation defects in budding yeast (Cuylen et al., 2011, 2013), suggesting that topological entrapment of DNA within the condensin ring might be required for chromosome condensation. However, it is not yet clearly established how the ring-like structure of SMC contribute to their functions.

Prokaryote and eukaryote SMC share functional properties in chromosome organization. Eukaryotes have at least six SMC proteins that interact with different partners to form the SMC complexes. SMC complexes have a conserved structure but play different roles in chromosome architecture. Many efforts are focused on understanding what features of eukaryote SMC specify their functions. Bacteria have a single SMC, and even if it has not been formally demonstrated that bacterial SMC is the functional counterpart of the eukaryotic condensin complex, the analysis of this bacterial protein would contribute to our understanding of the more sophisticated eukaryotic complexes (Hirano, 2005).

### C) Prokaryote SMC

Prokaryotes have only a single type of SMC that forms a homodimer. *Bacillus Subtilis* and *Caulobacter Crescentus* contain a homodimer of Smc and the kleisin protein ScpA. The additional regulatory protein ScpB, which contains a WHD domain, associates with ScpA (Figure 8C). On the other hand, the structure of the *Escherichia Coli* SMC-like protein comprises the MukB homodimer, and its accessory proteins, MukE and MukF. MukE contains tandem WHDs and MukF is the kleisin subunit (Figure 8C). The structure of MukBEF differs slightly from those of the other SMC complexes in the fact that the MukF kleisin domain forms dimers that allows the formation of multimers of SMC complexes (Hirano, 2016), at least *in vitro* (Matoba et al., 2005).

Disruption of Smc and MukB results in increased anucleate cells (Moriya et al., 1998; Yamazoe et al., 1999). These anucleate cells form after abnormal cell division at the ends of elongated cells that

result from defective segregation. These studies suggest that bacterial SMC play a role in chromosome partitioning.

The roles of Smc and MukB in chromosome organization have been investigated at the molecular level. Recent experiments have shown that upon chromosomal loading at *parS*, Smc-ScpAB moves onto flanking DNA aligning the two chromosomal arms progressively from *ori* to *ter*. Hi-C maps of thermosensitive Smc mutants in *B. Subtilis* at the non-permissive temperature showed the loss of the interactions between the arms, suggesting that Smc promotes the colinearity of chromosomal arms (Wang et al., 2017). *C. Crescentus* SMC is not essential for cell survival, suggesting that other mechanisms act to ensure proper chromosome segregation. Consistent with data in *B. Subtilis*, Hi-C maps of  $\Delta smc$ ,  $\Delta scpA$ , and  $\Delta scpB$  cells in *C. Crescentus* revealed a decrease in inter-arm interactions (Tran et al., 2017).

In *E. Coli*, the function of MukBEF differs from Smc-ScpAB. Chromosome conformation capture experiments indicate that MukBEF may generate a series of DNA loops covering most parts of the chromosome instead of co-aligning the two chromosome arms. This process would therefore be more similar to what happens in eukaryotes. A contact map of cells depleted for *mukB* showed a reduction in long-range interactions, suggesting that MukBEF is needed to establish chromosomal contacts between distant DNA regions (Lioy et al., 2018).

Despite differences in loading and action, both bacterial SMC complexes play an essential role in chromosome organization. Smc-ScpAB seems to act as a translocation factor moving through chromosome arms. On the other hand, MukBEF appears to be required for the formation and maintenance of loops.

Through this thesis I will mention several works in *B. subtilis* and *C. crescentus* regarding SMC loading and translocation on chromosomes that contribute to my study on condensin function.

## D) Eukaryote SMC

Eukaryote SMC complexes, contrary to prokaryote, are built from heterodimers of different SMC subunits. There are three different SMC complexes in eukaryotes, cohesin, condensin and SMC5/6 complexes (Figure 8D). Despite their structural homology, each of these SMC complexes performs specific, non-redundant functions in the maintenance of chromosomes. Current models predict that this is because they associate with different types of regulatory subunits and exhibit different localization pattern on chromosomes (Baxter et al., 2019).

### i. The SMC5/6 complex

SMC5-6 complex was initially identified as a complex involved in DNA repair (Lehmann et al., 1995; Nasim and Smith, 1975). However, several studies now show that SMC5/6 could play a role in regulating gene transcription during plant development and in inhibiting transcription of viral genomes (reviewed in Aragón, 2018).

SMC5/6 is composed of a SMC5–SMC6 heterodimer and at least four additional non-SMC elements (Nse proteins). In addition to the ATPase activity (Bermúdez-López et al., 2015; Kanno et al., 2015), the SMC5/6 complex possess the Nse2 subunit, a SUMO E3 ligase that facilitates the SUMOylation of substrates by the SMC5/6 complex and also self-SUMOylation (Andrews et al., 2005). Nse4 is the kleisin subunit that bridges the SMC5/6 heads. Nse1 and Nse3 contain tandem winged-helix domains (WHD) (Figure 8D). Two additional HEAT-containing repeats, Nse5 and Nse6 form a subcomplex and associate with SMC5/6 (not depicted in Figure 8D) (Aragón, 2018).

A study in budding yeast shows the role of Smc5/6 in reducing topological stress accumulated downstream of replication forks (Kegel et al., 2011). This work indicates that SMC5/6 via its interplay with topology can impact chromosome dynamics, however a role in chromosome organization has not yet been reported. In order to pursue this study in SMC-dependent genome organization, I will stay focused on cohesin and condensin.

### ii. The cohesin complex

Cohesin was first discovered for its vital role in holding sister chromatids together from the time of DNA replication until anaphase onset to ensure proper chromosome segregation in anaphase (Michaelis et al., 1997).

Cohesin complexes consist of typical SMC ring structures (Figure 8D) that interact with several accessory subunits. Mammalian cohesin is composed of SMC1, SMC3 and two non-SMC subunits, the kleisin subunit SCC1 (Scc1 in budding yeast and Rad21 in fission yeast) and a HEAT-repeat subunit, SA (stromal antigen) (Scc3 in budding yeast and Psc3 in fission yeast) (Figure 8D). The hinge domains of SMC1 and SMC3 bind tightly to each other, whereas the ATPase heads of both proteins are physically connected by SCC1. The ATPase activity of cohesin is required for its loading (Arumugam et al., 2003) and function (Hu et al., 2011; Petela et al., 2018). The loader NIPBL-MAU2 (Scc2-Scc4 in budding yeast) recruits cohesin to chromosomes (Ciosk et al., 2000; Watrin et al., 2006). PDS5 (a HEAT repeat-containing subunit) and WAPL are two other proteins that associate with the four canonical cohesin subunits and act to dissociate cohesin from chromosomes (Haering and Gruber, 2016a; Uhlmann, 2016).

Apart from its prominent role in sister chromatid cohesion, cohesin takes part in many other chromosomal processes, including organization of the genome into chromatin loops (reviewed in van Ruiten and Rowland, 2018).

Cohesin and the insulator protein CTCF were found to colocalize in mammalian cells on chromatin (Wendt et al., 2008), at the anchors of loops (Rao et al., 2014) and at boundaries of TADs (Lieberman-Aiden et al., 2009; Nora et al., 2012; Rao et al., 2014).

CTCF is an essential protein that is highly conserved from fly to human but absent in yeast, *C. elegans* and plants. CTCF binds genomic DNA through a central 11-zinc-finger DNA binding domain and its binding sites contain a directional sequence consensus of 11 to 15 bp (Ong and Corces, 2014). It was suggested that DNA loops correlate with the presence of pairs of CTCF sites arranged in a convergent orientation (Rao et al., 2014). This was supported by a study in mammals, showing that inversion of CTCF-binding sites alters looping and expression of a locus (Guo et al., 2015).

In yeast, (CTCF is absent) cohesin is enriched at sites of convergent transcription that represent the boundaries of the domains (Gullerova and Proudfoot, 2008; Lengronne et al., 2004; Mizuguchi et al., 2014).

Consistent with a role for CTCF and cohesin in genome organization, depletion of CTCF results in fewer intra-TAD contacts (Zuin et al., 2014). Loss of cohesin function causes a complete loss of interphase loops in mouse liver cells (Schwarzer et al., 2017) and human cell lines (Rao et al., 2017). In fission yeast, cohesin is required for maintaining interacting domains ~50-100 kb in size, since a loss of function mutation of cohesin causes the disruption of local contacts (Mizuguchi et al., 2014).

It has been proposed that CTCF defines contact points for cohesin-mediated chromosomal interactions. A current model suggests that, once loaded onto DNA, cohesin extrudes chromatin loops until it encounters convergent CTCF sites or convergent genes (Busslinger et al., 2017; Fudenberg et al., 2016; Wutz et al., 2017). This “loop extrusion” model will be presented in detail in the section II.E.

Cohesin remains the most studied SMC complex. During this thesis I will bring elements regarding cohesin loading, translocation and loop formation that contribute to my study on the role of condensin in mitotic chromosome organization.

### iii. The condensin complex

Condensin is essential for chromosome assembly and segregation during mitosis. Inactivation of condensin in organisms from bacteria to human cells leads to failure in chromosomes condensation. As a consequence, chromosomes remain entangled leading to the formation of anaphase

chromosome bridges (Figure 7) (Cuylen et al., 2013; Hirano et al., 1997; Saka et al., 1994; Woodward et al., 2016).

The five subunits of condensin form the ring and contrary to cohesin, no other accessory proteins have been found. The condensin complex contains a SMC2–SMC4 heterodimer, a kleisin subunit and two HEAT repeats subunits (Figure 8D). Mammals possess two different condensin complexes: condensin I and II, that share the SMC subunits but have different non-SMC subunits. Condensin I contains HEAT subunits called CAP-D2 and CAP-G, and the CAP-H kleisin subunit. Condensin II has CAP-D3, CAP-G2 (HEAT-repeat subunits) and the CAP-H2 kleisin subunit (Ono et al., 2003).

Without condensin, mitotic chromosomes retain their interphase structure with local interactions. Two studies in fission yeast analysed the contribution of condensin to large mitotic loops, using different tools to inhibit condensin. *Kakui et al.* arrested cells in metaphase by repressing the expression of the Slp1 activator of the anaphase-promoting complex. Condensin was depleted by repressing the expression of Cut14 and promoting its degradation via an auxin-inducible degron. *Kakui et al.* observed by Hi-C that under these conditions, long-range interactions were lost, suggesting that condensin is responsible for contact interactions in mitosis (Kakui et al., 2017). *Tanizawa et al.* performed Hi-C in thermosensitive mutants of condensin *cut14-208* and *cut3-477* at 36°C and found that large domains interactions were reduced (Tanizawa et al., 2017). These studies suggest that condensin mediates the formation of long-range contacts during mitosis and predict that this organization could explain chromosome compaction.

*Gibcus et al.* investigated the differing contributions of condensin I and II to mitotic chromosome formation in vertebrates. They depleted condensin I or II in G2-arrested cells and Hi-C was performed as cells progressed through mitosis. They showed that either condensin can mediate the formation of arrays of chromatin loops, however, they play distinct roles at different structural levels in mitotic chromosome formation. Condensin II, is nuclear in interphase and associates with chromosomes as early as prophase; it was shown to be centrally located on chromosomes and to compact chromosomes into arrays of consecutive loops. On the other hand, condensin I only gains access to chromosomes after nuclear envelope breakdown and it was shown to subdivide large condensin II-mediated loops into smaller loops (see Figure 5, condensin I is shown in red and condensin II in blue ) (Gibcus et al., 2018).

It has been proposed that condensin, like cohesin, can extrude DNA to form mitotic loops (Ganji et al., 2018; Kong et al., 2019). This “loop extrusion” model, and the evidences supporting it, will be explained in the section II.E.

To further understand condensin function, I detail in the next sections what is currently known of *in vitro* condensin-DNA interactions and activities of condensin.

#### a) The interaction of condensin with DNA

A prerequisite for chromosome condensation is the association of condensin with chromatin. Thermosensitive mutants of fission yeast condensin, in which condensin association with chromosomes is reduced fail to properly segregate chromosomes in anaphase (Nakazawa et al., 2015; Sutani et al., 2015; Tada et al., 2011).

To study the association of condensin with chromatin, I will address two main points: the nature of condensin-DNA interactions, and the cell-cycle regulated association of condensin with chromosomes.

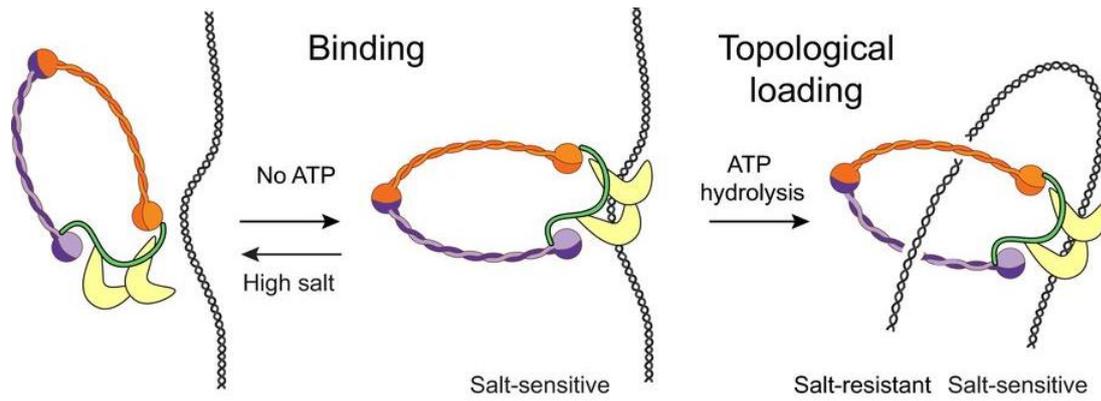
How does condensin interact with DNA? Three types of condensin-DNA interactions have been described *in vitro*: topological entrapment of DNA (Cuylet et al., 2011), binding of SMC subunits to ssDNA (Akai et al., 2011; Piazza et al., 2014) and binding of HEAT-repeat subunits to dsDNA (Kschonsak et al., 2017; Piazza et al., 2014).

An attractive multistep binding of condensin to DNA has been recently described (Figure 9). Using magnetic tweezers, it has been shown that the association of condensin with DNA can take place in the absence of ATP. This ATP-independent interaction is able to survive washing steps with physiological salt concentrations, but it does not survive in buffer conditions of high ionic strength, which indicates that the ATP-independent interaction of condensin with DNA may be electrostatic in nature. When condensin is added to DNA in the presence of ATP, it is, however, able to survive high-salt conditions. This suggests that the ATP-dependent mode of DNA binding must be exceptionally stable. These evidences suggest a model in which condensin first interacts electrostatically through the HEAT-repeat subunits to then topologically entrap DNA in an ATP-dependent manner (Figure 9) (Eeftens et al., 2017).

When does condensin associate with chromosomes? In higher eukaryotes, condensin II is located within the nucleus during interphase and associates with chromosomes in prophase. Condensin I only contact chromosomes after nuclear envelope breakdown in prometaphase (Haering and Gruber, 2016b; Hirota et al., 2004; Ono et al., 2003). In organisms with a closed mitosis like fission yeast, condensin has to be imported into the nucleus before mitotic chromatin compaction. In the case of fission yeast, condensin requires the CDK-dependent phosphorylation of Smc4/Cut3 for nuclear localization (Thadani et al., 2012).

#### b) The activities of condensin *in vitro*

SMC2 and SMC4 subunits provide the ATPase activity to the condensin complex, required for chromosomes condensation. However, how the energy of ATP hydrolysis promotes chromosome compaction is still unclear.



**Figure 9: Multistep binding mechanism of condensin with DNA.** Condensin first binds to DNA electrostatically, presumably through the HEAT-repeat subunits. Upon ATP hydrolysis, condensin embraces the DNA topologically. High salt or high force can disrupt the electrostatic interactions *in vitro*. Adapted from Eeftens et al, 2017.

*In vitro* studies have uncovered several activities of condensin, namely, the ability to introduce positive supercoiling into DNA, ssDNA reannealing, DNA translocation and DNA loop extrusion (Akai et al., 2011; Ganji et al., 2018; Kim et al., 2019; Kimura and Hirano, 1997; Kong et al., 2019; Sutani and Yanagida, 1997; Sutani et al., 2015; Terakawa et al., 2017).

*In vitro* studies revealed that condensins are able to introduce ATP-dependent positive supercoils on circular DNA in the presence of topoisomerase I (Kimura and Hirano, 1997). Yet it is not clear if condensin supercoiling activity is required for *in vivo* chromosome condensation.

Using magnetic-tweezers, a study with *Xenopus* condensin showed that condensin can bind to DNA in the absence of ATP, but it only compacts the DNA in the presence of hydrolysable ATP (Strick et al., 2004). Similar results were obtained in another magnetic-tweezers study on the *S. cerevisiae* complex that examined how the rate of compaction depends on protein concentration, ATP concentration, and the force applied on the DNA (Eeftens et al., 2017). Interestingly, both magnetic-tweezers studies on eukaryotic condensin failed to detect a supercoiling activity for condensin, which disagree with the observations of Hirano (Kimura and Hirano, 1997).

It has been shown *in vitro* that SMC subunits of condensin promote DNA renaturation reactions (the rewinding of single-strand DNA into double helical DNA) (Akai et al., 2011; Sutani and Yanagida, 1997). *In vivo* studies in fission yeast demonstrated, that an RPA (Replication protein A, the major protein that binds to ssDNA) mutant with reduced affinity to ssDNA restores the growth of condensin mutants (Akai et al., 2011). This observation suggests that ssDNA stabilized by RPA could hinder chromosome segregation. Additionally, it was shown in fission yeast that condensin accumulation sites are sensitive to nuclease P1 (which digests ssDNA or ssRNA), suggesting that these sites contain ssDNA (Sutani et al., 2015). The authors of this study proposed that unwound DNA produced by transcription, detrimental for chromosomes condensation, is recognized and rewound by condensin (Sutani et al., 2015).

To what extent these biochemical activities of condensin contribute to chromosome condensation *in vivo* is unclear.

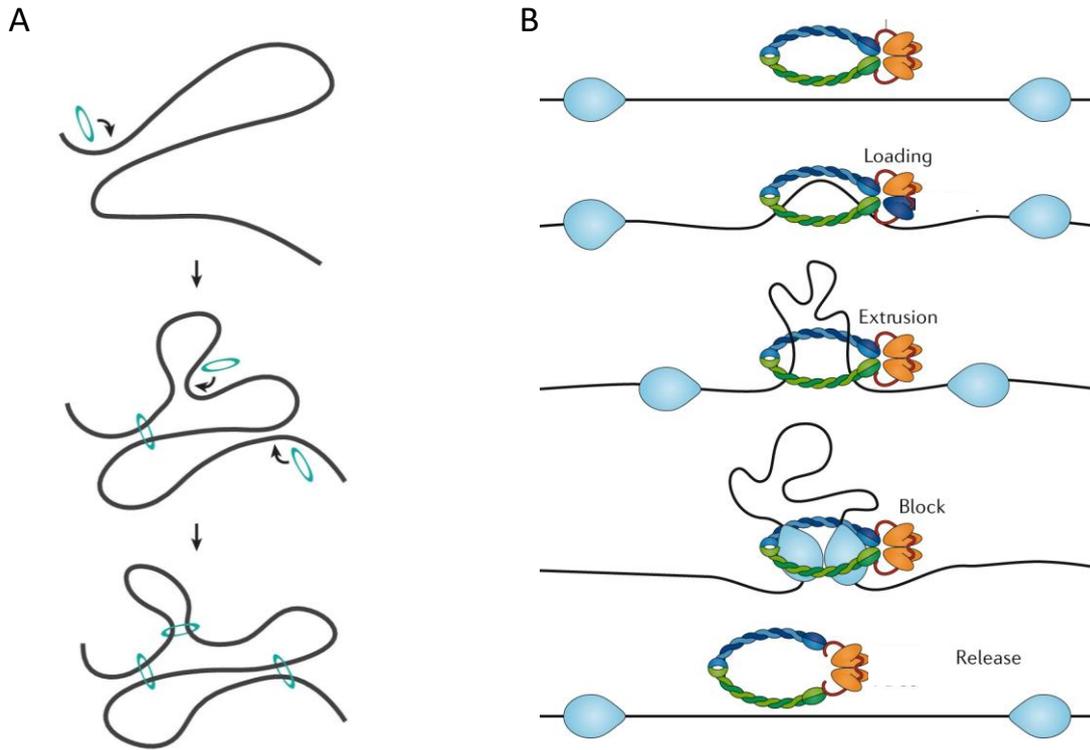
Very recently, DNA translocation and DNA extrusion activities of condensin have been demonstrated *in vitro* (Ganji et al., 2018; Terakawa et al., 2017). These evidences, together with *in vitro* evidences for cohesin translocation on DNA (Davidson et al., 2016; Stigler et al., 2016), support the proposed “loop extrusion model”.

## E) The loop extrusion model to explain SMC-driven chromatin loop formation

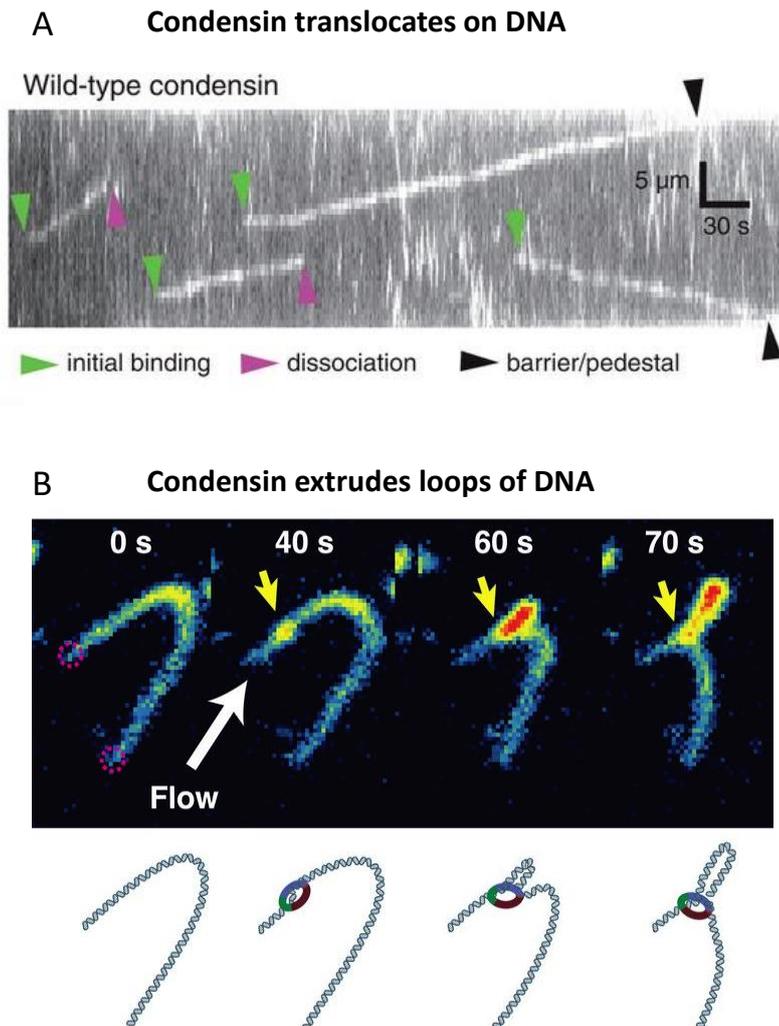
How do cohesin and condensin function to organize chromosomes into chromatin loops? Currently, two models have been proposed to explain how SMC complexes bring together distal

elements (Figure 10). The stochastic crosslinking model proposes that SMC complexes entrap DNA elements that by chance are in close proximity and thereby stabilize stochastic interactions (Figure 10A) (Cuylen et al., 2013; Thadani et al., 2012). In this model, the topological entrapment of two DNA strands within condensin would explain the long-range interactions mediated by condensin. Alternatively or additionally (Sakai et al., 2018), another model has recently gained a lot of attention: the loop extrusion model (Figure 10B). An increasing amount of evidence supports the notion that the common molecular mechanism that underlies the action of SMC complexes is their ability to create and progressively enlarge loops of DNA (Alipour and Marko, 2012; Goloborodko et al., 2016; Nasmyth, 2001). In this model, an SMC initially binds DNA. Then DNA then bends to form an initial loop. The SMC ring reels DNA generating a loop (Figure 10B). Cohesin extrusion would be blocked by convergent CTCF sites in human, and convergent genes in yeast (Rowley and Corces, 2018). There is no evidence yet that condensin extrusion could be blocked by DNA-bound proteins or transcription. Condensin II does not accumulate at CTCF sites in interphase cells (Downen et al., 2013), suggesting that CTCF can not block condensin II.

Several *in vitro* experiments performed on purified condensin support the loop extrusion model. An elegant DNA-curtain study showed that the *S. cerevisiae* condensin complex is a mechanochemical molecular motor that translocates on naked DNA (Figure 11A) (Terakawa et al., 2017). Translocation was ATP dependent, persisted for very long distances (>10 kb), and showed an average velocity of ~60 base pairs per second (Figure 11A) (Terakawa et al., 2017). Subsequent single-molecule experiments further demonstrated that the linear translocation observed for condensin could be converted into DNA loop extrusion. Addition of purified budding yeast condensin to DNA molecules, which had been tethered under low tension, allowed the real-time visualization of ATP-dependent formation and gradual expansion of DNA loops of several kbp in size. Condensin-dependent loop extrusion was strictly asymmetric, suggesting that condensin may anchor onto DNA and reels it in from only one side (Figure 11B) (Ganji et al., 2018). The same group has recently published that under the same conditions, higher concentrations of budding yeast condensin result in multiple loop formation on the same DNA molecule (Kim et al., 2019). Surprisingly, they observed a structure containing three dsDNA stretches, connected in parallel. The in-depth study of these structures led them suggest that condensin complexes are able to traverse each other (Kim et al., 2019). These observations challenge the posited model of single looping by condensin and open a new perspective in the mechanisms of chromosome organization. Condensin might create complex loops in which more than a single condensin molecule is implicated. Another very recent work using DNA-curtains and U-shaped DNA molecules (low tension) demonstrated that both human condensin I and II can translocate and extrude loops of DNA in an ATP-dependent manner, as yeast condensin (Kong et al., 2019). In contrast, human condensins DNA loops seem to form symmetrically. The



**Figure 10: Models of SMC function. (A)** The random cross-linking model. An SMC complex links DNA together by trapping two DNA strands inside its ring. Looping can be accomplished either by a single SMC complex or by two interacting SMC complexes. Adapted from van Ruiten & Rowland, 2018. **(B)** The extrusion process involves SMC loading, DNA bending to form the initial loop, and extrusion. Extrusion could be blocked by DNA-bound proteins (CTCF likely blocks cohesin sliding) or other SMC. A final step could involve release of SMC.. Adapted from Rowley & Corces, 2018.



**Figure 11: *In vitro* condensin activities that support the loop extrusion model. (A)** ATP-dependent translocation of condensin on dsDNA. DNA curtain assay in which dsDNA is tightly tethered at both ends. Adapted from Terakawa et al, 2017. **(B)** Condensin extrudes loops of DNA asymmetrically. Double-tethered DNA molecule in which the ends are attached at a distance much shorter than its contour length, in order to keep it loose. Application of a flow allows the visualization of the loop. Adapted from Ganji et al, 2018.

authors also showed that both condensins proceed without stopping upon encountering nucleosomes on single-tethered DNA curtains, and compact DNA (Kong et al., 2019). However, they were only able to assemble 3-4 nucleosomes on a 48,5 kb DNA molecule, which is a much lower density than *in vivo* chromatin.

Data about cohesin movement on naked DNA seem to be more controversial. Studies using DNA-curtains demonstrated diffusion of fission yeast and human cohesin on DNA but found no evidence for ATP-dependent translocation (Davidson et al., 2016; Stigler et al., 2016). Mobility of fission yeast cohesin can be restricted by nucleosomes, nucleosomes arrays, and DNA-bound proteins (Stigler et al., 2016). Human cohesin can pass over some DNA-bound proteins and nucleosomes but is constrained in its movement by transcription and CTCF (Davidson et al., 2016). A third study probed the dynamics of *Xenopus* cohesin on flow-stretched DNA. In contrast to the above reports, these authors claim that cohesin diffusion is dependent on both ATP and the cohesin-loading complex Scc2–Scc4 (Kanke et al., 2016).

*In vitro* data are very useful and informative to decipher the mechanism of function of SMC and to test the loop extrusion model, but they are performed on naked DNA, so they do not reflect the situation *in vivo*. Several questions remain to be addressed: How do SMC first contact DNA and associate with chromatin? Is DNA entrapment required for translocation? How do SMC catch the first loop of DNA? How do SMC behave *in vivo* when they encounter DNA bound proteins that could act as obstacles/roadblocks?

#### F) How could cohesin and condensin in interphase and in mitosis form different types of chromatin loops?

Chromatin loops are the structural unit of genome organization in interphase and in mitosis, however, their nature is different (see below section I.C) (Naumova et al., 2013). Short-range interphase loops depend on cohesin and long-range mitotic loops are dependent on condensin (Tanizawa et al., 2017). It is proposed that both machineries drive chromatin looping by extruding DNA (Fudenberg et al., 2016; Ganji et al., 2018). How can we explain the differences in loop sizes between interphase and mitosis?

Cohesin and condensin share similar structures, however they have specific interactors that could differentially regulate their function in loop formation. Non-SMC regulatory proteins could provide specific DNA contacts that would influence SMC translocation. Differences in processivity (speed of loop extrusion and residence time on chromatin) of SMC complexes could be linked to the size of loops generated. For example, condensin I and II have different residence times, with condensin II being more stably bound to chromatin and condensin I more dynamic (Walther et al.,

2018). And it was demonstrated (as mentioned in section II.D.iii) that condensin II mediates large interactions and condensin I form small loops (Gibcus et al., 2018). No comparative data is available for dynamics of cohesin and condensin association with chromatin.

Different ATPase activities could also be an explanation for differences in loops mediated by cohesin and condensin. Stable association of condensin with DNA (Eeftens et al., 2017) and condensin translocation (Terakawa et al., 2017) require its ATPase activity. ATP binding to SMC heads of cohesin changes its conformation, creating different compartments within the ring (Chapard et al., 2019). These evidences are consistent with the idea that regulation of SMC ATPase activity can directly impact their function.

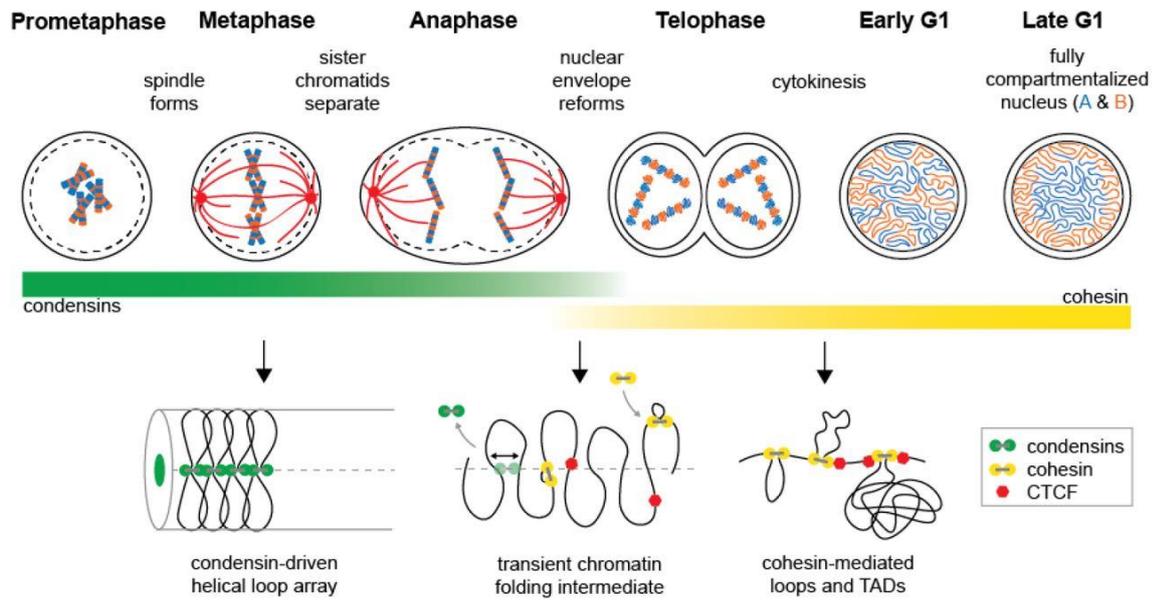
It is interesting to identify similarities and differences between cohesin and condensin structures and activities to better understand how these two machines shape different loops at different stages of the cell cycle.

Interesting studies analysed in detail the transition from interphase genome organization to mitotic chromosomes.

Single-cell Hi-C experiments in mouse embryonic stem cells allowed to phase thousands of single-cell maps, *in silico*, to study the cell cycle dynamics of genome organization (Nagano et al., 2017). Their analysis showed that interphase compartmentalization and local looping are lost in mitosis and contact distances evolve from <1 Mb in interphase to 1-10 Mb in mitosis (Nagano et al., 2017), consistent with other studies (Gibcus et al., 2018; Naumova et al., 2013). This work validated previous studies using single-cell experiments which do not need drugs or the use of modified cell lines for cell population synchronization, avoid the use of synchronized cell populations contaminated with interphase cells and allow the investigation of cell variability.

A very recent study in Hela cells analysed the transition from mitosis to interphase using Hi-C, microscopy and chromatin fractionation (Abramo et al., 2019). Prometaphase-arrested cells showed as expected, long-range contacts and the absence of locus specific features. From prometaphase to G1, Hi-C maps revealed that TADs and loops form with fast kinetics and compartment identity is established relatively quickly, but development and strengthening of long-range interactions continues for several hours (Abramo et al., 2019). Interestingly, they described the existence of a transient intermediate folding state observed during the anaphase-telophase transition, in which condensin and cohesin association with chromosomes is low and no condensin-mediated loops and only a very low density of cohesin loops are observed (Figure 12) (Abramo et al., 2019).

As condensin and cohesin mediate, respectively, large and small domain organization, cohesin-dependent loops are eliminated when condensin-dependent loops form, a study in fission yeast investigated whether condensin and cohesin might inhibit each other. They observed that a condensin mutant did not facilitate small range interactions and a cohesin mutant did not enhance



**Figure 12 : Chromatin loops at the mitosis-interphase transition.** Cellular and chromosomal events as cells exit mitosis and enter G1. Models of chromosome conformation during mitosis (condensin-dependent-arrays of nested loops, helically arranged around a spiral central axis), telophase (intermediate folding state with low levels of condensin and cohesin), and interphase (cohesin-dependent small loops). Green bar indicates abundance of condensins I and II on the chromatin at the corresponding cell cycle stages. Yellow bar indicates cohesin abundance on the chromatin at the corresponding cell cycle stages. Adapted from Abramo et al, 2019.

large domains. These observations suggest that condensin and cohesin independently organize large and small range interactions (Tanizawa et al., 2017).

Finally, a recent study investigated whether the loss of TADs during mitosis is due to regulation of CTCF (Oomen et al., 2019). One possibility could be that in mitosis condensin is not blocked by CTCF, therefore, condensin forms larger loops compared to cohesin. The second possibility is that CTCF and cohesin dissociates from mitotic chromatin. The authors demonstrated that CTCF binding is lost in mitosis and they proposed that CTCF dissociation from chromatin together with the known loss of cohesin during prophase, could explain why TADs and CTCF loops are not observed in mitosis (Oomen et al., 2019). It would be interesting to test whether CTCF removal from chromatin depends on condensin. For example, translocation of condensin through a CTCF site could induce CTCF dissociation. One could deplete condensin in mitosis and assess CTCF binding. If condensin translocation is able to remove CTCF from its binding sites, CTCF would remain bound to its sites under depletion of condensin in mitosis.

How cohesin and condensin form different loops is still not clear. The transition between cohesin-mediated interphase loops to condensin-mediated loops in mitosis is also unknown.

During my PhD project I focused my research on condensin and its role in the reorganization of the genome during mitotic chromosome condensation. In the next section I analyse the function of condensin in the context of chromatin. I will discuss the mechanisms driving the loading and positioning of condensin on mitotic chromosomes. I will end this general introduction by defining my research aims.

## G) Condensin function in the context of chromatin

When cells enter mitosis, the genome is dramatically reorganized, the hierarchical structure is lost, and chromatin fibres are arranged into large loops disposed consecutively around a central axis. These condensin-mediated loops are uniform and explain the linear and axial mitotic compaction of chromosomes (Abramo et al., 2019; Gibcus et al., 2018; Kakui et al., 2017; Nagano et al., 2017; Naumova et al., 2013; Tanizawa et al., 2017). To better understand how condensin binds and manipulates chromatin fibres, it is important to investigate the behaviour of condensin on chromatin.

### i. Genomic distribution of condensin on chromosomes

Based on the consecutive arrays of uniform loops formed by condensin, one could predict that condensin association along chromosomes is homogenous, i.e. condensin would show enrichment

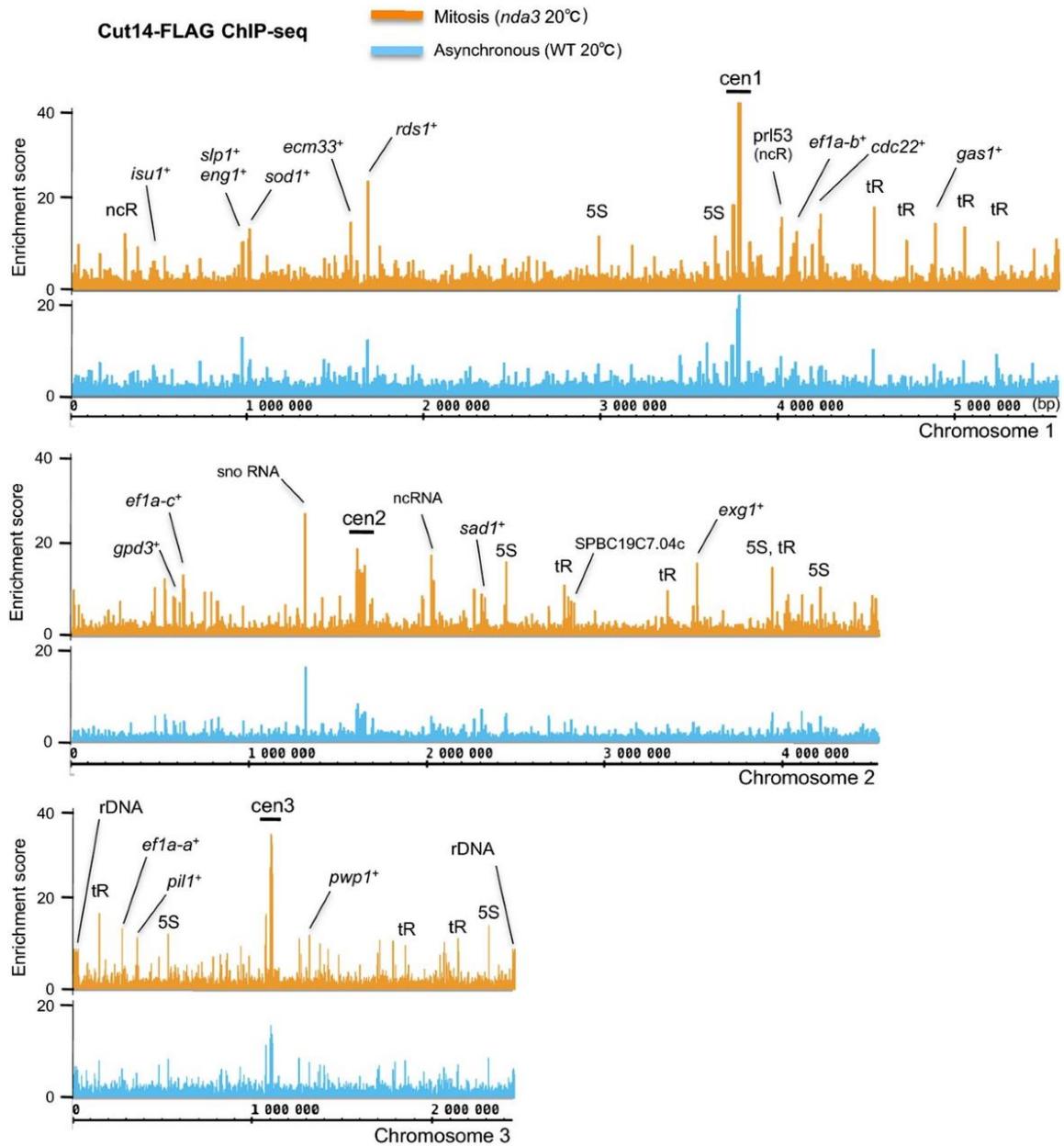
sites evenly spaced along chromosomes. Instead, genome-wide assays based on Chromatin Immunoprecipitation (ChIP) of condensin in mitosis in several organisms (from bacteria to human), have revealed that condensin is not evenly distributed on chromosomes. Condensin maps show a broad basal distribution of condensin along chromosomes, prominent peaks at centromeres and rDNA and discrete peaks along chromosome arms (Figure 13). These discrete peaks on chromosome arms correlate with highly expressed genes whatever the RNA polymerase involved (RNAPI, RNAPII or RNAPIII) (Table1) (D'Ambrosio et al., 2008; Downen et al., 2013; Gruber and Errington, 2009; Kim et al., 2013, 2016; Kranz et al., 2013; Nakazawa et al., 2015; Sutani et al., 2015). Condensin recruitment at centromeres and rDNA has been already investigated. The recruitment of condensin to rDNA depends on the factor Fob1 (Johzuka and Horiuchi, 2009). Fob1 is a polar replication fork barrier in budding yeast. In fission yeast and in mammalian cells blockage of replication forks at rDNA is conserved but it relies on different proteins (Castán et al., 2017). Fission yeast monopolin associates with condensin and contributes to its localization at the kinetochore and rDNA repeats (Tada et al., 2011).

The conserved correlation between condensin enrichment and high rates of transcription suggests that transcription plays a role in determining condensin positioning on chromosomes. As mentioned in the preface, this is intriguing since in vertebrates, transcription is repressed in mitosis (Gottesfeld and Forbes, 1997) when condensin associates with chromosomes. However, it was shown recently that transcription remains active but at low levels in mitosis (Palozola et al., 2017), or even that transcription is activated at the onset of mitosis before mitotic transcription inhibition (Liang et al., 2015). These studies support the idea that transcription could contribute to determine condensin positioning on chromosomes.

ChIP experiments do not allow to distinguish whether condensin peaks are loading or accumulation sites of condensin. Condensin peaks at highly expressed genes could indicate either recruitment sites or sites where condensin would be positioned after translocating from its loading sites.

Two main questions emerge from these genome-wide studies: 1) Are condensin peaks at highly expressed genes loading or accumulation sites of condensin? 2) How does transcription specify these condensin accumulation sites?

I will address these two questions in the following sections. I present data about loading and translocation of SMC. Then I introduce studies investigating the link between gene transcription and condensin positioning on chromosomes.



**Figure 13: Genomic distribution of condensin-binding sites in fission yeast.** FLAG-tagged *S. pombe* condensin subunit SMC2 (Cut14-FLAG) enrichment profiles along *S. pombe* chromosomes 1, 2, and 3 by ChIP-seq. Mitotically arrested, cold-sensitive (cs) beta-tubulin mutant *nda3-KM311* at the restrictive temperature 20 °C (shown in orange), and asynchronously grown wild type at 20 °C (shown in blue). Representative genes are marked: cen, centromere; tR, tRNA gene; 5S, 5S rRNA gene; ncR, noncoding RNA gene; snoRNA, small nucleolar RNA; rDNA, ribosomal DNA repeats. Only 2 and 1 rDNA repeats are shown on the left and right ends of chromosome 3, respectively, because of its repetitive sequences. Condensin binds to RNAPI, RNAPII and RNAPIII-transcribed genes and centromeric regions. From Nakazawa et al, 2015.

**Table 1: High occupancy condensin binding sites across evolution.** From Robellet et al, 2016.**Table 1** High-occupancy condensin binding sites across evolution

Organism	Condensin isoform	Method	Cen	Tel	Non-protein coding genes	Enhancers	Promoters of highly expressed genes	Gene body	References
<i>B. subtilis</i>	n.a.	ChIP-chip	Cen-like par-S	n.a.	tRNA, rRNA	n.a.	n.d.	n.d.	Gruber and Errington (2009)
<i>S. pombe</i>	n.a.	ChIP-chip ChIP-seq ChIA-PET	Yes	n.a.	tRNA, rRNA, ncRNA, snoRNA	n.a.	Yes	3'end	D'Ambrosio et al. (2008); Schmidt et al. (2009); Nakazawa et al. (2015); Sutani et al. (2015); Kim et al. (2016)
<i>S. cerevisiae</i>	n.a.	ChIP-chip	Yes	Sub-tel	tRNA, rRNA, ncRNA, snoRNA	n.a.	n.d.	~No	D'Ambrosio et al. (2008)
<i>C. elegans</i>	I, II and DC	ChIP-seq	n.d.	n.d.	tRNA, ncRNA	n.d.	Yes	~No	Kranz et al. (2013)
Chicken DT40 cells	Focus on I	ChAP-seq	Yes	Yes	tRNA, rRNA, ncRNA	n.d.	Yes	~No	Kim et al. (2013)
Mouse ES cell	II	ChIP-seq	n.d.	n.d.	n.d.	Yes and super-enhancers	Yes	n.d.	Downen et al. (2013)
Human HELA cells	I	ChIP-seq	n.d.	n.d.	tRNA	n.d.	Yes	n.d.	Sutani et al. (2015)

*n.a.* non-applicable, *n.d.* not determined, *Cen* centromere, *Tel* telomeres

## ii. Loading of condensin on chromosomes

Do condensin peaks detected by ChIP at highly expressed genes represent loading sites? How is condensin recruited on chromosomes?

Condensin binds to DNA in a sequence-independent manner. Our lab reported that in fission yeast, condensin accumulates in the vicinity of nucleosome depleted regions of highly expressed genes in mitosis (Toselli-Mollereau et al., 2016). Nucleosome depleted regions facilitate also the loading of cohesin in budding yeast (Muñoz et al., 2019). Consistent with these observations, mitotic histone-free chromosomes can be efficiently reconstituted *in vitro* (Shintomi et al., 2017), suggesting that condensin association with DNA do not require histones. In agreement with this study, magnetic-tweezers, DNA-curtains and U-shaped DNA assays (introduced above) show that purified condensin directly binds naked DNA (Eeftens et al., 2017; Ganji et al., 2018; Strick et al., 2004). A recent study tested the binding of condensin I and II to nucleosomes. Condensins bound nucleosomes prepared with a 183 bp DNA substrate with an affinity similar to naked DNA but binding was impaired with a 147 bp DNA substrate, indicating that condensin complexes do not bind to core nucleosomes but prefer the flanking DNA (Kong et al., 2019). Together, these studies suggest that condensin binds naked DNA and its loading on chromosomes is facilitated at nucleosome free regions.

In contrast to the direct binding of condensin with naked DNA, condensin has been shown to be recruited onto chromosomes by interaction with transcription factors. Two studies in budding and in fission yeasts have reported that the association of condensin with tRNA genes relies upon TFIIC (a transcription factor that recognizes internal promoter sequences within tRNA genes and recruits RNAPIII) (D'Ambrosio et al., 2008; Iwasaki et al., 2010). In human cells, TFIIC recruits specifically condensin II to tRNA genes (Yuen et al., 2017). A different study in fission yeast shows that condensin is recruited to tRNA genes and highly transcribed RNAPII genes by Tbp1 (the TATA-box binding protein) (Iwasaki et al., 2015). Another study in fission yeast revealed that the transcription factors Ams2 and Ace2 recruit condensin to their target genes (Kim et al., 2016). The studies in fission yeast, performed by the same group, show that different transcription factors could load condensin at different loci. Supporting the possibility that condensin could be recruited on chromosomes by physical interaction with a sequence-specific DNA-binding protein, in bacteria, the ParB protein is required to recruit Smc-ScpAB to *parS* sequences located at the origin of replication (Sullivan et al., 2009) and cohesin interacts with the conserved NIPBL-MAU2 (name in human) loading complex that recruits cohesin on chromosomes (Ciosk et al., 2000; Watrin et al., 2006).

This model of transcription factors (TF) as recruiters of condensin implies that a wide variety of TF would constantly interact with condensin to load it at specific sites. The finding that condensin is recruited to highly expressed genes transcribed by all classes of RNAP, in many different organisms,

suggests a conserved pathway for condensin recruitment through a chromatin structure rather than a dependency on several transcription factors for specific sites. There is no evidence for the moment of a conserved recruiter that could load condensin at all strong condensin binding sites.

We could re-interpret the observations of TF-dependent recruitment of condensin and postulate that condensin is rather attracted to chromosomes by a chromatin structure and when it encounters a TF bound to its site, the TF stabilizes or maintain the association of condensin at this site.

In conclusion, it is not yet clear how is condensin recruited on chromosomes. The observations that nucleosome free regions facilitate its binding, and the fact that no conserved loaders have been identified, suggest that condensin could directly recognize chromatin features.

Current evidences do not allow to know whether genome-wide condensin peaks represent sites where condensin is loaded. I provide evidences in the next section about SMC relocation on chromosomes.

### iii. Translocation of condensin on chromosomes

As mentioned above, it was shown *in vitro* that condensin can actively translocate on naked DNA. Little is known however about condensin translocation on chromatin.

Cohesin translocation after loading has been well described. It has been shown in budding yeast by ChIP-chip that cohesin accumulates at sites of convergent transcription, suggesting that RNAPII is able to push cohesin through genes (Lengronne et al., 2004). The localization of cohesin at places of convergent transcription was also observed in fission yeast (Gullerova and Proudfoot, 2008; Lengronne et al., 2004; Mizuguchi et al., 2014). In mammals, it was shown that in the absence of both CTCF and Wapl, cohesin accumulates at the 3' end of active genes and at sites of convergent transcription (Busslinger et al., 2017). This suggests that either that RNAPII can push cohesin along chromatin or, convergent genes represent an obstacle for the translocation of cohesin. These observations *in vivo* fits well with the finding that *in vitro* a motor protein (the FtsK translocase) can push cohesin along DNA (Stigler et al., 2016). Two studies, in budding yeast and in human cells, show that transcription elongation dissociates cohesin from chromosomes (Bausch et al., 2007; Heinz et al., 2018). These conclusions are slightly different, since RNAPII do not push, but remove cohesin.

Work on bacterial SMC complexes shows that the transcriptional machinery slows down SMC translocation when it meets convergent transcription (Tran et al., 2017; Wang et al., 2017).

Collectively these studies demonstrate that transcription play a role in positioning cohesin at convergent genes or at CTCF convergent sites by transcription, and in bacteria, transcription block the progression of SMC when they encounter head on.

If condensin can translocate along chromatin and creates loops, one could think that condensin peaks reflect loop boundaries where translocation stalls, and condensin accumulates. To test this possibility, one needs to compare genome-wide ChIP with Hi-C data. In DT40 cells, around 16,000 loops are detected by Hi-C (Gibcus et al., 2018), while ChIP data detected only 289 condensin sites with more than a fivefold enrichment, and 4617 sites where condensin is more than twofold enriched (Kim et al., 2013). A study in fission yeast shows that condensin binding sites determined by ChIP have more probability of engaging in intra-arm DNA interactions (determined by Hi-C) than sites not binding condensin (Kakui et al., 2017). However, condensin accumulation sites as well as sites not enriched on condensin engage specifically in mitotic long-range interactions. In the same study, the authors show that most of the mitotic boundaries contain a condensin accumulation site (Kakui et al., 2017). On the other hand, a ChIA-PET (chromatin interaction analysis by paired-end tag sequencing) study of condensin in fission yeast, in which site-specific looping between condensin-binding sites have been determined, showed that condensin mediates the association of mitotically-active genes (Kim et al., 2016). Another work in fission yeast compares these condensin ChIA-PET sites with Hi-C data. They found that strong condensin-accumulation sites tend to mediate long-range contacts (Tanizawa et al., 2017). Collectively these data suggest that condensin accumulation sites are implicated in long-range mitotic loops. However, there seems to be more loops than condensin accumulation sites. One could speculate that different pools of condensin and different types of mitotic loops exist, some of the loops anchored at highly transcribed genes. It would be interesting to test whether condensin-mediated mitotic loops are dependent on transcription. In fission yeast, it has been shown that inhibiting transcription results in reduced condensin accumulation at highly expressed genes (Sutani et al., 2015). One could reduce transcription genome wide with the RNAP inhibitors phenanthroline or thiolutin used in the latter study, which would result in reduced condensin accumulation at highly expressed genes, and assess genome-wide interactions frequencies. If condensin accumulation sites at highly expressed genes are somehow related to condensin-mediated loops in mitosis, one could expect to observe a change in the pattern of mitotic interactions when transcription is inhibited.

How transcription can position condensin on chromosomes is not clear. High rates of transcription could facilitate loading of condensin or, alternatively, block translocation.

At the time I started my PhD, a substantial amount of publications addressing this point was available. I collected and confronted these data in the next section.

iv. How does gene transcription contribute to position condensin on mitotic chromosomes?

The conserved localization of condensin at highly transcribed genes led to the proposal that transcription could somehow determine the position of condensin on chromosomes. Despite the well described links between transcription and cohesin and transcription and bacterial SMC, current data addressing the link between transcription and condensin are contradictory (Bernard and Vanoosthuyse, 2015; Robellet et al., 2016).

On one hand, it has been shown in fission yeast that, in prometaphase arrested cells treated with transcription inhibitors, the accumulation of condensin at highly expressed genes is reduced (Sutani et al., 2015), as mentioned above. This observation suggests that condensin association with chromosomes depends on transcription. Consistent with this observation, the authors placed a reporter gene under the control of a mitotically active promoter and observed that transcription was induced and condensin association with chromatin was enhanced, suggesting that a mitotically active promoter is sufficient to cause an accumulation of condensin on chromosomes (Sutani et al., 2015). The authors then inhibited transcription in the thermosensitive *cut14-208* mutant of condensin that shows segregation defects when grown at a restrictive temperature. Inhibition of transcription pharmacologically or genetically in this mutant resulted in the partial suppression of the chromosome segregation defects in anaphase, suggesting that when condensin function is impaired, active transcription impedes chromosome segregation (Sutani et al., 2015). The authors proposed that ssDNA forms at highly transcribed genes in mitosis, since they observed that Ssb1 (ssDNA binding protein 1) binds preferentially at condensin sites (Sutani et al., 2015). Based on the *in vitro* ssDNA reannealing activity of condensin (Akai et al., 2011), they posited that condensin could be targeted to highly transcribed genes to rewind transcription-dependent ssDNA and ensure proper chromosome segregation. Together, these results suggest that transcription is detrimental for chromosome segregation, likely because it produces ssDNA that needs to be removed from chromatin by condensin in order to achieve proper chromosome condensation.

In line with this study, it has been recently shown in fission yeast that defective segregation of a strong condensin binding site (*ecm33*) in condensin mutants is suppressed when transcription is shut-off (Nakazawa et al., 2019). What is missing in this experiment is to know whether condensin binding is reduced at *ecm33* under transcription inhibition.

Other works support this positive correlation between transcription and condensin. A work in fission yeast demonstrate that condensin accumulates at heat-shock protein genes when cells are shifted to 36°C to induce their expression (Nakazawa et al., 2015). Furthermore, a study in mouse embryonic stem cells analysed the localization of condensin II by microscopy at an inducible

transgene (Dowen et al., 2013). The authors demonstrated that upon transcription activation of the transgene, the condensin II signal was enhanced and colocalized with the active transgene (Dowen et al., 2013). These results are consistent with the idea that transcription generates a stress on chromatin that causes an accumulation of condensin. Condensin could be recruited to these sites or alternatively, these sites might represent obstacles for the translocation of condensin.

It is important to note, however, that it is still unclear why left-over ssDNA or chromatin-associated RNA would be harmful for chromosome condensation and segregation. As such, this model seems highly speculative at this stage.

Strikingly, in human cells and in DT40 chicken cells, condensin I also accumulates at highly expressed genes in mitosis (Kim et al., 2013; Sutani et al., 2015), even if transcription is largely switched off in mitosis (Gottesfeld and Forbes, 1997). In human cells, condensin I peaks in prometaphase cells were associated with strong RNAPII peaks in asynchronous cells, and the degree of condensin I binding at these peaks correlated with the expression level of the corresponding genes in interphase cells (Sutani et al., 2015). This observation strongly supports the idea that transcription deposits some sort of epigenetic or structural mark that facilitates the accumulation of condensin (reviewed in Bernard and Vanoosthuyse, 2015).

On the other hand, several studies have reported an antagonism between transcription and condensin association with chromosomes. In budding yeast, the inhibition of RNAPI by the phosphatase Cdc14 during anaphase is necessary for the association of condensin with the rDNA (Clemente-Blanco et al., 2009; Wang et al., 2006). Similarly, transcription by RNAPII of sub-telomeric regions prevents the association of condensin (Clemente-Blanco et al., 2011). Contrary to the previous model, in these studies, transcription inhibition is required to facilitate the enrichment of condensin on chromosomes.

To summarise, existing data on the functional links between transcription and condensin can be classified into two contradictory models:

1) *Transcription facilitates the loading of condensin.* Transcription creates a structure on chromatin that is deleterious for chromosome segregation in mitosis; this structure, perhaps ssDNA, is recognized and disassembled by condensin, which accumulates where the structure forms; the removal of this toxic structure by condensin allows proper chromosome segregation in anaphase. Condensin therefore plays a specific and active role in correcting a mark left by transcription. Transcription and condensin accumulation are positively correlated.

2) *Transcription opposes the loading of condensin.* Inhibition of transcription in mitosis by dedicated mechanisms facilitates the function of condensin. Transcription and condensin accumulation are inversely correlated.

To investigate the link between transcription and condensin, we use in our lab the fission yeast *Schizosaccharomyces pombe*. Fission yeast is a model of choice to study chromatin processes and cell cycle regulated mechanisms, as many different tools are available to manipulate the cell cycle. Its relatively small genome (13.8 Mb) is packed into 3 chromosomes and can be easily manipulated using genetic approaches. Finally, *S. pombe* only possess a single condensin isoform whose amino acid sequence is similar to condensin I.

### III. Genetic experiments indicate that the transcription-associated factors Sen1 and Swd22 act as negative regulators of condensin

In order to decipher the functional links between transcription and condensin, the lab previously carried out genetic screens to identify components of the transcription machinery that either assist or negatively regulate the function of condensin. *cut3-477* is a temperature-sensitive mutant of the Smc4/Cut3 condensin subunit that fails to grow when cells are incubated at the restrictive temperature of 34°C. This mutation weakens the association of condensin with chromatin, resulting in defective chromosome condensation and chromosome segregation defects (Figure 7) (Robellet et al., 2014; Saka et al., 1994; Tada et al., 2011).

A group in the lab screened for mutations synthetically lethal with *cut3-477* at 32°C, the semi permissive temperature at which condensin mutant cells continue to proliferate (Robellet et al., 2014). The *gcn5-47* mutant was co-lethal with *cut3-477* at 32°C, suggesting that Gcn5 collaborates with condensin. Gcn5 is the histone acetyl-transferase subunit of the SAGA complex, a conserved transcription coactivator that acetylates nucleosomes (Koutelou et al., 2010). Nucleosome eviction promoted by Gcn5 was shown to be necessary for condensin accumulation to chromosomes in mitosis (Toselli-Mollereau et al., 2016).

In order to identify potential negative regulators of condensin, our group isolated deletions of non-essential components of the transcription machinery that could restore growth of *cut3-477* mutant cells at 34°C. Two of the regulators identified were Swd22 and Sen1 (Vanoosthuyse et al., 2014). At 34°C, the single mutant *cut3-477* failed to grow while the double mutant *cut3-477 swd22Δ* or *cut3-477 sen1Δ* did form colonies (Legros et al., 2014; Vanoosthuyse et al., 2014). This suppressor effect suggests that Swd22 and Sen1 are, directly or indirectly, negative regulators of condensin function.

The fact that Swd22 and Sen1 could act as negative regulators of condensin was considerably strengthened by the observation that the accumulation of condensin was specifically increased at least at a subset of RNAPIII-transcribed genes in the absence of both Swd22 and Sen1. This observation suggested that Swd22 and Sen1 act either directly or indirectly to limit the accumulation of condensin at RNAPIII-transcribed genes (Legros et al., 2014).

Swd22 is a non-essential component of the Cleavage and Polyadenylation Factor (CPF), the protein complex that is responsible for the 3' end processing of RNA polymerase II transcripts in yeast (Richard and Manley, 2009). The best-characterized function of budding yeast Sen1 and of its human homologue Senataxin is in transcription termination of a subset of RNAPII transcripts (Mischo et al., 2011; Porrua and Libri, 2013). Both Swd22 and Sen1 were therefore previously implicated in the process of transcription termination. I use the term transcription termination to refer to the step during transcription in which the RNAP reaches the termination site, the transcript is cleaved and released and the RNAP falls off the template, allowing it to restart a new cycle of transcription. Transcription termination mechanisms will be introduced in detail in Chapter 2.

Of Swd22 and Sen1, only Sen1 was conclusively shown to associate with RNAPIII-transcribed genes. Sen1 was shown to form a stable complex with RNAPIII (Legros et al., 2014), suggesting that it could regulate condensin positioning by modulating RNAPIII transcription in *-cis*. This constitutes the founding hypothesis of my PhD project and I sought to use the *sen1Δ* mutant to better understand the links between transcription and the positioning of condensin.

Why is the *sen1Δ* mutant a good model to study the impact of transcription on condensin positioning? Preliminary data indicated that Sen1 plays a role in RNAPIII transcription in fission yeast. RNAPIII transcription units are well studied and their promoter and terminator sequences are well characterized. Because they are present in multiple copies in the genome, deletions of most of RNAPIII-transcribed genes are viable. As a result, RNAPIII transcription units are relatively easy to manipulate and well-characterized mutations are known to modulate promoter and terminator functions. Moreover, RNAPIII-transcribed genes harbour features that were previously implicated in the positioning of condensin. For instance, specific RNAPIII transcription factors have been implicated in the regulation of condensin binding (D'Ambrosio et al., 2008; Iwasaki et al., 2010, 2015; Yuen et al., 2017).

#### IV. Research aims

During my PhD, my aim was to understand how transcription could influence the positioning of condensin in mitosis.

I used fission yeast strains deleted for the conserved DNA/RNA helicase Sen1 to try to elucidate the mechanism that explains the accumulation of condensin specifically at RNAPIII-transcribed genes in these strains. I tested a model in which Sen1 modulates condensin positioning by regulating RNAPIII transcription.

To address the intriguing links between Sen1, RNAPIII transcription and condensin accumulation on chromosomes, I divided my project into three main research axes that constitute the three chapters of my manuscript:

*Chapter 1) The RNAPIII-associated factor Sen1 regulates the accumulation of condensin in the vicinity of RNAPIII-transcribed genes*

The first aim of my project was to improve the results previously obtained in the lab. ChIP experiments to monitor the binding of condensin at tRNA genes had been previously carried out in asynchronous cells and in the double mutant *swd22Δ sen1Δ* (Legros et al., 2014). Of both proteins, only Sen1 was shown to associate with RNAPIII, suggesting that only Sen1 could directly modulate transcription at RNAPIII-transcribed genes. Lack of such Sen1-dependent regulation of RNAPIII would somehow facilitate the accumulation of condensin in mitosis in *-cis*. It was therefore important to improve the published observations by investigating the distribution of condensin in the sole absence of Sen1 and in mitotic cells.

In this first chapter, I demonstrate that the sole absence of Sen1 results in the accumulation of condensin at a subset of RNAPIII-transcribed genes in mitosis. To strengthen this observation, I used two different systems to synchronize the cells in mitosis. I show that the impact of Sen1 on the distribution of condensin is specific as lack of the related helicase Dbl8 or of Pso2, a physical interactor of Sen1 previously identified in the lab, failed to impact the distribution of condensin. In addition, I provide evidence that Sen1 acts in *-cis* on the distribution of condensin at RNAPIII-transcribed genes. Moreover, I show that the impact of lack of Sen1 on the accumulation of condensin cannot be explained by an accumulation of the RNAPIII-transcription factors Tbp1 and TFIIC. This shows that lack of Sen1 impacts the distribution of condensin by a novel pathway. Finally, I describe the efforts I put in to demonstrate that Sen1 regulates condensin accumulation in a transcription-dependent manner.

Interestingly, I also demonstrate that Topoisomerase 1 (Top1) also accumulates at RNAPIII-transcribed genes in the absence of Sen1, and this accumulation correlates with the accumulation of condensin. Note however that Top1 also accumulates in the vicinity of RNAPIII-transcribed genes in the absence of Sen1 when cells are in interphase and condensin is not on chromatin. This establishes that the accumulation of Top1 does not result from the accumulation of condensin. Our data are consistent with the following model: lack of Sen1 modulates RNAPIII transcription throughout the

cell-cycle, resulting in a Top1-sensitive DNA structure that enhances the accumulation of condensin at RNAPIII-transcribed genes in mitosis.

*Chapter 2) Sen1 is required for robust RNAPIII transcription termination.*

I then investigated how Sen1 could modulate RNAPIII transcription. Based on: 1) the known function of Sen1 in budding yeast and in human cells in the regulation of RNAPII transcription termination and on, 2) the observation that fission yeast Sen1 binds to RNAPIII-transcribed genes and associates with RNAPIII, we tested whether Sen1 could be important for RNAPIII transcription termination.

In this second chapter, I show that Sen1 does indeed promote RNAPIII transcription termination. In the absence of Sen1, RNAPIII accumulates downstream of RNAPIII-transcribed genes and produces long exosome-sensitive 3'-extended transcripts. This establishes that, contrary to accepted models, RNAPIII requires a dedicated cofactor for efficient transcription termination. Our results were strengthened by genome-wide data from the Bachand lab and were successfully published in *EMBO Journal*. I include this manuscript on which I am the first author, in Chapter 2 of my manuscript. I also describe in Chapter 2 additional results that were not included in the article published in *EMBO J*.

*Chapter 3) The control of RNAPIII transcription termination by Sen1 determines condensin positioning in the vicinity of RNAPIII-transcribed genes.*

I show in Chapter 1 that Sen1 regulates the transcription-dependent positioning of condensin at RNAPIII-transcribed genes in mitosis and in Chapter 2 that Sen1 is required for robust RNAPIII transcription termination. In Chapter 3, we asked whether these two phenotypes are linked.

By manipulating the sequences of the primary terminators of two different tRNA genes, we demonstrate that the RNAPIII transcription termination defects in the absence of Sen1 underlie the accumulation of condensin. We also provide evidence that the sole production of read-through transcripts is not sufficient to alter the positioning of condensin. I end this last chapter with a final discussion to confront my results to current models about transcription-dependent positioning of condensin on chromosomes. I propose a model in which lack of Sen1 interferes with RNAPIII removal from the DNA template, resulting in a wide and dense accumulation of RNAPIII that blocks the translocation of condensin in mitosis, thereby creating an accumulation of condensin at the 3' of RNAPIII-transcribed genes.



## Materials and Methods

### I. Fission yeast strains and culture

All the strains used in this thesis are listed in Table 2. Yeast cultures were grown from fresh plates overnight in YES+A (Yeast Extract + Supplements + Adenine) or in synthetic PMG (Pombe Minimal Glutamate) at 30°C (Forsburg and Rhind, 2006; Moreno et al., 1991). Cell concentration was determined by counting cells using Thoma chambers. Experiments were always carried out on exponentially-growing cells (concentration of  $8 \cdot 10^6$  to  $10^7$  cells/ml).

To synchronize cells in metaphase, cells expressing the gene *slp1+* under a thiamine-repressible promoter (*nmt41*) (Maundrell, 1990) were incubated with 40µM of thiamine for 3 hours.

To block cells in G2, cells carrying the analogue-sensitive *cdc2asM17* allele (Aoi et al., 2014) were incubated for 3 hours with 2µM 3-BrB-PP1.

The expression of the *dis3* gene driven by the *nmt81* promoter was repressed by the addition of 60 µM of thiamine to the PMG medium. To induce the expression of *E. coli* RnhA from by the *nmt1* promoter, cells were grown in PMG minimal medium lacking thiamine for a minimum of 18h.

### II. Genetic methods

#### A) Crosses

Fission yeast has two mating types:  $h^+$  and  $h^-$ , which are alleles of the *mat1* locus (*mat1-M* for  $h^-$ , *mat1-P* for  $h^+$ ) (Moreno et al., 1991). Mating between two haploid strains of interest are induced on SPA (Sporulation Agar), a solid medium poor in nitrogen. Equal amount of strains,  $h^+$  and  $h^-$ , are mixed on SPA plates. Following conjugation, newly formed zygotes immediately enter meiosis and sporulate to produce four spores in a linear tetrad ascus. The presence of spores is checked under the microscope. The cross is treated with 0,5% v/v cytohelicase, which digest remaining cells and release the spores from the asci. Cell lysis is completed by the addition of SDS 0,1%. Spores are counted using Thoma chambers, and 500 and 1000 spores are plated. Replicate plating onto selective media plates allow to select for markers

#### B) Tetrads dissection

To test lethality of the double mutant *sen1Δ X rpc37-V189D*, tetrads from crosses on SPA plates were plated on YES+A plates. Spores were isolated using a microscope equipped with a micromanipulator. Phenotypes of the spores were then identified by replica plating on selective media.

### III. Mutagenesis

#### A) SPCTRNAARG.10 and SPCTRNATHR.10 mutants

The *arg10TATA-less* and super-terminator mutants of *SPCTRNAARG.10* and *SPCTRNATHR.10* were first synthesized (GeneCust Europe). The mutagenized *SPCTRNAARG.10* and *SPCTRNATHR.10* genes were then transformed into fission yeast and its correct integration was selected by counter-selecting on FOA (5-Fluoroorotic acid) the loss of the *ura4* gene previously integrated at *SPCTRNAARG.10* and *SPCTRNATHR.10*. The correct integration of the mutations were confirmed by sequencing.

#### B) *sen1-G1534D* and *rpc37-V189D* mutants

To obtain *sen1-G1534D-gfp* and *rpc37-V189D-flag*, the 3' end of the corresponding gene was amplified by PCR using the primers listed below. The PCR product was cloned into pCR Blunt II-TOPO using the Zero Blunt TOPO PCR Cloning Kit (Life Technologies). Site-directed PCR mutagenesis was then carried out to mutate the codon corresponding to Glycine 1534 in Sen1 and to Valine 189 in Rrpc37, using the QuikChange II Site-Directed Mutagenesis Kit (Agilent Technologies) according to the manufacturer's instructions. The presence of the point mutations was then verified by sequencing. Overlapping PCR was then used to add the sequence of an epitope tag (GFP to *sen1-G1534D* and 3Flag to *rpc37-V189D*) and a selection marker (hygromycin (Hyg) to *sen1-G1534D* and nourseothricin (Nat) to *rpc37-V189D*) to the mutated sequence of the gene. The resulting PCR product was used to transform yeast using routine protocols. Proper integration of the construct at the endogenous locus was verified by PCR and sequenced to verify the presence of the mutations.

Primers used for mutagenesis of Sen1 and Rrpc37:

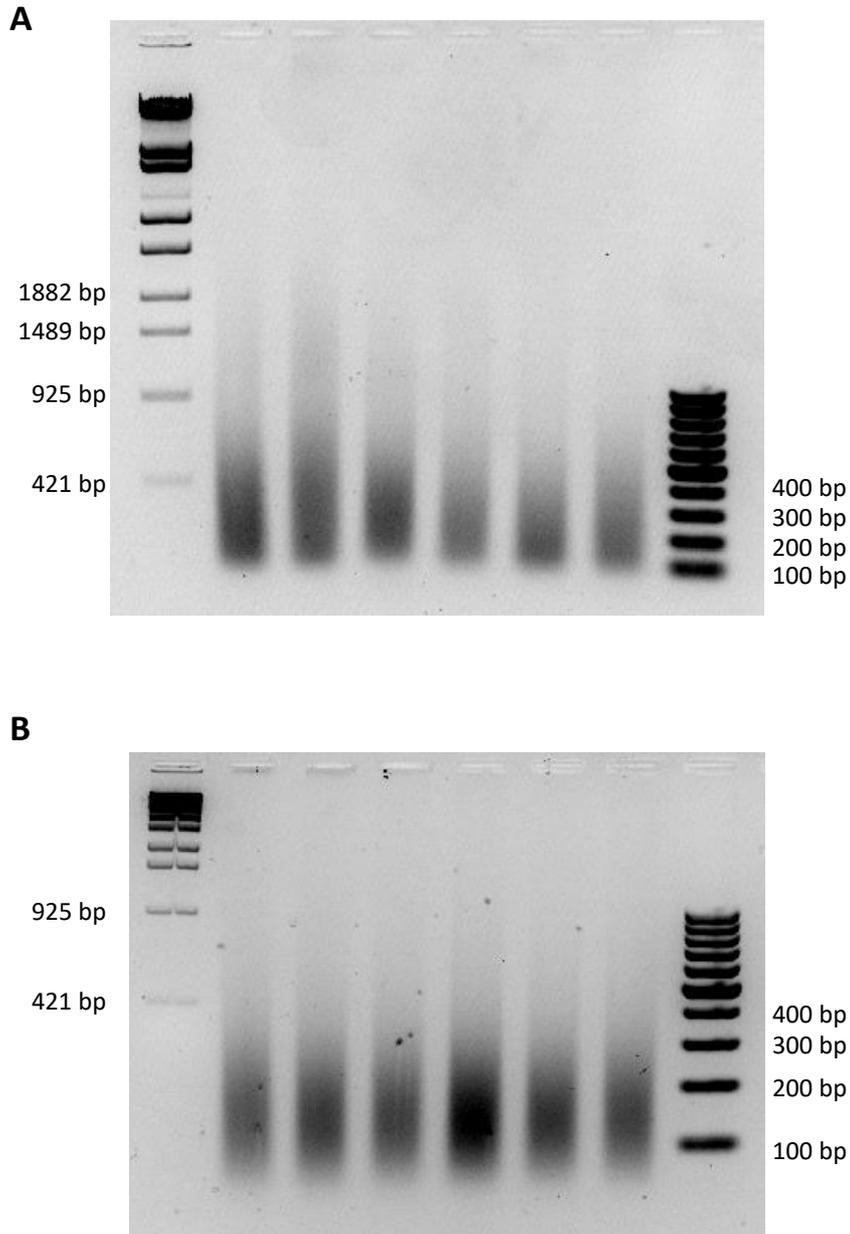
Amplification of 3' end of Sen1	Sen1 4501 FW	ctatgtccacgtataacctgg
	Sen1 STOP RV Bahler	ttaattaaccggggatccgtgatcgTTGTCTGATTTCTTAGAAGG
Mutation of 3' end of Sen1	Sen1 MUT FW	gacgttaatttcaccgggcgaataGACgttattacccatcgatcccaa
	Sen1 MUT RV	ttgggatcgatattgggtaataacGTCtattcgccgggtaattaacgtc
Sequencing of Sen1	Sen1 4368 FW	aagaagtctacgattcag
Amplification of 3' end of Rrpc37	Rrpc37 308 FW	gacccaaacactttctgg
	Rrpc37 STOP RV Bahler	ttaattaaccggggatccgAATAAAGGAGTAATCTTCATCAACAGC
Mutation of 3' end of Rrpc37	Rrpc37 V189D FW	acagaagcaccacaaaGACTccaccactcacata
	Rrpc37 V189D RV	tatgtgagtgggtgaGTCtttgggtctctgt
Sequencing of Rrpc37	Rrpc37 213 FW	actggctggatggaggtc

#### IV. Chromatin Immunoprecipitation (ChIP)

$1.5 \cdot 10^8$  cells were cross-linked with 1% formaldehyde (Sigma) at 18°C for 30 minutes. After 3 washes with cold PBS, the cells were frozen in liquid nitrogen. Frozen cells were then lysed in cold lysis buffer (Hepes-KOH 50mM [pH 7.5], NaCl 140mM, EDTA 1mM, Triton 1%, Na-deoxycholate 0.1%, PMSF Phenylmethanesulfonyl fluoride 1 mM) with glass beads using a Precellys 24 mill (Bertin Technology). To fragment the chromatin, the lysates were sonicated at 4°C using a Covaris S220 (200W 20% Duty factor for 15', or 140W 5% Duty factor for 15') or Diagenode Bioruptor sonicator (10 cycles 30''ON/30''OFF). Covaris sonication at these conditions, generate DNA fragments of around 100-200 bp, which smaller than those produced when Diagenode is used (300-400 bp). Smaller fragments allow to reach a better resolution to be able to determine proteins enrichments at sites 300 bp apart. Highest resolution was essential for the scanning of loci by ChIP. Sonication efficiency was tested by migration of phenol-chloroform purified DNA on 1% or 2% agarose gels. (Figure 14). Immuno-precipitation was done overnight at 4°C using Protein A-coupled or Protein G-coupled Dynabeads previously incubated with anti-GFP A11122 antibody (Invitrogen), anti-Flag antibody (M2 Sigma), anti-Myc 9E10 (Sigma) and anti-H3 1791 (Abcam). The immunoprecipitated complexes were washed for 5' successively with: Wash I buffer (20mM Tris pH 8, 150 mM NaCl, 2mM EDTA, 1% Triton-X100, 0.1% SDS), Wash II buffer (20mM Tris pH 8, 500mM NaCl, 2 mM EDTA, 1% Triton-X100, 0.1% SDS) and Wash III buffer (20mM Tris pH 8, 1mM EDTA, 0.5% Na-deoxycholate, 1% Igepal, 250mM LiCl). After two additional washes in Tris EDTA pH 8, the beads were resuspended in 10% Chelex resin (Biorad) and incubated at 98°C for 10'. After addition of 2 µL of 10 mg/mL proteinase K, the mixture was incubated at 43°C for 1 hour, then for another 10' at 98°C. After centrifugation, the supernatant was collected and analyzed by qPCR in a thermocycler Rotor Gene (Qiagen) using the primers listed in Table 3.

#### V. Immunofluorescence (IF)

$5 \cdot 10^7$  cells were fixed with methanol. After centrifugation 2' at 6000 rpm, cells were resuspended in 1 ml of PEM (100mM PIPES; 1mM EGTA, 1mM MgSO<sub>4</sub>, pH6,9). Three consecutive washes on PEM were done before treatment with Zymoliase 100T at a final concentration of 0,4mg/ml in PEMS, at 37°C. The cells were washed three times with PEMS (PEM, 1,2 Sorbitol). Cells were incubated in 1ml of 1% Triton X-100 in PEMS. After washing in PEM, cells were resuspended in PEMBAL (PEM, 1% BSA 100mM Lysine-HCl). Permeabilized cells were incubated in 100µl with the antibody TAT1 in a 1/200 dilution overnight at 4°C. After three washes in PEMBAL, cells were incubated with the secondary antibody 1/400 in 100 µl for 30 minutes. Cells were washed three time with PEM and resuspended PEM+DAPI.



**Figure 14: Shearing of chromatin for ChIP.** Sonication patterns of chromatin are verified on agarose gels after phenol-chloroform extraction, ethanol precipitation and RNase A digestion of DNA. **(A)** Chromatin sonication using Diagenode Bioruptor, 10 cycles 30''ON/30''OFF. Migration on agarose gel 1%. Note the size of DNA fragments approximately at 300-400 bp. **(B)** Chromatin sonication using Covaris S220 (200W, 20% duty factor, 15'). Migration on agarose gel 2%. Note the size of fragments around 100-200 bp.

## VI. Western Blot

Protein extraction was performed using the TCA (trichloroacetic acid)-glass beads method.  $10^8$  cells were centrifuged 3' at 3400 rpm, resuspended in 20% TCA and then lysed using glass beads in a Precellys 24 mill (Bertin Technology). After centrifugation (4' at 13 krpm), the resulting pellets were resuspended in sample buffer (0,1M Tris-HCl pH 9.5, 20% Glycerol, 4% SDS (sodium dodecyl sulphate), 0.2% bromophenol blue, 715mM  $\beta$ -mercaptoethanol), incubated 5' at 100°C and centrifugated again at 13 krpm for 4'. The resulting supernatants were separated using SDS-PAGE on 7,5 % polyacrylamide gels and transferred onto nitrocellulose using a semi-dry transfer system. Anti-GFP JL8 and anti-Flag (M2 Sigma) antibodies were used for immunodetection of proteins and revealed using ECL-based reagents. An anti-tubulin antibody (TAT1), courtesy of Prof. Keith Gull (Oxford) was used as loading control.

## VII. Strand specific and random hexamers RT-qPCR

Total RNA was extracted from logarithmical growing cells ( $2 \cdot 10^8$ ) by the standard hot-phenol method. The remaining traces of genomic DNA digest were digested with DNase I (Ambion) and the integrity of RNAs was verified by electrophoresis on 0,8% agarose gels. Total RNA was reverse-transcribed using SuperScript III (Invitrogen) according to the manufacturer's instructions using the strand-specific primers listed in Table 3. To quantify read-through transcripts at specific tRNA genes, two independent RT reactions were carried out in parallel: RT1 used a priming oligonucleotide placed downstream of the primary terminator and RT2 used a priming oligonucleotide placed in the gene body upstream of the primary terminator. For both RT1 and RT2, an act1-specific priming oligonucleotide was also used as internal control. The resulting cDNAs were quantified by quantitative PCR (qPCR) using a Rotor Gene machine (Qiagen) and primers specific for act1 and the gene body of the tRNA of interest. The proportion of read-through transcripts was expressed using the ratio RT1/RT2 and expressed as a percentage (read-through tRNA transcripts/total tRNA transcripts).

## VIII. Northern blot

For Northern blots, 10  $\mu$ g of total RNAs were separated on 10% polyacrylamide-8M urea gels and transferred onto a nylon membrane (GE Healthcare Amersham Hybond -N+). The membrane was then UV cross-linked and dried at 80°C for 30 minutes. After incubation with Church Buffer for 30 minutes at 37°C, the membrane was hybridized overnight at 37°C with a  $^{32}$ P-labeled DNA oligo

antisense to the intron of *SPATRNaPRO.02*. The blot was then washed 4 times with 1X SSC + 0.1% SDS and scanned using a Phosphorimager Typhoon FLA 9500 - GE Healthcare.

#### IX. Mapping of the 3' end of read-through transcripts at *SPATRNaPRO.02*.

20 µg of total RNA were separated on a 10% polyacrylamide–8M urea gel. The 200 to 600 bp-long RNAs were extracted from the gel and purified, before a preadenylated RNA adaptor was ligated in 3' as described previously (Heyer et al., 2015). This 3' adaptor was used for retro-transcription as described (Heyer et al., 2015) and *SPATRNaPRO.02*-derived cDNAs were amplified by PCR using the primer Pro.02 qL1 (5'-ACATACCTCTTTCGGGTAATCC-3'). The PCR fragment obtained was cloned into pCR Blunt II TOPO using the Zero Blunt II TOPO PCR Cloning Kit (Invitrogen Life Technologies) and sequenced.

#### X. Transcription termination assay

Strains carrying the *ade6-704* mutation and the DRT5T dimeric construct (see diagram of the DRT5T construct in Figure 5A in the publication) were obtained from the Marais laboratory (Iben et al., 2011). Standard genetic crosses were employed to introduce these reporter constructs in the strains of interest. At least two independent strains for each genotype were then plated on YES medium depleted or not of adenine for 3 days at 30°C.

**Table 2. Strains used in this thesis.**

Strain n°	Mat. type	Leu Ura Ade His	Genotype	Origin
LY5	<i>h+</i>	<i>leu1-32</i>		lab stock
LY6	<i>h-</i>	<i>leu1-32</i>		lab stock
LY11	<i>h+</i>	<i>leu1-32 ura4D18</i>		lab stock
LY12	<i>h-</i>	<i>leu1-32 ura4D18</i>		lab stock
LY112	<i>h+</i>	<i>leu1-32 ura4D18 ade6-210</i>		lab stock
LY113	<i>h-</i>	<i>leu1-32 ura4D18 ade6-210</i>		lab stock
LY1052	<i>h-</i>	<i>leu1-32</i>	<i>rrp6Δ::KanR</i>	lab stock
LY1053	<i>h+</i>	<i>leu1-32</i>	<i>rrp6Δ::KanR</i>	lab stock
LY1334	<i>h-</i>	<i>leu1-32</i>	<i>sen1Δ::KanR</i>	lab stock
LY1613	<i>h-</i>		<i>psm3-GFP-NatR</i>	lab stock
LY2615	<i>h-</i>	<i>leu1-32 ura4- ade6-210</i>	<i>sen1-GFP-KanR</i>	lab stock
LY2616	<i>h+</i>	<i>leu1-32 ura4- ade6-210</i>	<i>sen1-GFP-KanR</i>	lab stock
LY2528	<i>h-</i>	<i>leu1-32 ura? ade?</i>	<i>rnh1D129N-GFP-KanR</i>	lab stock
LY2529	<i>h-</i>	<i>leu1-32 ura? ade?</i>	<i>rnh1D129N-GFP-KanR</i>	lab stock
LY2544	<i>h+</i>	<i>leu1-32 ura4- ade6-210</i>	<i>rnh1D129N-GFP-KanR ARS::nmt1-RnhA::LEU2+</i>	lab stock
LY2545	<i>h-</i>	<i>leu1-32 ura4- ade6-216</i>	<i>rnh1D129N-GFP-KanR ARS::nmt1-RnhA::LEU2+</i>	lab stock
LY2638	<i>h+</i>	<i>leu1-32 ura4D18</i>	<i>sen1Δ::NatR</i>	lab stock
LY2639	<i>h-</i>	<i>leu1-32 ura4D18 ade6-210</i>	<i>sen1Δ::NatR</i>	lab stock
LY3159	<i>h+</i>	<i>leu1-32 ura4D-</i>	<i>rpc25-13myc-KanR sen1Δ::NatR</i>	lab stock
LY3160	<i>h+</i>	<i>leu1-32 ura4D- ade6-210</i>	<i>rpc25-13myc-KanR sen1Δ::NatR</i>	lab stock
LY3161	<i>h+</i>	<i>leu1-32 ura4D- ade6-210</i>	<i>rpc25-13myc-KanR sen1Δ::NatR</i>	lab stock
LY3681	<i>h90?</i>	<i>leu1-32 ura4D- ade6-216</i>	<i>rpc25-13myc-KanR rnh1Δ::HygroR rnh201Δ::KanR</i>	lab stock
LY3747	<i>h-?</i>	<i>leu1-32 ura- ade6-216</i>	<i>rrp6Δ::KanR</i>	lab stock
LY3790	<i>h+</i>	<i>leu1-32 ura4-</i>	<i>sen1Δ::NatR rrp6Δ::KanR</i>	lab stock
LY3791	<i>h-</i>	<i>leu1-32 ura4- ade6-216</i>	<i>sen1Δ::NatR rrp6Δ::KanR</i>	lab stock
LY3811	<i>h-</i>	<i>leu1-32 ura4-</i>	<i>dbl8Δ::NatR</i>	this study
LY3812	<i>h+</i>	<i>leu1-32 ura4-</i>	<i>dbl8Δ::NatR</i>	this study
LY3815	<i>h-</i>	<i>leu1-32 ura4D18 ade6-210</i>	<i>dbl8-3flag-NatR</i>	this study
LY3843	<i>h-</i>	<i>leu1-32 ura4-</i>	<i>sen1Δ::KanR</i>	lab stock
LY3844	<i>h+</i>	<i>leu1-32 ura4-</i>	<i>sen1Δ::KanR</i>	lab stock
LY3859	<i>h-</i>	<i>leu1-32 ura4- ade6-210</i>	<i>sen1-GFP-KanR dbl8Δ::NatR</i>	this study
LY3860	<i>h-</i>	<i>leu1-32 ura4- ade6-210</i>	<i>sen1-GFP-KanR dbl8Δ::NatR</i>	this study
LY3861	<i>h+</i>	<i>leu1-32 ura4- ade6-210</i>	<i>sen1-GFP-KanR dbl8Δ::NatR</i>	this study
LY3921	<i>h+</i>	<i>leu1-32 ura4- ade6-210</i>	<i>rpa43-GFP-KanR</i>	this study
LY3923	<i>h-</i>	<i>leu1-32 ura4- ade6-210</i>	<i>rpa43-GFP-KanR dbl8-3flag-NatR</i>	this study
LY3929	<i>h-</i>	<i>leu1-32 ura4- ade6-210</i>	<i>sen1-3flag-NatR</i>	lab stock
LY3930	<i>h+</i>	<i>leu1-32 ura4- ade6-210</i>	<i>sen1-3flag-NatR</i>	lab stock
LY3966	<i>h-</i>	<i>leu1-32 ura4D18 ade6? his3D1</i>	<i>P81nmt1-dis3::KanR</i>	F. Bachand
LY4125	<i>h-</i>	<i>ura4D18 ade6-704</i>	<i>leu1-32::[DRT6T::leu1+]</i>	R. Maraia
LY4127	<i>h-</i>	<i>leu1-32 ura4D18 ade6-704</i>		R. Maraia
LY4130	<i>h-</i>	<i>ura4D18 ade6-704</i>	<i>leu1-32::[DRT5T::leu1+]</i>	R. Maraia
LY4156	<i>h-</i>	<i>leu1-32 ade6-704</i>	<i>sen1Δ::KanR</i>	this study
LY4157	<i>h+</i>	<i>leu1-32 ade6-704</i>	<i>sen1Δ::KanR</i>	this study
LY4164	<i>h+</i>	<i>ade6-704</i>	<i>sen1Δ::KanR leu1-32::[DRT5T::leu1+]</i>	this study
LY4165	<i>h-</i>	<i>ade6-704</i>	<i>sen1Δ::KanR leu1-32::[DRT5T::leu1+]</i>	this study
LY4166	<i>h+</i>	<i>ura4- ade6-704</i>	<i>sen1Δ::KanR leu1-32::[DRT6T::leu1+]</i>	this study
LY4167	<i>h-</i>	<i>ade6-704</i>	<i>sen1Δ::KanR leu1-32::[DRT6T::leu1+]</i>	this study
LY4174	<i>h-</i>	<i>leu1-32 ura4- ade6-704</i>	<i>dbl8Δ::NatR</i>	this study
LY4175	<i>h+</i>	<i>leu1-32 ura4- ade6-704</i>	<i>dbl8Δ::NatR</i>	this study
LY4183	<i>h+</i>	<i>leu1-32 ura4- ade6-704</i>	<i>dbl8Δ::NatR leu1-32::[DRT5T::leu1+]</i>	this study
LY4184	<i>h-</i>	<i>leu1-32 ura4- ade6-704</i>	<i>dbl8Δ::NatR leu1-32::[DRT5T::leu1+]</i>	this study
LY4195	<i>h+</i>	<i>ade6-704 ura4D-</i>	<i>dbl8Δ::NatR leu1-32::[DRT6T::leu1+]</i>	this study
LY4196	<i>h-</i>	<i>ade6-704</i>	<i>dbl8Δ::NatR leu1-32::[DRT6T::leu1+]</i>	this study
LY4222	<i>h+</i>	<i>leu1-32</i>	<i>sen1-G1534D-GFP-HygroR</i>	this study
LY4223	<i>h+</i>	<i>leu1-32</i>	<i>sen1-G1534D-GFP-HygroR</i>	this study
LY4232	<i>h-</i>		<i>KanR-Pnmt41-slp1+</i>	this study
LY4233	<i>h-</i>		<i>KanR-Pnmt41-slp1+ sen1Δ::HygroR</i>	this study
LY4234	<i>h-</i>		<i>KanR-Pnmt41-slp1+</i>	this study

LY4235	<i>h+</i>		<i>KanR-Pnmt41-slp1+</i>	this study
LY4237	<i>h+</i>		<i>KanR-Pnmt41-slp1+ sen1Δ::HygroR cnd2-GFP-LEU2</i>	this study
LY4238	<i>h+</i>		<i>KanR-Pnmt41-slp1+ sen1Δ::HygroR cnd2-GFP-LEU2</i>	this study
LY4267	<i>h+</i>	<i>leu1-32 ade6-704</i>	<i>sen1-G1534D-GFP-HygroR</i>	this study
LY4268	<i>h-</i>	<i>leu1-32 ade6-704</i>	<i>sen1-G1534D-GFP-HygroR</i>	this study
LY4285	<i>h-</i>	<i>leu1-32 ura4D18 ade6-704</i>	<i>rpc37-3flag-NatR</i>	this study
LY4290	<i>h-</i>	<i>leu1-32 ura4D18 ade6-704</i>	<i>rpc37-V189D-3flag-NatR</i>	this study
LY4294	<i>h+</i>	<i>leu1-32 ura4D18 ade6-704</i>		this study
LY4315	<i>h-</i>	<i>leu1-32 ura4D18 ade6-704</i>	<i>rpc37-V189D-3flag-NatR</i>	this study
LY4316	<i>h+</i>	<i>leu1-32 ura4D18 ade6-704</i>	<i>rpc37-V189D-3flag-NatR</i>	this study
LY4331	<i>h+</i>	<i>ura4D18 ade6-704</i>	<i>rpc37-3flag-NatR leu1-32::[DRT5T::leu1+]</i>	this study
LY4332	<i>h+</i>	<i>ura4D18 ade6-704</i>	<i>rpc37-3flag-NatR leu1-32::[DRT5T::leu1+]</i>	this study
LY4333	<i>h+</i>	<i>ura4D18 ade6-704</i>	<i>rpc37-3flag-NatR sen1Δ::KanR leu1-32::[DRT5T::leu1+]</i>	this study
LY4334	<i>h+</i>	<i>ura4D18 ade6-704</i>	<i>rpc37-3flag-NatR sen1Δ::KanR leu1-32::[DRT5T::leu1+]</i>	this study
LY4335	<i>h+</i>	<i>ura4D18 ade6-704</i>	<i>rpc37-3flag-NatR leu1-32::[DRT6T::leu1+]</i>	this study
LY4336	<i>h+</i>	<i>ura4D18 ade6-704</i>	<i>rpc37-3flag-NatR leu1-32::[DRT6T::leu1+]</i>	this study
LY4339	<i>h-</i>		<i>KanR-Pnmt41-slp1+ cut3-GFP-ura4+</i>	this study
LY4341	<i>h-</i>		<i>KanR-Pnmt41-slp1+ cut3-GFP-ura4+</i>	this study
LY4349	<i>h+</i>	<i>leu1-32 ade6-704</i>	<i>rpc37-3flag-NatR</i>	this study
LY4350	<i>h-</i>	<i>leu1-32 ade6-704</i>	<i>rpc37-3flag-NatR</i>	this study
LY4355	<i>h-</i>	<i>ade6-704</i>	<i>rpc37-V189D-3flag-NatR leu1-32::[DRT5T::leu1+]</i>	this study
LY4356	<i>h-</i>	<i>ade6-704</i>	<i>rpc37-V189D-3flag-NatR leu1-32::[DRT5T::leu1+]</i>	this study
LY4357	<i>h-</i>	<i>ade6-704</i>	<i>rpc37-V189D-3flag-NatR leu1-32::[DRT6T::leu1+]</i>	this study
LY4358	<i>h+</i>	<i>ade6-704</i>	<i>rpc37-V189D-3flag-NatR leu1-32::[DRT6T::leu1+]</i>	this study
LY4413	<i>h-</i>		<i>KanR-Pnmt41-slp1+ cut3-GFP-ura4+</i>	lab stock
LY4414	<i>h-</i>		<i>KanR-Pnmt41-slp1+ cut3-GFP-ura4+ sfc3-1</i>	lab stock
LY4415	<i>h-</i>		<i>KanR-Pnmt41-slp1+ cut3-GFP-ura4+ sfc3-1</i>	lab stock
LY4416	<i>h+</i>		<i>KanR-Pnmt41-slp1+ cut3-GFP-ura4+ sfc3-1</i>	lab stock
LY4417	<i>h+</i>		<i>KanR-Pnmt41-slp1+ cut3-GFP-ura4+ sfc3-1</i>	lab stock
LY4443	<i>h+</i>	<i>leu1-32 ura4D ade6-21? arg3D4</i>	<i>ura4-Pnmt41-slp1+</i>	lab stock
LY4444	<i>h+</i>	<i>leu1-32 ura4D ade6-21? arg3D4</i>	<i>ura4-Pnmt41-slp1+</i>	lab stock
LY4483	<i>h-</i>	<i>leu1-32 ura4D</i>	<i>ura4-Pnmt41-slp1+ cnd2-GFP-LEU2</i>	this study
LY4484	<i>h+</i>	<i>leu1-32 ura4D</i>	<i>ura4-Pnmt41-slp1+ cnd2-GFP-LEU2</i>	this study
LY4488	<i>h?</i>		<i>KanR-Pnmt41-slp1+ cnd2-GFP-LEU2 sen1Δ::HygroR</i>	this study
LY4523	<i>h-</i>	<i>leu1-32 ura4D</i>	<i>sen1Δ::NatR</i>	lab stock
LY4524	<i>h+</i>	<i>leu1-32 ura4D ade6-210</i>	<i>sen1Δ::NatR</i>	lab stock
LY4547	<i>h-</i>	<i>leu1-32 ura4D ade6-210</i>	<i>ura4-Pnmt41-slp1+ sen1Δ::KanR</i>	this study
LY4591	<i>h?</i>	<i>leu1-32 ura4D ade6-</i>	<i>ura4-Pnmt41-slp1+ cnd2-GFP-LEU2 pso2Δ::KanR</i>	lab stock
LY4592	<i>h?</i>	<i>leu1-32 ura4D ade6-</i>	<i>ura4-Pnmt41-slp1+ cnd2-GFP-LEU2 pso2Δ::KanR</i>	lab stock
LY4593	<i>h?</i>	<i>leu1-32 ura4D ade6-</i>	<i>ura4-Pnmt41-slp1+ cnd2-GFP-LEU2 pso2Δ::KanR</i>	lab stock
LY4681	<i>h+</i>	<i>leu1-32 ura4D18</i>	<i>cdc2asM17</i>	I. Hagan
LY4682	<i>h-</i>	<i>leu1-32 ura4D18</i>	<i>cdc2asM17</i>	I. Hagan
LY4684	<i>h+</i>	<i>leu1-32 ura4D</i>	<i>ura4-Pnmt41-slp1+ tbp1-GFP-KanR</i>	lab stock
LY4685	<i>h+</i>	<i>leu1-32 ura4D</i>	<i>ura4-Pnmt41-slp1+ tbp1-GFP-KanR</i>	lab stock
LY4687	<i>h-</i>	<i>leu1-32 ura4D</i>	<i>ura4-Pnmt41-slp1+ tbp1-GFP-KanR sen1Δ::KanR</i>	lab stock
LY4757	<i>h-</i>	<i>ura4D- ade6-704</i>	<i>cdc2asM17 rpc37-3flag-NatR</i>	lab stock
LY4768	<i>h+</i>	<i>leu1-32 ura4D</i>	<i>ura4-Pnmt41-slp1+ cnd2-GFP-LEU2 dbl8Δ::NatR</i>	lab stock
LY4769	<i>h+</i>	<i>leu1-32 ura4D</i>	<i>ura4-Pnmt41-slp1+ cnd2-GFP-LEU2 dbl8Δ::NatR</i>	lab stock
LY4781	<i>h-</i>		<i>cdc2asM17 sen1Δ::KanR</i>	lab stock
LY4793	<i>h+</i>	<i>leu1-32 ura4D18 ade6-210</i>	<i>arg10-2T</i>	this study
LY4795	<i>h+</i>	<i>leu1-32 ura4D18 ade6-210</i>	<i>arg10-2T</i>	this study
LY4799	<i>h?</i>	<i>leu1-32 ura4D</i>	<i>ura4-Pnmt41-slp1+ tbp1-GFP-KanR sen1Δ::KanR</i>	this study
LY4800	<i>h+</i>	<i>leu1-32 ura4D</i>	<i>ura4-Pnmt41-slp1+ tbp1-GFP-KanR sen1Δ::KanR</i>	this study
LY4801	<i>h+</i>	<i>leu1-32 ura4D</i>	<i>ura4-Pnmt41-slp1+ tbp1-GFP-KanR sen1Δ::KanR</i>	this study
LY4880	<i>h+</i>	<i>leu1-32 ura4D18 ade6-210</i>	<i>sen1Δ::NatR arg10-2T</i>	this study
LY4881	<i>h+</i>	<i>leu1-32 ura4D18 ade6-210</i>	<i>sen1Δ::NatR arg10-2T</i>	this study
LY4882	<i>h-</i>	<i>leu1-32 ura4D18 ade6-210</i>	<i>sen1Δ::NatR arg10-2T</i>	this study
LY4894	<i>h-</i>	<i>leu1-32 ura4- ade6-210</i>	<i>cdc2asM17 top1-3flag-NatR cnd2-GFP-LEU2</i>	this study
LY4903	<i>h+</i>		<i>cdc2asM17 top1-3flag-NatR cnd2-GFP-LEU2 sen1Δ::KanR</i>	this study
LY4938	<i>h-</i>	<i>leu1-32 ura4D18 ade6-704/210?</i>	<i>rpc37-3flag-NatR arg10-2T</i>	this study
LY4939	<i>h+</i>	<i>leu1-32 ura4D18 ade6-704/210?</i>	<i>rpc37-3flag-NatR arg10-2T</i>	this study
LY4946	<i>h-</i>	<i>leu1-32 ura4- ade6-210</i>	<i>cdc2asM17 top1-3flag-NatR cnd2-GFP-LEU2 [Ser09 Arg10] TERMA</i>	this study

LY4975	<i>h+</i>	<i>leu1-32 ade6-704</i>	<i>cdc2asM17 rpc37-3flag-NatR</i>	this study
LY4976	<i>h-</i>	<i>leu1-32 ade6-704</i>	<i>cdc2asM17 rpc37-3flag-NatR</i>	this study
LY4977	<i>h-</i>	<i>leu1-32 ade6-704</i>	<i>cdc2asM17 rpc37-3flag-NatR sen1Δ::KanR</i>	this study
LY4978	<i>h-</i>	<i>leu1-32 ade6-704</i>	<i>cdc2asM17 rpc37-3flag-NatR sen1Δ::KanR</i>	this study
LY4980	<i>h-</i>	<i>leu1-32 ade6-704</i>	<i>cdc2asM17 cnd2-GFP-LEU2 rpc37-3flag-NatR</i>	this study
LY4981	<i>h+</i>	<i>leu1-32 ade6-704</i>	<i>cdc2asM17 cnd2-GFP-LEU2 rpc37-3flag-NatR sen1Δ::KanR</i>	this study
LY4982	<i>h-</i>	<i>leu1-32 ade6-704</i>	<i>cdc2asM17 cnd2-GFP-LEU2 rpc37-3flag-NatR sen1Δ::KanR</i>	this study
LY5034	<i>h?</i>	<i>leu1-32 ura4- ade6-</i>	<i>ura4-Pnmt41-slp1+ his7::LacI-GFP</i>	this study
LY5035	<i>h-</i>	<i>leu1-32 ura4- ade6-</i>	<i>ura4-Pnmt41-slp1+ his7::LacI-GFP sen1Δ::KanR</i>	this study
LY5036	<i>h?</i>	<i>leu1-32 ura4- ade6-</i>	<i>ura4-Pnmt41-slp1+ nda3-KM311 his7::LacI-GFP</i>	this study
LY5037	<i>h?</i>	<i>leu1-32 ura4- ade6-</i>	<i>ura4-Pnmt41-slp1+ nda3-KM311 his7::LacI-GFP</i>	this study
LY5051	<i>h+</i>	<i>leu1-32 ura4- ade?</i>	<i>cdc2asM17 cnd2-GFP-LEU2 rpc37-3flag-NatR arg10Δ::ura4+</i>	this study
LY5052	<i>h-</i>	<i>leu1-32 ura4- ade?</i>	<i>cdc2asM17 cnd2-GFP-LEU2 rpc37-3flag-NatR arg10Δ::ura4+</i>	this study
LY5053	<i>h-</i>	<i>leu1-32 ura4- ade?</i>	<i>cdc2asM17 cnd2-GFP-LEU2 rpc37-3flag-NatR arg10Δ::ura4+ sen1Δ::KanR</i>	this study
LY5218	<i>h+</i>	<i>leu1-32 ura4D-</i>	<i>rpc25-13myc-KanR sen1Δ::NatR ARS::nmt1-RnhA::LEU2+</i>	this study
LY5219	<i>h+</i>	<i>leu1-32 ura4D-</i>	<i>rpc25-13myc-KanR sen1Δ::NatR ARS::nmt1-RnhA::LEU2+</i>	this study
LY5220	<i>h-</i>	<i>leu1-32 ura4D- ade6-210</i>	<i>rpc25-13myc-KanR sen1Δ::NatR ARS::nmt1-RnhA::LEU2+</i>	this study
LY5221	<i>h+</i>	<i>leu1-32 ura4D- ade6-210</i>	<i>rpc25-13myc-KanR sen1Δ::NatR ARS::nmt1-RnhA::LEU2+</i>	this study
LY5242	<i>h-</i>	<i>leu1-32 ura4D- ade6-210 his2-Mat1M</i>	<i>rpc25-13myc-KanR</i>	K. Noma
LY5271	<i>h+</i>	<i>leu1-32</i>	<i>psm3-GFP-NatR sen1Δ::KanR</i>	lab stock
LY5272	<i>h-</i>		<i>psm3-GFP-NatR sen1Δ::KanR</i>	lab stock
LY5277	<i>h+</i>	<i>leu1-32 ura4D-</i>	<i>rpc25-13myc-KanR ARS::nmt1-RnhA::LEU2+</i>	this study
LY5278	<i>h-</i>	<i>leu1-32 ura4D- his2-Mat1M</i>	<i>rpc25-13myc-KanR ARS::nmt1-RnhA::LEU2+</i>	this study
LY5357	<i>h-</i>	<i>leu1-32 ura4D- ade6-210/216?</i>	<i>rnh1-D129N-GFP-KanR sen1Δ::NatR</i>	this study
LY5358	<i>h-</i>	<i>leu1-32 ura4D- ade6-210/216?</i>	<i>rnh1-D129N-GFP-KanR sen1Δ::NatR</i>	this study
LY5359	<i>h+</i>	<i>leu1-32 ura4D- ade6-210/216?</i>	<i>rnh1-D129N-GFP-KanR sen1Δ::NatR</i>	this study
LY5360	<i>h+</i>	<i>leu1-32 ura4D- ade6-210/216?</i>	<i>rnh1-D129N-GFP-KanR sen1Δ::NatR ARS::nmt1-RnhA::LEU2+</i>	this study
LY5361	<i>h-</i>	<i>leu1-32 ura4D- ade6-210/216?</i>	<i>rnh1-D129N-GFP-KanR sen1Δ::NatR ARS::nmt1-RnhA::LEU2+</i>	this study
LY5362	<i>h+</i>	<i>leu1-32 ura4D- ade6-210/216?</i>	<i>rnh1-D129N-GFP-KanR sen1Δ::NatR ARS::nmt1-RnhA::LEU2+</i>	this study
LY5465	<i>h-</i>	<i>leu1-32 ura4D- ade6-210/704?</i>	<i>rpc37-3flag-NatR sen1Δ::KanR arg10-2T</i>	this study
LY5466	<i>h+</i>	<i>leu1-32 ura4D- ade6-210/704?</i>	<i>rpc37-3flag-NatR sen1Δ::KanR arg10-2T</i>	this study
LY5467	<i>h-</i>	<i>leu1-32 ura4D- ade6-210/704?</i>	<i>rpc37-3flag-NatR sen1Δ::KanR arg10-2T</i>	this study
LY5521	<i>h-</i>	<i>leu1-32 ura4D- ade6-? his3D1</i>	<i>sen1Δ::NatR P81nmt1-dis3::KanR</i>	this study
LY5522	<i>h+</i>	<i>leu1-32 ura4D- ade6-?</i>	<i>sen1Δ::NatR P81nmt1-dis3::KanR</i>	this study
LY5524	<i>h-</i>	<i>leu1-32 ura4D- ade6-? his3D1</i>	<i>sen1Δ::NatR P81nmt1-dis3::KanR</i>	this study
LY5533	<i>h-</i>	<i>leu1-32 ura4D ade6-?</i>	<i>KanR-Pnmt41-slp1+ cnd2-GFP-LEU2 sen1Δ::NatR sfc3-1</i>	this study
LY5534	<i>h-</i>	<i>leu1-32 ura4D ade6-?</i>	<i>KanR-Pnmt41-slp1+ cnd2-GFP-LEU2 sen1Δ::NatR sfc3-1</i>	this study
LY5609	<i>h-</i>	<i>leu1-32</i>	<i>KanR-Pnmt41-slp1+</i>	this study
LY5610	<i>h-</i>	<i>leu1-32</i>	<i>KanR-Pnmt41-slp1+ sen1Δ::HygroR</i>	this study
LY5615	<i>h+</i>	<i>leu1-32</i>	<i>KanR-Pnmt41-slp1+ sfc6-13myc-KanR</i>	this study
LY5616	<i>h+</i>	<i>leu1-32</i>	<i>KanR-Pnmt41-slp1+ sfc6-13myc-KanR</i>	this study
LY5617	<i>h+</i>	<i>leu1-32</i>	<i>KanR-Pnmt41-slp1+ sfc6-13myc-KanR sen1Δ::HygroR</i>	this study
LY5618	<i>h+</i>	<i>leu1-32</i>	<i>KanR-Pnmt41-slp1+ sfc6-13myc-KanR sen1Δ::HygroR</i>	this study
LY5681	<i>h-</i>	<i>leu? ura4D18 ade6-210</i>	<i>cdc2-asM17 cnd2-GFP-LEU2 arg10-10xReb1</i>	this study
LY5724	<i>h+</i>	<i>leu1-32 ura4D18 ade6-210</i>	<i>arg10-23T</i>	this study
LY5756	<i>h+</i>	<i>leu1-32 ura4D ade6-210/704?</i>	<i>cdc2-asM17 cnd2-GFP-LEU2 rpc37-3flag-NatR arg10-TATAless sen1Δ::KanR</i>	this study
LY5757	<i>h-</i>	<i>leu1-32 ura4D ade6-210/704?</i>	<i>cdc2-asM17 cnd2-GFP-LEU2 rpc37-3flag-NatR arg10-TATAless sen1Δ::KanR</i>	this study
LY5762	<i>h-</i>	<i>leu1-32 ura4D- ade6-210/704?</i>	<i>cdc2asM17 rpc37-3flag-NatR sen1Δ::KanR arg10-23T</i>	this study
LY5763	<i>h+</i>	<i>leu1-32 ura4D- ade6-210/704?</i>	<i>cdc2asM17 rpc37-3flag-NatR sen1Δ::KanR arg10-23T</i>	this study
LY5764	<i>h+</i>	<i>leu1-32 ura4D ade6-210/704?</i>	<i>cdc2-asM17 cnd2-GFP-LEU2 rpc37-3flag-NatR arg10-23T sen1Δ::KanR</i>	this study
LY5765	<i>h-</i>	<i>leu1-32 ura4D ade6-210/704?</i>	<i>cdc2-asM17 cnd2-GFP-LEU2 rpc37-3flag-NatR arg10-23T sen1Δ::KanR</i>	this study
LY5766	<i>h-</i>	<i>leu1-32 ura4D- ade6-210/704?</i>	<i>cdc2asM17 rpc37-3flag-NatR arg10-23T</i>	this study

LY5767	<i>h+</i>	<i>leu1-32 ura4D- ade6-210/704?</i>	<i>cdc2asM17 rpc37-3flag-NatR arg10-23T</i>	this study
LY5768	<i>h-</i>	<i>leu1-32 ura4D ade6-210/704?</i>	<i>cdc2-asM17 cnd2-GFP-LEU2 rpc37-3flag-NatR arg10-23T</i>	this study
LY5955	<i>h+</i>	<i>leu1-32 ura4D- ade6-210</i>	<i>sen1-3flag-NatR arg10-TATAless</i>	this study
LY5956	<i>h-</i>	<i>leu1-32 ura4D- ade6-210</i>	<i>sen1-3flag-NatR arg10-TATAless</i>	this study
LY5961	<i>h-</i>	<i>leu1-32 ura4D- ade6-210</i>	<i>sen1Δ::NatR arg10-23T</i>	this study
LY5962	<i>h-</i>	<i>leu1-32 ura4D- ade6-210</i>	<i>sen1Δ::NatR arg10-23T</i>	this study
LY5965	<i>h-</i>	<i>leu1-32 ura4D- ade6-</i>	<i>rpc37-3flag-NatR arg10-TATAless</i>	this study
LY5966	<i>h-</i>	<i>leu1-32 ura4D- ade6-</i>	<i>rpc37-3flag-NatR arg10-TATAless</i>	this study
LY6095	<i>h-</i>	<i>leu1-32 ura4D ade6-210/704</i>	<i>cdc2-asM17 cnd2-GFP-LEU2 rpc37-3flag-NatR thr10Δ::ura4+</i>	this study
LY6097	<i>h+</i>	<i>leu1-32 ura4D ade6-210/704</i>	<i>cdc2-asM17 cnd2-GFP-LEU2 rpc37-3flag-NatR thr10Δ::ura4+</i>	this study
LY6180	<i>h+</i>	<i>leu1-32 ura4D ade6-210/704?</i>	<i>rpc37-3flag-NatR sen1D::KanR thr10-20T</i>	this study
LY6201	<i>h-</i>	<i>leu1-32 ura4D ade6-210/704</i>	<i>cdc2-asM17 cnd2-GFP-LEU2 rpc37-3flag-NatR thr10-20T sen1Δ::KanR</i>	this study
LY6202	<i>h+</i>	<i>leu1-32 ura4D ade6-210/704</i>	<i>cdc2-asM17 cnd2-GFP-LEU2 rpc37-3flag-NatR thr10-20T sen1Δ::KanR</i>	this study
LY6203	<i>h+</i>	<i>leu1-32 ura4D ade6-210/704</i>	<i>cdc2-asM17 cnd2-GFP-LEU2 rpc37-3flag-NatR thr10-20T sen1Δ::KanR</i>	this study
LY6211	<i>h+</i>	<i>leu1-32 ura4D ade6-210/704?</i>	<i>sen1D::KanR thr10-20T</i>	this study
LY6233	<i>h+</i>	<i>leu1-32 ura4D ade6-210/704</i>	<i>cdc2-asM17 cnd2-GFP-LEU2 rpc37-3flag-NatR arg10 TATAless</i>	this study
LY6234	<i>h-</i>	<i>leu1-32 ura4D ade6-210?</i>	<i>cdc2-asM17 cnd2-GFP-LEU2 rpc37-3flag-NatR arg10 TATAless</i>	this study

**Table 3. Primers used in this thesis.**

Chromosome site	Name	Experiment	Primer name	Primer sequence
5S ribosomal RNA	5S	ChIP	5SqL1 5SqR1	TAGGCGAAAACACCAGTTCC TTCCCATGTTGTCTCCAACC
SPRRNA.20	5S20	ChIP	5S20qL2 5S20qR2	CATGGCTAAATTGTTCAAATCC GTGGTAATTCGCCATTGTC
18S ribosomal RNA	18S	ChIP	18SqL1 18SqR1	tttctaggaccgccgtaatg tgctttcgcagtagtctgctc
28S ribosomal RNA	28S	ChIP	28sqL1 28sqR1	cccttgcgcttaattggac tacattccggcaccttaacc
act1	act1	RTqPCR	act1qL0 act1qR0	aacgctcgtttccgatagtg acgtcgctttggactttgag
adh1	adh1	ChIP	adh1ORFqL1 adh1ORFqR1	tcaccatggtcactgtttc cgggaaacgtattcaagagc
aes1	aes1	ChIP	aes1 qL2 aes1 qR2	GCCGACAATCGATAAAGCAG CGATCCAATTGTTGGGTGAC
SPBTRNAARG.04	arg04	ChIP	arg4qL1 arg4qR1	cattaatccgccgtggatag ttcacctaataagttgccaaacg
SPBTRNAARG.05	arg05	RTqPCR	arg05qL2 arg05qR2	ATGGTAGCGCATCTCATTCC ACAATCTTACACGACGGGACTC
SPCTRNAARG.09	arg09	ChIP	arg9qL2 arg9qR2	aattcgagtcaaaaaggatg ggaatgtaaatcccgcctcag
SPBTRNAARG.05	arg05	RTqPCR	arg05RTT2	CATGAGCAAAAACAAAATACCACA
SPCTRNAARG.10	arg10.1	ChIP/RTqPCR	arg10qL1 arg10qR1	GGTGTGTAGCCTAATGGTTAAGG GAGTGTGACAGGACTCGAACC
SPCTRNAARG.10	arg10.2	ChIP	arg10qL2 arg10qR2	GGAAGGATACAATATCCACAACG GCTGTTATCCATCCACTTACGG
SPCTRNAARG.10	arg10.3	ChIP	arg10 -300 FW arg10qR3	TGCGACTCAGCATAAAGGTG GGCATTCTGTCGATTTTGC
SPCTRNAARG.10	arg10.4	ChIP	arg10qL4 arg10qR4	AAGCCGCCTTTCGTTAACAC CTGCTTGACCAGCTTTTGTG
SPCTRNAARG.10	arg10.5	ChIP	arg10qL5 arg10qR5	ACCAAAGTCCCGATAACAAC GCAAACAGAGTCCAATTGAGG
SPCTRNAARG.10	arg10	RTqPCR	arg10RTT1	CAATATTTCTATTGCATGTTGACAG
cnt	cnt1	ChIP	cnt1qL1 cnt1qR1	accgttgcaacttacatcagc ggtcgccaatagcaatgag
Chromosome-Organizing Clamp 3*	COC3	ChIP	COC3qL COC3qR	CGAATCGCCCTTATGGATTTC ATGCTACATCCCGATGATCC
Chromosome-Organizing Clamp 4*	COC4	ChIP	COC4qL COC4qR	TGTCGATATTAGACCGAGCAAC TTCGAACCCACAGACTTTGC
Chromosome-Organizing Clamp 5*	COC5	ChIP	COC5qL COC5qR	CCGAATTGACGCTAGTCTCC TTGTTGCTACCAGCGTATCC
ecm33	ecm33	ChIP	ecm33qL4 ecm33qR4	TTGGCAAAGATGAGACATAAGG AATAAACTCGGTAGTCTTGCAATC
fba1	fba1	ChIP	fba1qL1 fba1qR1	tcaagaccaccaacgacaag aggcgaattgggtatcagtg
gas1	gas1	ChIP	gas1qL3 gas1qR3	AATAGCATGTCGAGGTTGTATGG TGTCATCGCGAAACCTTACC
gdh2	gdh2	ChIP	gdh2 qL1 gdh2 qR1	GCCGTTTGTCAACATTAGCC CGCACGATGTAAATGAGGTG
SPCTRNAME.07	met07/c 417	ChIP	c417qL1 c417qR1	AGGTTCAAATCCTGCTGGTG TGGGACCTACGGGTTATGAG
SPCTRNA.09	pro09	ChIP	pro9 qL2 pro9 qR2	GCCGTTTGGTCTAGTGGTATG TTGGGCTGTTGTGGGAATC

rds1	rds1	ChIP	rds1s-2qL1 rds1s-2qR1	tctggctcctcgctcattttc tcatcgctccaacccttac
SPCTRNASER.13	ser13	ChIP	ser13 qL2 ser13 qR2	CGAGTGGTTTTAAGGCGTTC TCGACAACGGCAGGATTC
SPCTRNATHR.10	thr10.1	ChIP	thr10qL1 thr10qR1	CAATCAACGTTGCCCTATG ATACAAATTGCCCCACTCG
SPCTRNATHR.10	thr10.2	ChIP	thr10qL2 thr10qR2	ATTAGGAGGAGCATCGTACAGC CACATAAAAGTGATATGCAAAAAC G
SPCTRNATHR.10	thr10.4	ChIP	thr10qL4 thr10qR4	AGTCACGGACTTGGTCTTTACC TTCTTTGCACCCCATAGCAC
SPCTRNATHR.10	thr10.5	ChIP	thr10qL5 thr10qR5	AATGAGATTCATCCAGCGTTC GCAGGTACCGTAATTAGCCTTC
SPCTRNATHR.10	thr10.6	ChIP	thr10qL6 thr10qR6	GTGGGATGTACTAAACCACGTC ACGGATGACAGTAAAAGGAATG
SPBTRNATYR.04	tyr04	ChIP/RTqPCR	tyr04FW tyr04RV	TGGTGTAGTTGGTTATCACATCC AATCTCCTGAGCCAGAATCG
SPBTRNATYR.04	lys09	ChIP	lys09qL1 lys09qR1	GCTTTATGAGCGGGCGTAAAC GAGCATTAGGTTTTTGCGTAG
SPBTRNATYR.04	tyr04.2	ChIP	tyr04qL2 tyr04qR2	ttgcccttgcacacctatctc tgaatttagcacgtttctctcaa
SPBTRNATYR.04	tyr04.3	ChIP	tyr04qL3 tyr04qR3	TCCTTTCACGCCCTTTC ACAAATCCTGTTCAAAATTAGCC
SPBTRNATYR.04	tyr04	RTqPCR	tyr04RTT2	TACCACAAGTAGCCAGGGTG
SPATRNASPRO.02	pro02	ChIP/RTqPCR	pro02qL1 pro02qR1	ACATACCTCTTTCGGGTAATCC GGGCCTAACCAAGGATTCG
SPATRNASPRO.02	pro02	RTqPCR	pro02RTT1	GGTAATCAAGCAAGGTGTAAGG
SPATRNASPRO.02	pro02	Northern	pro02qR3	TCTAAACTCAGCATACAAGTGGGG
snu6	snu6	ChIP	snu6qL1 snu6qR1	GATCTTCGGATCACTTTGGTC ATGTCGCAGTGTATCCTTG
srp7	srp7	ChIP	srp7 qL1 srp7 qR1	TACCGATGGAGGTTGGAAAC ACATCCTGCGAAGGTGAATC

# Chapter 1: The RNAPIII-associated factor Sen1 regulates the accumulation of condensin in the vicinity of RNAPIII-transcribed genes.

## 1.1. Introduction

### 1.1.1. Identification of negative regulators of condensin that function in transcription termination

In order to investigate the link between gene transcription and condensin, our group previously carried out a targeted suppressor screen in fission yeast to identify factors associated with transcription that could negatively regulate condensin function (Vanoosthuysse et al., 2014). For this, our group used the thermo-sensitive hypomorphic allele *cut3-477* of the *cut3* subunit of condensin (SMC4 in human) in which chromosome condensation is defective (Robellet et al., 2014; Saka et al., 1994; Tada et al., 2011). Cells carrying this mutation fail to grow at the restrictive temperature of 34°C (Saka et al., 1994). Among others, single deletions of *Swd22*, *Ppn1*, *Ssu72* and *Sen1* were shown to partially rescue the growth defects of *cut3-477* mutant cells at 34°C (Legros et al., 2014; Vanoosthuysse et al., 2014). This observation suggested that these proteins, either directly or indirectly, could act as negative regulators of condensin. It is striking that these proteins have all been implicated in the 3' end processing of RNAPII transcripts. *Swd22* and *Ppn1*, together with the Protein Phosphatase 1 *PP1<sup>Dis2</sup>* form a sub-complex of the Cleavage and Polyadenylation Factor (CPF) complex that is responsible for the 3' end maturation of RNAPII transcripts (Vanoosthuysse et al., 2014). *Ssu72* is another phosphatase associated with the CPF. *Sen1* is a highly conserved ATP-dependent DNA&RNA helicase, whose homologues in *Saccharomyces cerevisiae* (budding yeast *Sen1*) and in vertebrate cells (*Senataxin*) have been implicated in transcription termination of at least a subset of RNAPII-transcribed RNAs (Grzechnik et al., 2015; Mischo et al., 2011; Porrua and Libri, 2013; Skourti-Stathaki et al., 2011; Steinmetz et al., 2001). In budding yeast, *Sen1* participates in transcription termination of non-coding RNAs such as snRNA and snoRNAs as part of the NNS complex (*Nrd1-Nab3-Sen1*) (Porrua and Libri, 2013; Steinmetz et al., 2001). In human cells, *Senataxin* is implicated in transcription termination of genes that carry G-rich terminator sequences (Skourti-Stathaki et al., 2011). Strikingly, condensin is particularly enriched in the vicinity of transcription termination sites of mitotically-expressed RNAPII genes in fission yeast (Sutani et al., 2015; Toselli-Mollereau et al., 2016), suggesting a possible co-localization with the transcription termination factors that are enriched at the 3' end of genes. Taken together, these observations strengthened the possibility of functional links between transcription termination mechanisms and condensin

function and/or localization. I have investigated this possibility throughout my PhD, focusing particularly on the genes transcribed by RNA Polymerase III (RNAPIII) for the reasons explained below.

### 1.1.2. The concomitant deletions of Sen1 (*sen1Δ*) and Swd22 (*swd22Δ*) result in the increased accumulation of condensin around some RNAPIII-transcribed genes

The idea that Sen1 and Swd22 could actually modulate the function of condensin was considerably strengthened by the observation that the concomitant absence of Swd22 and Sen1 resulted in the increased accumulation of condensin on chromatin, specifically around RNAPIII-transcribed genes (Legros et al., 2014). This was a striking observation as it described for the first time a situation where the levels of condensin would increase in a site-specific manner. It was however also a surprising observation because the literature suggested that both Swd22 and Sen1 should function predominantly at RNAPII-transcribed genes and not at RNAPIII-transcribed genes. But our group obtained evidence that in fission yeast Sen1 actually associates predominantly with RNAPIII-transcribed genes and forms a stable complex with RNAPIII. This suggested that Sen1 could act in *-cis* at RNAPIII-transcribed genes to modulate the association of condensin. The team also showed previously that, in the concomitant absence of Swd22 and Sen1, the amount of RNAPIII increased on chromatin specifically on its target genes, suggesting that Swd22 and Sen1 might indeed modulate RNAPIII transcription in *-cis* (Legros et al., 2014). As Sen1 but not Swd22 associates physically with RNAPIII, we postulated that Sen1 could directly regulate the transcription cycle of RNAPIII, thereby contributing to modulate the enrichment of condensin at RNAPIII-transcribed genes. We reasoned that the Sen1 deletion mutant could therefore provide an interesting model to study the impact of transcription on the distribution of condensin.

It is important to note that this hypothesis was very different from the model accepted at the time to explain the accumulation of condensin at RNAPIII-transcribed genes (see below).

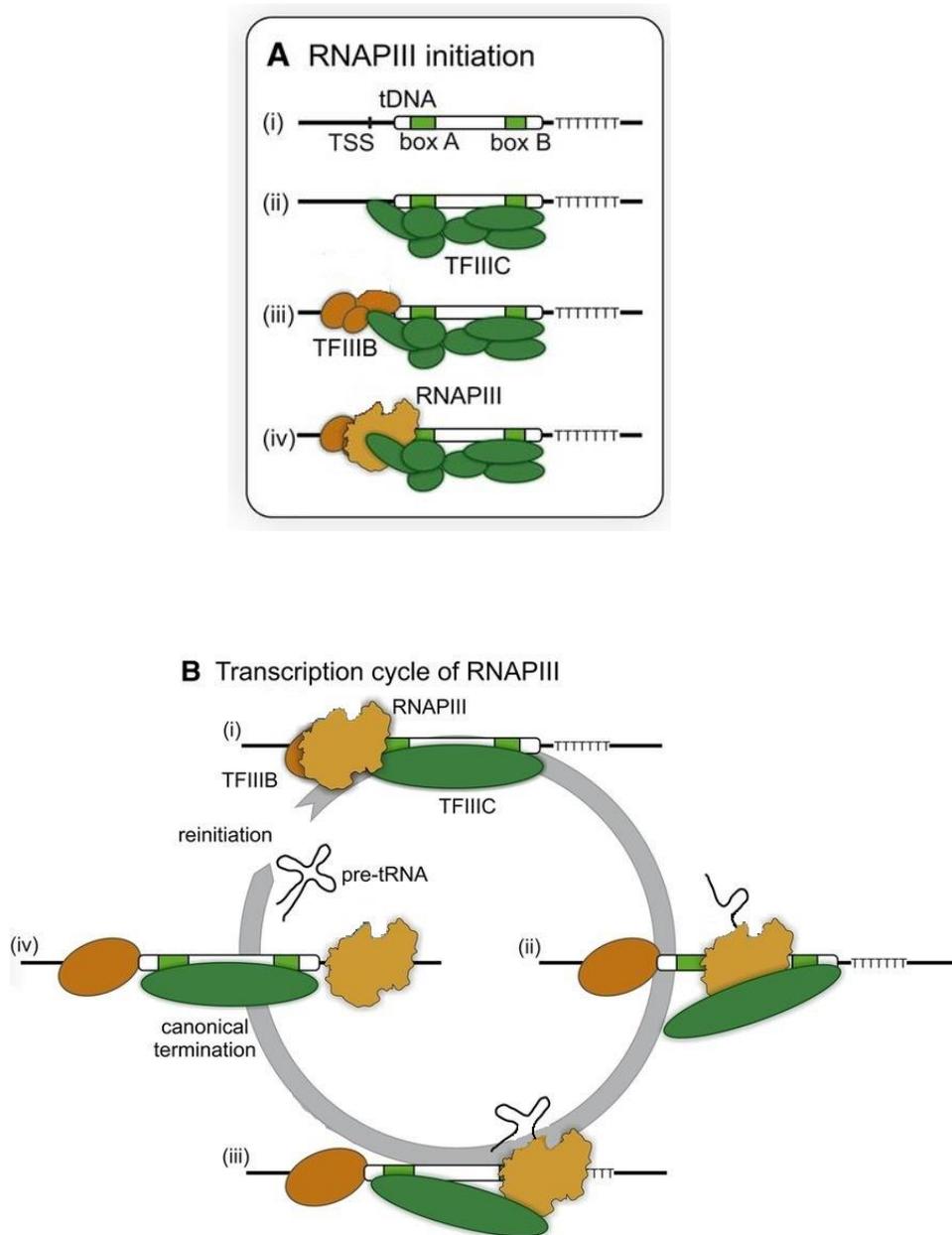
### 1.1.3. The accepted model to explain the accumulation of condensin at RNAPIII-transcribed genes: direct recruitment by the transcription factors TFIIC and Tbp1.

There are currently two models in the literature to explain the accumulation of condensin at highly expressed genes: 1) condensin may directly recognize one or more transcription-dependent chromatin features (Legros et al., 2014; Nakazawa et al., 2019; Sutani et al., 2015; Toselli-Mollereau et al., 2016), or 2) transcription factors (TF) could be responsible for anchoring condensin onto DNA

(D'Ambrosio et al., 2008; Iwasaki et al., 2010, 2015; Kim et al., 2016). In the case of RNAPIII-transcribed genes, published data argue that TF help to anchor condensin in *-cis*.

RNAPIII is specialized for transcription of short, abundant nonprotein-coding RNA transcripts (Dieci et al., 2013). In fission yeast, RNAPIII transcribes tRNA genes, 5S rRNA genes, the U6 small nucleolar RNA (*snu6*) and the 7SL component of signal recognition particle (*srp7*). The transcription cycle by RNAPIII is well described and relies on dedicated transcription factors (Schramm and Hernandez, 2002). The promoter elements of RNAPIII-transcribed genes lie within the transcribed region (Figure 15A). tRNA genes have two conserved internal elements, termed the A box and B box. The transcription factor TFIIA (Sfc2) is specific for transcription of 5S rRNA genes and recognizes a specific internal C box (Camier et al., 1995). TFIIC (consisting of Sfc1, Sfc3, Sfc4, Sfc6 and Sfc9 subunits) recognizes and binds the internal promoter elements and subsequently recruits TFIIB upstream of the transcription start site (Baker et al., 1987; Brun et al., 1997; Deprez et al., 1999). TFIIB contains 3 subunits: TATA-binding protein (Tbp1), TFIIB-related factor 1 (Brf1) and B double prime 1 (Bdp1). In fission yeast, an upstream TATA box assists TFIIC in recruiting TFIIB and is essential for the proper recruitment of RNAPIII (Figure 15A) (Hamada et al., 2001). The transcription termination signal for RNAPIII consists of a tract of T on the non-template strand (A residues on the template DNA strand) (Figure 15B) (Arimbasseri and Maraia, 2015; Mishra and Maraia, 2018; Turowski et al., 2016). In fission yeast the minimal length for efficient termination of RNAPIII is 5T (Hamada et al., 2000).

Models explaining how condensin accumulates in the vicinity of tRNA genes present some contradictions but they all envisage direct recruitment of condensin by TF anchoring. *D'Ambrosio et al.* first showed in budding yeast that condensin co-localizes with TFIIC at RNAPIII-transcribed genes and that an isolated B-box sequence element is sufficient to trigger the accumulation of condensin in *-cis*. Consistent with this, the association of condensin was reduced in a mutant of TFIIC that shows reduced TFIIC binding on chromatin (D'Ambrosio et al., 2008). It was also proposed that budding yeast condensin associates with and clusters tRNA genes through an interaction with TFIIC (Haeusler et al., 2008). In fission yeast, the Noma group engineered the same TFIIC mutation as the one described by *D'Ambrosio et al.* but they argued that the mutation had an opposite effect on both TFIIC and condensin: they presented evidence that the TFIIC mutant *sfc3-1* stabilizes both the binding of the TFIIC subunit Sfc6 and the binding of condensin but they only looked at one RNAPIII-transcribed region (Iwasaki et al., 2010). Nevertheless, the authors reached the same conclusion as *D'Ambrosio et al.* and suggested that TFIIC binds to condensin and facilitates its association with RNAPIII-transcribed genes (Iwasaki et al., 2010). In human cells, TFIIC recruits specifically condensin II to tRNA genes (Yuen et al., 2017). Together, these data in yeast and human cells suggested that



**Figure 15: The RNAPIII transcription cycle. (A)** (i) and (ii) Transcription initiation on tRNA genes. The internal A box and B box elements present within tRNA genes (tDNA) are bound by the multiprotein TFIIC complex. (iii) This recruits the TFIIB complex. In fission yeast, a TATA box is located ~30 bp upstream of the transcription unit (iv) RNAPIII is recruited and transcription initiates. **(B)** RNAPIII transcription cycle on tRNA genes. (i) Transcription elongation (ii and iii) TFIIC remains associated with the tDNA, possibly because it is not simultaneously displaced from both the A and B boxes. (iv) In canonical termination, RNAPIII terminates following transcription of an oligo(U) tract. Adapted from Turowski & Tollervey, 2016.

TFIIIC might play a role in recruiting condensin to RNAPIII-transcribed genes by direct protein-protein interactions. It is important to note however that our lab could not reproduce the data published by the Noma lab (Iwasaki et al., 2010): in our hands, the recruitment of TFIIIC was in fact considerably reduced in the TFIIIC mutant *sfc3-1* (Vanoosthuyse et al., 2014) and we could not detect an impact of the *sfc3-1* mutation on the accumulation of condensin (see below).

Another study from the Noma team brought some more complexity into the picture: they showed that Tbp1 interacts with condensin through the C-terminus of the kleisin subunit and recruits condensin to RNAPIII- and also to highly expressed RNAPII-transcribed genes. Disrupting the Tbp1-Cnd2 interaction reduced the association of condensin with chromosomes (Iwasaki et al., 2015). Note however that in human cells the mitotic depletion of TBP did not alter condensin I binding (Sutani et al., 2015). Taken together, these observations suggest that the TBP-dependent recruitment of condensin is unlikely to be a conserved mechanism.

#### 1.1.4. An alternative model: condensin recognizes one or more transcription-associated chromatin feature(s)

The fact that condensin accumulates in the vicinity of highly-expressed genes in every organism where it has been looked at suggested to us that condensin might be attracted primarily by a physical feature associated with transcription rather than by protein-protein interactions with dedicated transcription factors. In addition, the model proposed by Noma that such condensin-anchoring transcription factors are locus-specific (Iwasaki et al., 2010, 2015) seemed over complicated. Nevertheless, it is conceivable that protein-protein interactions with locally-enriched proteins might stabilize condensin locally.

What transcription-associated feature(s) might help to recruit/stabilize condensin? Because the hinge domain of condensin displays significant affinity for single-stranded DNA (ssDNA) *in vitro* (Akai et al., 2011; Piazza et al., 2014), my supervisor first made the hypothesis that ssDNA produced by transcription could be a receptor for condensin. There are at least two ways by which transcription might produce ssDNA: through DNA melting associated with negative topological stress or through the formation of R-loops. R-loops are conserved chromatin structures that result from the hybridization of the nascent RNA to its DNA template (reviewed in Aguilera and García-Muse, 2012). Interestingly, fission yeast Sen1 was shown to disassemble DNA-RNA hybrids at least *in vitro* (Kim et al., 1999) and R-loops are considered as key substrates of budding yeast Sen1 and human Senataxin *in vivo* (Mischo et al., 2011; Skourti-Stathaki et al., 2011). Based on these observations, the team first speculated that R-loops might be stabilized at RNAPIII-transcribed genes in the absence of Sen1, thereby attracting/stabilizing condensin. Although the team demonstrated that R-loops do indeed

form at RNAPIII-transcribed genes (Hartono et al., 2018; Legros et al., 2014), the idea that R-loops could recruit condensin was quickly dismissed when it was shown that the strong expression of RNase H1 *in vivo* was sufficient to disassemble R-loops at RNAPIII-transcribed genes but had no effect on the accumulation of condensin, whether or not Sen1 and Swd22 were present (Legros et al., 2014). This showed that R-Loops play no part in recruiting/positioning condensin (Legros et al., 2014).

To explain the accumulation of condensin in the vicinity of RNAPIII-transcribed genes in the concomitant absence of Swd22 and Sen1, the team proposed instead that transcription-induced topological stress might contribute to the accumulation of condensin in *-cis*. To support this hypothesis, it was shown that both Topoisomerase 1 and 2 (Top1 and Top2) accumulate at RNAPIII-transcribed genes in the absence of Swd22 and Sen1, suggesting that lack of Swd22 and Sen1 results in greater topological stress at RNAPIII-transcribed genes. Moreover, deletion of Top1 further enhanced both the suppressor effect of *swd22Δsen1Δ* on the condensin-defective *cut3-477* and the accumulation of condensin around RNAPIII-transcribed genes in *swd22Δsen1Δ* cells (Legros et al., 2014). Taken together, these observations suggested that in the concomitant absence of Swd22 and Sen1, a Top1-sensitive chromatin structure would form in the vicinity of RNAPIII-transcribed genes and contribute to the enrichment of condensin in *-cis*.

Although potentially attractive, this hypothesis would need to be substantiated further however. In particular, it would be very important to demonstrate that Top1 is actually active in these conditions (i.e that it genuinely cleaves the DNA) and that short-term and mitotic-specific inhibition of Top1 (i.e at the time when condensin is loaded on chromosomes) could indeed stimulate the accumulation of condensin in the absence of Swd22 and Sen1. In addition, the data described in Legros et al (2014) were obtained on asynchronous cells, primarily because *swd22Δ* mutants proved difficult to synchronize in mitosis. My first aim in my PhD was to improve these data by working on mitotically-arrested cells.

The founding hypothesis of my PhD project was that Sen1, by associating with RNAPIII, impacts RNAPIII transcription directly, and that the transcription defects associated with lack of Sen1 create a chromatin environment that facilitate the local accumulation of condensin. This local enrichment could either reflect enhanced loading in *-cis*, or the accumulation of condensin molecules loaded elsewhere on the chromosome. With this hypothesis in mind, we decided that it was important: 1) to identify how RNAPIII transcription was affected in the absence of Sen1 and, 2) to evaluate whether these transcription defects could somehow be corrected. If possible, this would indeed help to establish whether or not the transcription changes observed in the absence of Sen1 were directly responsible for the changes in the localization pattern of condensin. The great advantage of working

with RNAPIII-transcribed genes is that RNAPIII transcription can easily be manipulated by introducing dedicated mutations in well-identified regulatory sequences.

In this first chapter, we asked two main questions:

1. Could the accumulation of condensin and Top1 around RNAPIII-transcribed genes that was previously observed in asynchronous *swd22Δ sen1Δ* cells be reproduced in single *sen1Δ* mutant cells synchronized in mitosis?
2. Is this accumulation due to an accumulation of either TFIIC or Tbp1 or is it more likely to be due to a change in RNAPIII transcription associated with lack of Sen1?

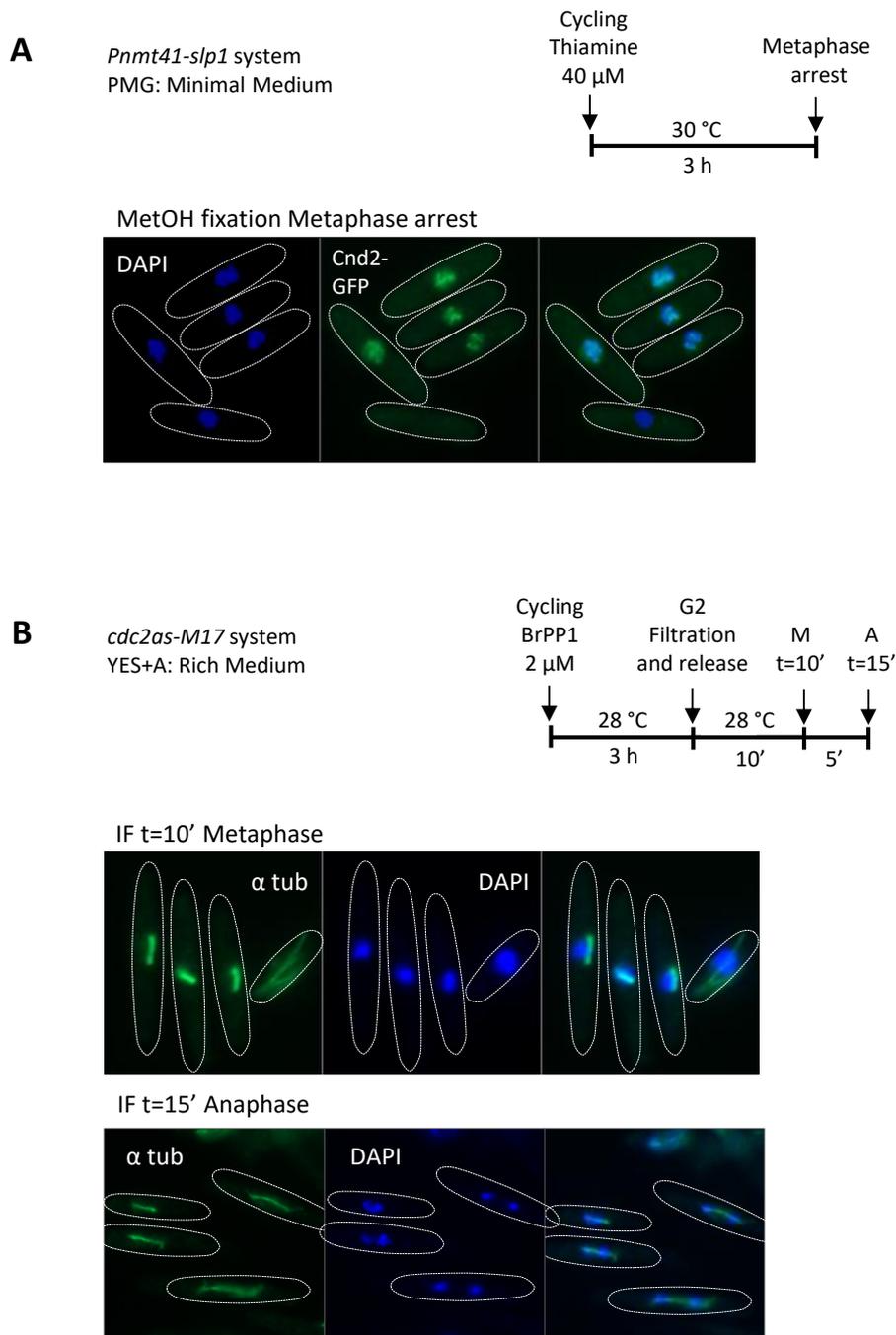
## 1.2. Results

### 1.2.1. Synchronization methods

To synchronize cells in mitosis, we used two different methods:

1. To synchronize cells in metaphase, we depleted Slp1, the activator of the anaphase-promoting complex (APC/C) in mitosis in fission yeast. Upon depletion of Slp1, cells are unable to enter anaphase and therefore remain blocked at the metaphase to anaphase transition. The expression of *slp1* is placed under the control of a promoter (Pnmt41) that can be repressed by the addition of thiamine (Maundrell, 1990). Cells were grown in PMG (Pombe Minimal Glutamate), to maintain the Pnmt41 promoter active. Addition of thiamine 40  $\mu$ M during one cell-cycle (3h) results in a cell population almost exclusively composed of metaphase cells. As condensin localizes in the nucleus in metaphase, mitotic indexes were measured as the percentage of mitotic cells showing nuclear localization of the GFP-tagged version of the Cnd2 subunit of condensin (Cnd2-GFP). Alternatively, IF (Immunofluorescence) using anti-Tubulin antibodies was used to label mitotic spindles and thereby quantify the percentage of cells in mitosis (Figure 16A).

2. Alternatively, we used an analogue-sensitive (as) mutant of the cyclin-dependent kinase *cdc2* (Cdk1 in humans) to block cells at the G2/M transition. The allele is called *cdc2-asM17* (Aoi et al., 2014). Substitution of a single amino acid in the ATP-binding pocket of this kinase renders the mutant kinase sensitive to ATP-analogue molecules (3-BrB-PP1). This confers specificity to the inhibitor, as genetically unmodified kinases are unaffected by ATP-analogues. Cells are cultured in rich medium, YES+A (Yeast Extract with Supplements + Adenine). Addition of 3-BrB-PP1 2  $\mu$ M during one cell-cycle (3h) results in blocking of cell population in G2. Filtration and recovery of cells in YES+A induce the



**Figure 16: Mitotic synchronization methods.** (A) *Pnmt41-slp1* system: the activator of the anaphase promoter complex, Slp1 is under the expression of a thiamine repressible promoter. Cells are grown in PMG. Addition of thiamine for 3h blocks cells in metaphase. Methanol (MeOH) fixation of cells is used to observe the localization of Cnd2-GFP. Cnd2 localizes in the nucleus in metaphase. (B) *cdc2as-M17* system: analogue sensitive mutant of Cdc2. Cells are grown in YES+A. Addition of BrPP1 for 3h blocks cells in G2. After filtration and recovery of cells in YES+A, cells are released into mitosis. At 28 $^{\circ}$ C, cells reach metaphase 10' post-release and anaphase 15' post-release. IF (Immunofluorescence) using anti-Tubulin antibody show the characteristic short metaphase and elongated anaphase mitotic spindles.

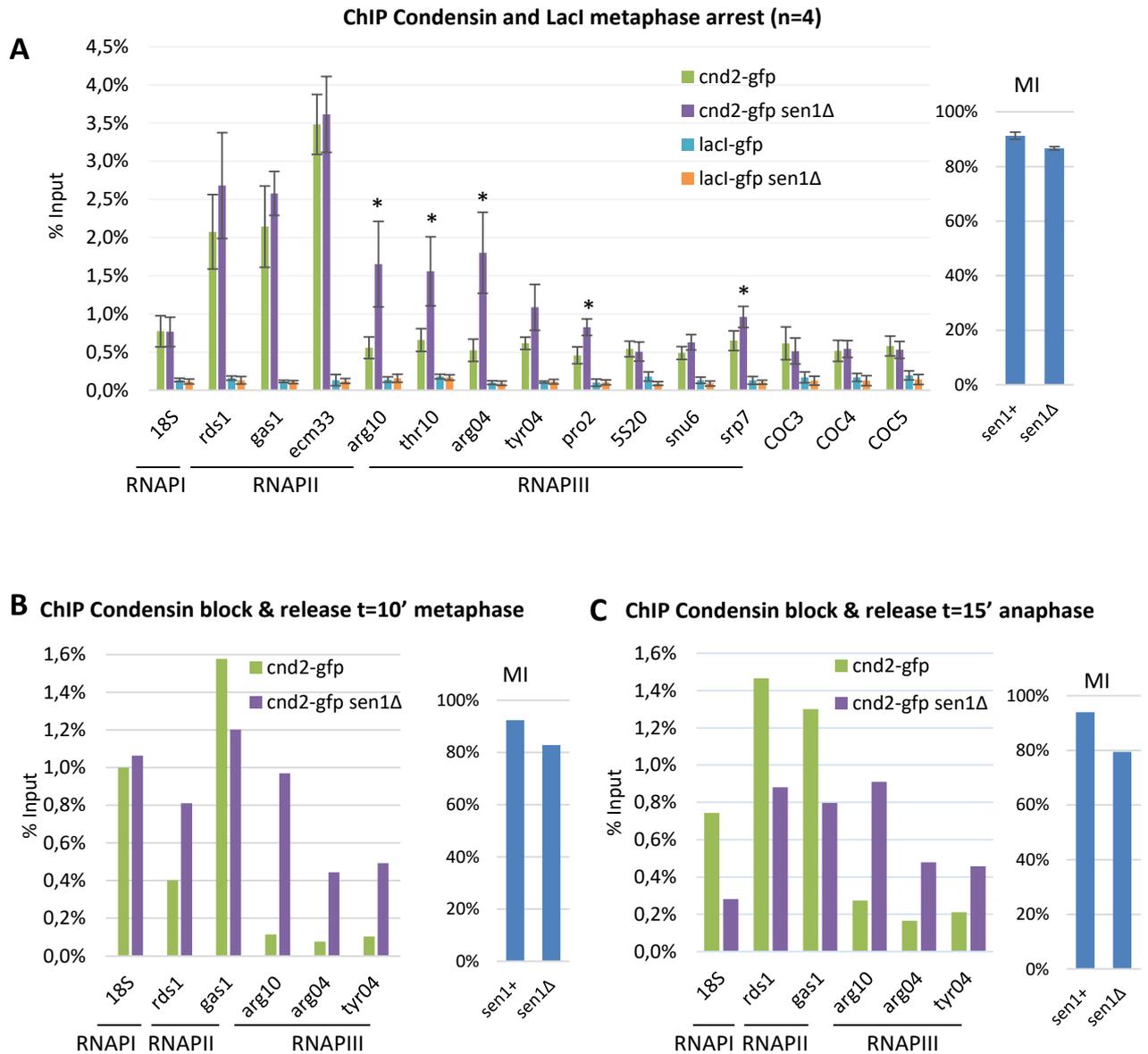
release of cells into the cell cycle. We set up the *cdc2-asM17* synchronization at 28°C. At this temperature, we showed that cell population reached metaphase within 10 minutes after release and anaphase at 15 minutes after release (Figure 16B).

### 1.2.2. Sen1 antagonizes the accumulation of condensin specifically at RNAPIII-transcribed genes in mitosis.

We tested whether the sole absence of Sen1 could impact the accumulation of condensin and Top1 at RNAPIII-transcribed genes in mitosis. First, *sen1+* and *sen1Δ* cells were synchronized in metaphase by addition of thiamine and processed to carry out Chromatin Immunoprecipitation (ChIP) analysis of the GFP-tagged Cnd2 subunit of condensin (Cnd2-GFP) to assess the distribution of condensin. Note that the percentage of metaphase-arrested cells was always slightly lower in the absence of Sen1 (86,6% ± 0,63% for *sen1Δ* and 91,3% ± 1,32% for *sen1+*). We do not have an explanation for this observation, but we think that the difference is too small to have a significant impact on the results of the ChIP experiments. In the absence of Sen1, the accumulation of condensin was significantly increased at a subset of RNAPIII-transcribed genes (tRNA genes: *arg10*, *thr10*, *arg04*, *tyr04*, *pro02*; and the RNAPIII-transcribed gene *srp7*) but remained unchanged at other RNAPIII-transcribed genes (5S rRNA gene: *5S20*; and the RNAPIII-transcribed gene U6 snRNA *snu6*) and at RNAPI-transcribed or RNAPII-transcribed genes (Figure 17A). Interestingly, the levels of condensin were not affected at COC sites (Chromosome-Organizing Clamps) which recruit TFIIC but not RNAPIII (Noma et al., 2006). The observation that condensin accumulates at a subset of tRNA genes but not at COC sites in the absence of Sen1 is consistent with the fact that Sen1 interacts with RNAPIII but not with TFIIC (Legros et al., 2014).

It has been reported that highly expressed genes are “hyper-ChIPable” sites. Nonspecific and meaningless enrichment of proteins are detected at these regions by ChIP, likely caused by high levels of RNAPII and RNAPIII transcription (Teytelman et al., 2013). To rule out that the accumulation of condensin at specific RNAPIII-transcribed genes in the absence of Sen1 was simply due to an increased “hyper-ChIPability” of these loci, we used a GFP-tagged and nuclear version of the unrelated LacI protein from *Escherichia coli* as control. The levels of LacI-GFP did not increase at RNAPIII-transcribed genes in the absence of Sen1, establishing that the enhanced binding of condensin in the absence of Sen1 is specific and not due to hyper-ChIPability (Figure 17B).

Importantly, the significant accumulation of condensin at RNAPIII-transcribed genes in the absence of Sen1 was also observed when cells were synchronized using the *cdc2-asM17* allele. With this synchronization protocol, cells do not arrest at a specific mitotic stage but instead go through metaphase and anaphase in a synchronous manner with a normal timing. As a result, this method is



**Figure 17: Sen1 regulates condensin binding specifically at RNAPIII genes in mitosis.** (A) ChIP-qPCR analysis of GFP-tagged Cnd2 and LacI at the indicated loci in a population of metaphase arrested cells by depletion of Slp1 (mean  $\pm$  standard deviation from 4 biological replicates). (B) ChIP-qPCR analysis of GFP-tagged Cnd2 at the indicated loci in a population of metaphase cells obtained after BrPP1 block and release in mitosis. One biological replicate. (C) Same ChIP experiment as in (B) in which cells were collected in anaphase. MI: Mitotic index. RNAPI: RNA polymerase I-transcribed genes. RNAPII: RNA polymerase II-transcribed genes. RNAPIII: RNA polymerase II-transcribed genes. COC: Chromosome-Organizing Clamps. \*p-value<0,05

more likely to represent a more physiological mitotic state. Interestingly, both in metaphase (Figure 17C) and in anaphase (Figure 17D), condensin accumulated at RNAPIII-transcribed genes in the absence of Sen1.

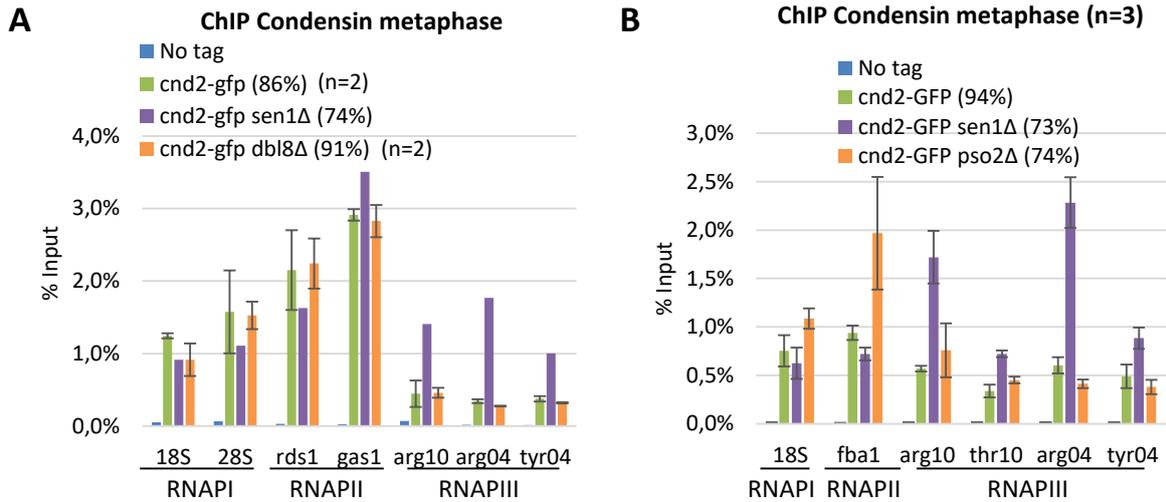
Together these results establish that, whatever the synchronization method or the medium used (poor or rich), condensin accumulates at least at a subset of RNAPIII-transcribed genes in mitosis in the sole absence of Sen1.

### 1.2.3. Lack of other proteins related to Sen1 function does not impact the distribution of condensin

We have shown above that the impact of lack of Sen1 on the distribution pattern of condensin was specific, as the association of the unrelated GFP-tagged LacI protein was not altered in the absence of Sen1 (Figure 17A). Here we tested whether the deletion of other proteins related to Sen1 function had a similar impact on the distribution of condensin.

Fission yeast expresses two non-essential homologues of Senataxin, Sen1 and Dbl8. While Sen1 interacts with RNAPIII and associates with RNAPIII-transcribed genes, Dbl8 interacts instead with RNAPI and is particularly enriched at the rDNA but not at RNAPIII-transcribed genes (Rivosecchi et al., 2019). To establish whether Dbl8, like Sen1, could impinge on the distribution pattern of condensin, we used ChIP to assess the association of condensin with chromatin in metaphase arrested cells lacking Dbl8 (*dbl8Δ*). Condensin levels were not affected in *dbl8Δ* (Figure 18A), suggesting that Sen1 function in regulating condensin is not shared with its close homologue Dbl8. This is consistent with the idea that Sen1 acts locally at RNAPIII-transcribed genes to modulate the association of condensin, either directly or indirectly, possibly through an interaction with RNAPIII.

The team published previously that Sen1, in addition to interacting with RNAPIII, interacts physically with the DNA 5' exonuclease Pso2 (Legros et al., 2014). Pso2 has been implicated in the repair of interstrand cross-links (Lambert et al., 2003). To test whether the Sen1-Pso2 interaction could underlie the Sen1-mediated regulation of condensin accumulation at RNAPIII-transcribed genes, we analyzed the distribution pattern of condensin in metaphase arrested cells lacking Pso2 (*pso2Δ*). Note that this experiment was performed by my colleague Amélie Malapert. No changes in condensin levels were detected in the absence of Pso2 at RNAPIII-transcribed genes (Figure 18B), suggesting that the role of Sen1 in regulating condensin at RNAPIII-transcribed genes is unlikely to rely on its interaction with Pso2.



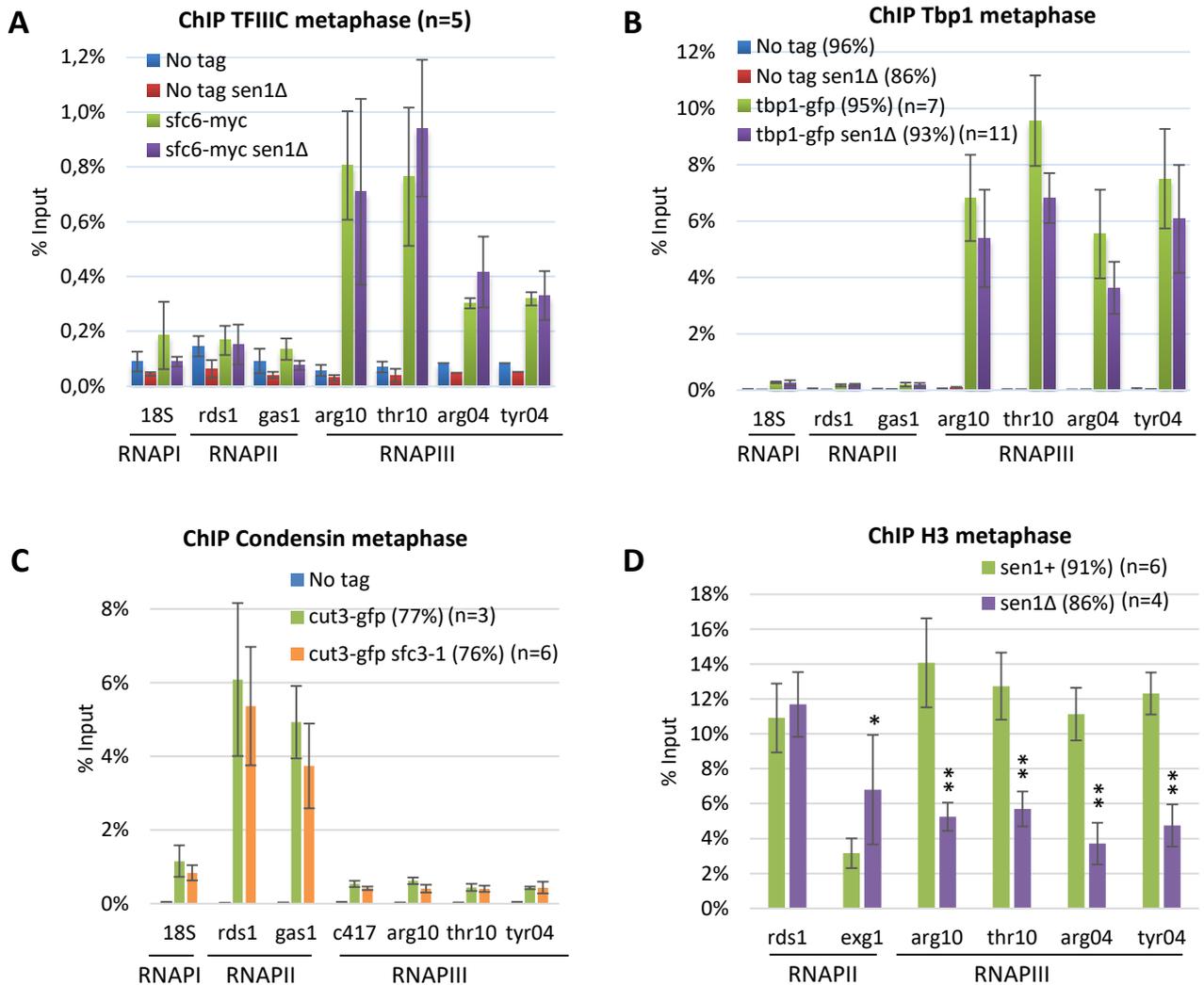
**Figure 18: The function of Sen1 on condensin binding is specific. (A)** ChIP-qPCR analysis of Cnd2 in the presence or absence of Sen1 or Dbl8 at the indicated loci in a population of metaphase arrested cells by depletion of Slp1 (mean  $\pm$  standard deviation from 1 or 2 biological replicates). **(B)** ChIP-qPCR analysis of Cnd2 in the presence or absence of Sen1 or Pso2 at the indicated loci in a population of metaphase arrested cells by depletion of Slp1 (mean  $\pm$  standard deviation from 3 biological replicates). This last experiment was performed by my colleague Amélie Malapert. Mitotic indexes are expressed in percentage between brackets. RNAPI: RNA polymerase I-transcribed genes. RNAPII: RNA polymerase II-transcribed genes. RNAPIII: RNA polymerase II-transcribed genes.

#### 1.2.4. Condensin accumulation at RNAPIII-transcribed genes cannot be explained by increased TFIIC or Tbp1-dependent recruitment

Published data indicate that condensin accumulates at tRNA genes in fission yeast through a physical interaction with two different transcription factors: TFIIC (Iwasaki et al., 2010) and Tbp1 (Iwasaki et al., 2015). We asked whether the accumulation of condensin at RNAPIII-transcribed genes in the absence of Sen1 could be explained by these pathways. Cells were arrested in metaphase and mitotic indexes were assessed using immuno-fluorescence experiments (IF) directed against  $\alpha$ Tubulin. ChIP enrichments showed that there is no increase in the levels of TFIIC (Sfc6 subunit) (Figure 19A) or Tbp1 (Figure 19B) in the absence of Sen1, suggesting that enhanced condensin association with RNAPIII-transcribed genes in the absence of Sen1 cannot be explained by enhanced accumulation of TFIIC or Tbp1.

*sfc3-1* is a thermosensitive mutant of the TFIIC subunit Sfc3 that was proposed to share several phenotypes with the deletion of Sen1 (*sen1 $\Delta$* ). *sfc3-1*, like *sen1 $\Delta$* , was shown previously to rescue the thermosensitive growth defects of the condensin-defective *cut3-477* mutant (Iwasaki et al., 2010; Tada et al., 2011). In addition, it was suggested that in this mutant, both TFIIC and condensin accumulate at a region of chromosome III (c417: 1689713-1691075) containing 2 tRNA genes (*SPCTRNASER.11* and *SPCTRNAMET.07*), and a 5SrRNA (*SPRRNA.05*) (Iwasaki et al., 2010). This again was reminiscent of our observations made on the *sen1 $\Delta$*  mutant. To try to confirm these observations, we assessed the accumulation of condensin in the *sfc3-1* mutant, using the same experimental conditions described in the original publication, where cells were cultured at 36°C for 2h to inactivate *sfc3-1*. We failed however to reproduce the published data, as we did not detect an increase in the accumulation of condensin at the c417 locus or other RNAPIII-transcribed genes (Figure 19C). As mentioned above, neither TFIIC nor condensin accumulates at RNAPIII-transcribed genes in the *sfc3-1* mutant in our hands (this data and Vanoosthuysen et al., 2014)). This suggests that, even if *sfc3-1* and *sen1 $\Delta$*  mutants are both deletions of factors related to the RNAPIII transcription machinery in fission yeast and both are suppressors of condensin mutant, they facilitate the function of condensin by different mechanisms.

If condensin accumulation at RNAPIII-transcribed genes in the absence of Sen1 is not due to enhanced recruitment by TFIIC or Tbp1, it is likely that chromatin around RNAPIII-transcribed genes is modified creating an accumulation of condensin. The lab has published that nucleosome free regions facilitate the binding of condensin at highly expressed RNAPII genes (Toselli-Mollereau et al., 2016). We tested the occupancy of nucleosomes by ChIP of histone H3 in the absence of Sen1, to see whether in our case condensin accumulation also correlates with reduced nucleosome occupancy.



**Figure 19: The function of Sen1 on condensin binding does not rely on Tbp1 and TFIIC dependent pathways.** ChIP-qPCR analysis in cells arrested in metaphase by depletion of Slp1, except for cohesin (e) which was performed in cycling cells. **(A)** ChIP-qPCR analysis of Sfc6 (subunit of TFIIC) in the presence or absence of Sen1 at the indicated (mean  $\pm$  standard deviation from 5 biological replicates). Mitotic indexes were not assessed. **(B)** ChIP-qPCR analysis of Tbp1 (subunit of TFIIB) in the presence or absence of Sen1 at the indicated loci (mean  $\pm$  standard deviation from 7 [*sen1+*] and 11 [*sen1Δ*] biological replicates). **(C)** ChIP-qPCR analysis of Cut3 (Condensin subunit) in the presence or absence of the *sfc3-1* mutation at the indicated loci. The 3h incubation with thiamine to arrest cells in metaphase was done at 36°C to inactivate the Sfc3 mutation. (mean  $\pm$  standard deviation from 3 [*sfc3+*] and 6 [*sfc3-1*] biological replicates). **(D)** ChIP-qPCR analysis of histone H3, using the antibody ab1791, in the presence or absence of Sen1 at the indicated loci (mean  $\pm$  standard deviation from 6 [*sen1+*] and 4 [*sen1Δ*] biological replicates). Mitotic indexes are expressed in percentage between brackets. RNAPI: RNA polymerase I-transcribed genes. RNAPII: RNA polymerase II-transcribed genes. RNAPIII: RNA polymerase II-transcribed genes. \*p-value<0,05, \*\*p-value<0,01

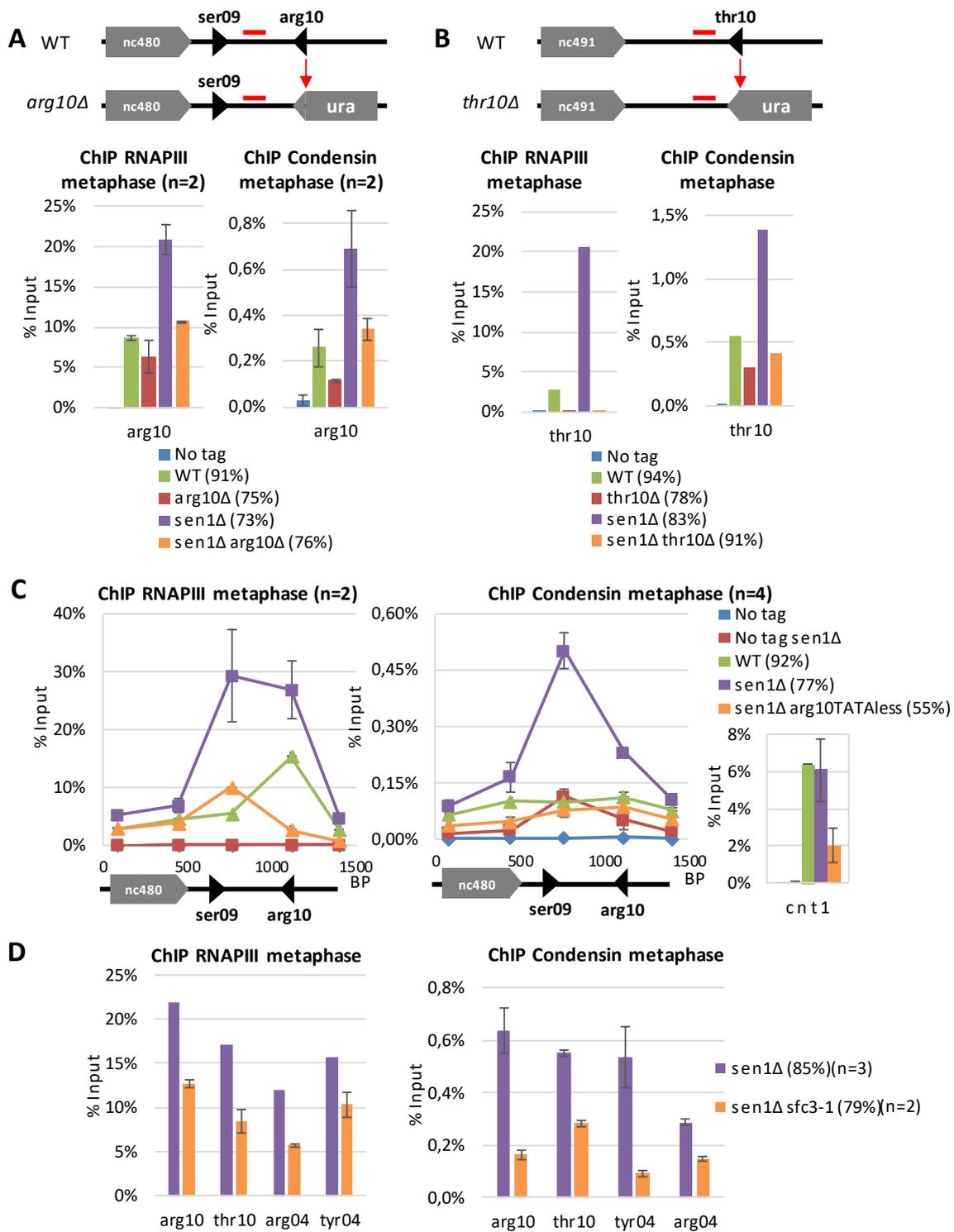
Interestingly, we observed that histone H3 association with RNAPIII-transcribed genes is reduced in mitosis in the absence of Sen1 (Figure 19D).

#### 1.2.5. Sen1 acts in *-cis* at RNAPIII-transcribed genes to regulate the positioning of condensin

Because Sen1 associates physically with RNAPIII and is particularly enriched on chromatin at RNAPIII-transcribed genes, we postulated that Sen1 acts locally at RNAPIII-transcribed genes to regulate the distribution of condensin. To test this, we engineered strains in which tRNA genes were replaced by a gene transcribed by RNAPII. We chose two tRNA genes where the levels of condensin are significantly increased in the absence of Sen1: *arg10* (*SPCTRNAARG.10*) (Figure 20A) and *thr10* (*SPCTRNATHR.10*) (Figure 20B). We replaced those genes by the RNAPII-transcribed *ura4* gene, making sure that we were preserving intact the loci at which we detected high condensin enrichment. We then assessed the association of RNAPIII (Rpc37 subunit) and condensin (Cnd2) in these strains after synchronizing cells in metaphase cells using the *cdc2-asM17* system. Deletion of tRNA genes in both *sen1+* and *sen1Δ* cells resulted in dramatically reduced RNAPIII occupancy (Figure 20AB). In the case of *arg10Δ*, we speculate that the remaining RNAPIII molecules that we detected had initiated transcription at the neighbouring *ser09* gene. Interestingly, the levels of condensin were also sharply reduced (Figure 20AB) and decreased to the basal level of condensin accumulation detected all along chromosome arms as described by ChIP-seq experiments. These experiments confirmed that lack of Sen1 only impacts the accumulation of condensin if a RNAPIII transcription unit is present and suggested that Sen1 might act in *-cis* at tRNA genes to modulate the accumulation of condensin.

These results do not formally demonstrate that the impact of *sen1Δ* on the accumulation of condensin requires RNAPIII transcription. To investigate the role of RNAPIII transcription on the accumulation of condensin when Sen1 is missing, one would need to impair specifically RNAPIII transcription without replacing the transcription unit. The simplest way to inhibit RNAPIII transcription would be pharmacological. *Iwasaki et al.* treated fission yeast cells with the RNAPIII transcription inhibitor ML-60218 (Iwasaki et al., 2010). However, we and others failed to successfully inhibit RNAPIII using this drug. Instead, we decided to try and prevent the loading of RNAPIII by mutating the TATA box of the tRNA gene *SPCTRNAARG.10* (thereafter referred simply as *arg10*). To mutate the TATA box would prevent the binding of Tbp1 locally and thereby interfere with the recruitment of RNAPIII. As we had shown previously that deleting the entire tRNA gene was viable, we did not anticipate any particular problems with the deletion of the TATA box.

We constructed a mutant tRNA gene *arg10* that lacks the TATA box and synchronized TATA+ and TATA- cells in mitosis using the *cdc2-asM17* system. ChIP enrichments of the Rpc37 RNAPIII subunit showed a significant reduction of RNAPIII levels at *arg10* in TATA- cells (Figure 20C). Similarly, the



**Figure 20: Sen1 acts in *-cis* at RNAPIII genes to modulate condensin binding.** (A) Diagram showing the replacement of the tRNA gene *arg10* by the *ura* gene (*arg10Δ*). The red bar shows the qPCR primer site. ChIP-qPCR analysis of Rpc37 (RNAPIII subunit) and Cnd2 in cells tagged for both proteins, arrested in metaphase by depletion of Slp1, in the indicated strains, at the indicated loci (mean  $\pm$  standard deviation from 2 biological replicates). (B) Diagram showing the replacement of the tRNA gene *thr10* by the *ura* gene (*thr10Δ*). The red bar shows the qPCR primer site. ChIP-qPCR analysis of Rpc37 and Cnd2 in cells tagged for both proteins, arrested in metaphase by depletion of Slp1, in the indicated strains, at the indicated loci (1 biological replicate). (C) ChIP-qPCR analysis of Rpc37 and Cnd2 in cells tagged for both proteins, synchronized in metaphase by the *Cdc2* mutation, in the indicated strains. The *ser09-arg10* loci was scanned. Condensin enrichment is also shown at the centromere (*cnt1*) (mean  $\pm$  standard deviation from 4 biological replicates for condensin and 2 for RNAPIII). (D) Two different ChIP-qPCR experiments of Rpc25 (subunit of RNAPIII) and Cnd2 in cells synchronized in metaphase by depletion of Slp1, in the indicated strains, at the indicated loci. Cells in both experiments were shifted 3h at 36°C and then 3h more at 36°C once thiamine was added. (ChIP RNAPIII: mean  $\pm$  standard deviation from 1 [*sen1Δ*] and 2 [*sen1Δ sfc3-1*] biological replicates. ChIP Condensin: mean  $\pm$  standard deviation from 3 [*sen1Δ*] and 2 [*sen1Δ sfc3-1*] biological replicates). Mitotic indexes are shown in brackets.

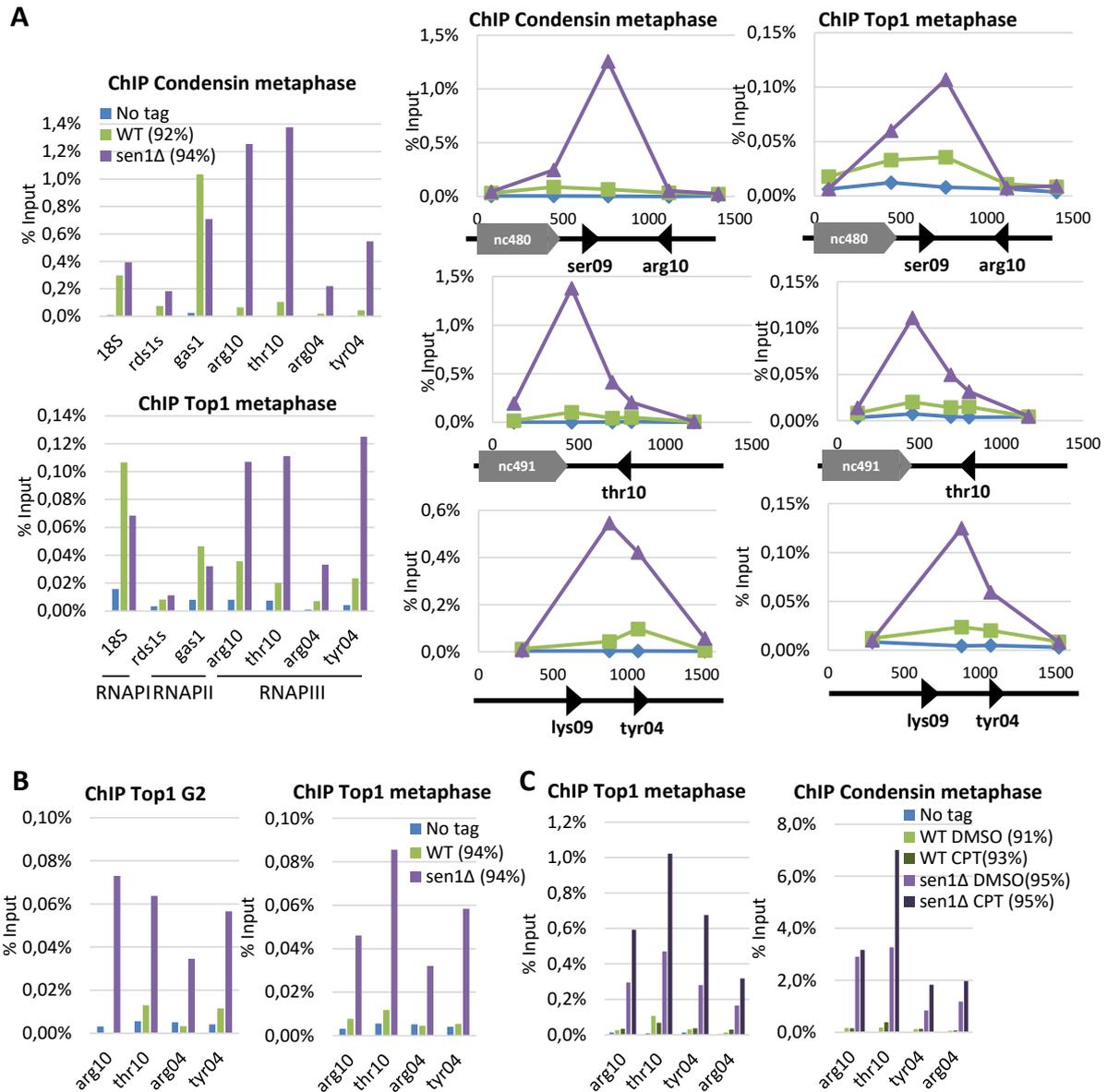
accumulation of condensin in the absence of Sen1 was also suppressed in the TATA-less mutant (Figure 20C). Unfortunately, we could not synchronize this strain in mitosis efficiently. 10 minutes after release from the G2 block, we only obtained 55% of metaphase cells in TATA- cells compared to 92% in the TATA+ cells. Consistent with this, the association of condensin with centromeres was also strongly reduced in the TATA- mutant. We do not yet have an explanation for this observation. But as this experiment currently stands, we cannot rigorously conclude that active RNAPIII transcription is required to promote the accumulation of condensin in the absence of Sen1.

To circumvent this issue, we sought another way of reducing specifically RNAPIII transcription. We turned to the *sfc3-1* mutant. Previous observations showed that the accumulation of RNAPIII is reduced in the *sfc3-1* mutant, probably reflecting diminished loading of RNAPIII at tRNA genes (Iwasaki et al., 2010; Vanoosthuyse et al., 2014). We therefore generated a double mutant *sen1Δ sfc3-1* in order to reduce the loading of RNAPIII in mitotic *sen1Δ* cells. We synchronized the cells in metaphase using the depletion of Slp1: we first inactivated *sfc3-1* during 3h at 36°C and then we added thiamine for 3 more hours at 36°C to synchronize cells in metaphase. This treatment reduced both RNAPIII and condensin loading (Figure 20D), suggesting that there is a correlation between the accumulation of RNAPIII and the accumulation of condensin in the absence of Sen1.

#### 1.2.6. Topological stress accumulates around RNAPIII-transcribed genes in the absence of Sen1 in G2 and in mitosis

Previous data suggested the accumulation of topological stress around tRNA genes in the concomitant absence of both Sen1 and Swd22 (Legros et al., 2014). To confirm these observations, we monitored the enrichment of both condensin and Top1 in mitotic cells deleted only of Sen1. We synchronized cells using the *cdc2-asM17* system and we ChIPed condensin and Top1 from the same extracts. In metaphase, Top1 significantly accumulates in the absence of Sen1 at RNAPIII-transcribed genes but not at RNAPI or RNAPII genes (Figure 21A). When we looked in details at three different tRNA loci (*ser29-arg10*; *thr10*; *lys09-tyr04*) we observed that condensin and Top1 accumulate at the same loci (Figure 21A). This strong correlation between Top1 and condensin accumulation is consistent with the hypothesis that in the absence of Sen1, a Top1-sensitive structure facilitates condensin accumulation. It is interesting to note that the scanning of three loci showed that condensin does not significantly accumulate in WT cells at the three RNAPIII-transcribed genes tested (Figure 21A). It is important to remark also that in the absence of Sen1, condensin accumulates 3' of RNAPIII-transcribed genes (Figure 21A).

The accumulation of Top1 could be due to the accumulation of condensin. This hypothesis could be discarded if we could show that in the absence of Sen1, Top1 also accumulates around RNAPIII-



**Figure 21: Topological stress accumulates in the absence of Sen1 in G2 and in mitosis at RNAPIII genes. (A)** ChIP-qPCR analysis of Top1 and Cnd2 in cells tagged for both proteins, in metaphase cells synchronized by the *cdc2as-M17* system, in the presence or absence of Sen1, at the indicated loci. Scanning of three tRNA loci. (1 biological replicate). **(B)** ChIP-qPCR analysis of Top1 in cells blocked in G2 by the *cdc2as-M17* system and cells collected 10' after release into mitosis, in the presence or absence of Sen1, at the indicated loci (1 biological replicate). **(C)** ChIP-qPCR analysis of Top1 and Cnd2 in metaphase cells synchronized by the *cdc2as-M17* system in which Camptothecin (CPT) 50  $\mu$ M was added 2' after release in mitosis (1 biological replicate).

Mitotic indexes are expressed in percentage between brackets. RNAPI: RNA polymerase I-transcribed genes. RNAPII: RNA polymerase II-transcribed genes. RNAPIII: RNA polymerase III-transcribed genes.

transcribed genes in interphase, i.e when condensin is not in the nucleus. To investigate this possibility, we monitored the accumulation of Top1 in the absence of Sen1 in G2 cells using *cdc2-asM17* cells blocked in G2 after addition of BrPP1 2  $\mu$ M for 3h or in metaphase (10 minutes after release into mitosis). Surprisingly, we observed that Top1 binding was already enhanced in G2 cells when condensin was not yet bound to chromatin (Figure 21B), suggesting that the accumulation of Top1 around RNAPIII-transcribed genes in the absence of Sen1 is not due to the accumulation of condensin. These observations are consistent with the idea that the deletion of Sen1 affects RNAPIII transcription in a way that generates topological stress.

To rigorously confirm the presence of topological stress however, it was important to show that the accumulated Top1 was actively cutting DNA strands. Camptothecin (CPT) is a topoisomerase 1 inhibitor that binds to the Top1-DNA covalent complex and blocks the rejoining step of the catalytic reaction. To demonstrate that CPT treatment increases the accumulation of Top1 in mitosis would therefore strongly suggest that Top1 actively cuts the DNA in that region and conclusively support the idea that topological stress accumulates around RNAPIII-transcribed genes in mitotic cells deleted of Sen1. We synchronized cells in G2 using the *cdc2as* system and released the cells in mitosis. 2 minutes after release in mitosis, we added 50  $\mu$ M of CPT and collected metaphase cells 10 minutes after release. Importantly, we showed that the amount of Top1 was further increased by CPT treatment in cells lacking Sen1 (Figure 21C), suggesting that Top1 was active.

Note that all the experiments presented in the last section need to be repeated. Taken together however, they are consistent with the idea that lack of Sen1 introduces Top1-sensitive topological stress around RNAPIII-transcribed genes.

### 1.3. Discussion

In this chapter, we provided evidence that Sen1 acts in *-cis* to regulate the accumulation of condensin at RNAPIII-transcribed genes in mitosis. Our results show that the accumulation of condensin around RNAPIII-transcribed genes in the absence of Sen1 is not due to a stoichiometric accumulation of either Tbp1 or TFIIC. Our results are more consistent with the idea that the positioning of condensin around RNAPIII-transcribed genes in the absence of Sen1 results from changes to the RNAPIII transcription cycle.

#### 1.3.1. Is the regulation of condensin by Sen1 dependent on RNAPIII transcription?

The observation that condensin accumulation at RNAPIII-transcribed genes occurs in poor and rich media, made us think that global changes to transcription rates do not alter the effect of Sen1 on condensin. So, it is not likely that changes in rate of transcription could explain the accumulation of

condensin at RNAPIII-transcribed genes, but instead some precise regulation of the RNAPIII transcription cycle by Sen1.

Our data is consistent with a new pathway of condensin accumulation at RNAPIII-transcribed genes in the Sen1 mutant, that cannot be explained by increased TFIIC (Sfc6) or Tbp1 recruitment. However, the observation that reducing RNAPIII loading (by mutating TFIIC) diminishes condensin peaks in the absence of Sen1, suggest that the effect of Sen1 on condensin needs the loading of RNAPIII by TFIIC. So, our data conclusively demonstrate that Sen1 needs an RNAPIII transcription unit and TFIIC-dependent RNAPIII loading to modulate condensin. Abolishing RNAPIII loading by the TATA box deleted mutant would demonstrate that RNAPIII transcription is required for the effect of Sen1 on condensin. Another way of inhibiting RNAPIII would be to use a degron system to induce a rapid degradation of RNAPIII.

Another way to definitely demonstrate this is to find a mutant of Sen1 that does not interact with RNAPIII, and test whether this mutant is able to create strong condensin accumulation sites at RNAPIII-transcribed genes.

We demonstrated that topological stress accumulates together with condensin at RNAPIII-transcribed genes in the absence of Sen1, and condensin and Top1 peaks strongly correlate. The observation that Top1 already accumulates in G2 suggests that deletion of Sen1 changes chromatin around tRNA genes already in interphase, creating topological stress, and then in mitosis condensin is attracted (recruited) or blocked (if condensin translocates through tRNA genes) at these loci. However, how is topological stress created in the absence of Sen1 at RNAPIII-transcribed genes, is not clear. Reduction of nucleosome occupancy and cohesin accumulation in the Sen1 mutant, are consistent with specific formation of chromatin structures around tRNA genes that could recruit SMC or block SMC movement.

### 1.3.2. Could the accumulation of condensin specifically at RNAPIII-transcribed genes in the absence of Sen1 explain the suppressor effect?

We detect a significant accumulation of condensin specifically at RNAPIII-transcribed genes when Sen1 is missing. However, condensin seems to be enriched at different extent over tRNA genes. The fission yeast genome contains 171 tRNA genes but using CHIP-qPCR we only analysed a few loci. We identified some tRNA loci in which condensin is strongly accumulated in the absence of Sen1, such as *arg10*, *thr10*, *arg04* and *tyr04*. Other tRNA genes show a mild increase such as *pro02*, *srp7* and *cys03* (not shown). We think that in most cases we are not able to detect the main peak of condensin around these loci, because qPCR primers are maybe not located at exact positions where condensin accumulates. To identify all the chromosomal loci where the association of condensin is

affected in the absence of Sen1, and the exact position of condensin peaks around RNAPIII-transcribed genes, we should use perform CHIP-seq. CHIP-seq would allow us to investigate whether condensin accumulates at most tRNA and 5S rDNA genes in the absence of Sen1 or whether it is rather a specific accumulation at some RNAPIII-transcribed genes. On the other hand, we cannot rule out the possibility that condensin enrichment at tRNA genes in the absence of Sen1 depends also on the genomic context of tRNA genes. This argument will be developed in Chapter 3.

Another way to answer this question is to investigate whether condensin peaks in the absence of Sen1 play a physiological role in condensation, contributing to condensin function and thus, improving growth of condensin mutant cells. For example, it would be interesting to test whether tRNA loci are better condensed (by using condensation assays) (Petrova et al., 2013), or if their frequency of interaction change by Hi-C. The problem with these methods is that tRNA genes are very small transcription units (around 70 bp), so it would be difficult to reach the sensitivity to detect changes in condensation or frequency of interaction at this scale. In the case of condensation assays, they measure the distances between two fluorescently labelled loci. The two loci are separated by 0.5 or 1.0 Mb, so to detect differences in condensation, one would need to enrich the region within the two loci with several tRNA genes in which condensin accumulation is enhanced in the absence of Sen1. Yet, it is not sure that differences in condensation could be detected.

We can not exclude the possibility that the suppressor effect of Sen1 deletion on condensin mutants is due to indirect mechanisms.

*sfc3-1* is also a suppressor of condensin mutants. The suppressor effect of *sfc3-1*, has been attributed to enhanced TFIIIC-mediated condensin binding. We failed to recapitulate these data. We detect reduced TFIIIC and no changes in condensin levels in *sfc3-1*. So, we cannot provide another explanation for the suppressor effect of *sfc3-1*. Our data show that *sfc3-1* and *sen1Δ* impact condensin in different ways.

In any case, even if we cannot answer the question whether condensin accumulation at RNAPIII-transcribed genes explains the suppressor effect of Sen1 deletion, what is important in our model is that we have concrete increase of condensin at specific sites. We can manipulate these sites in order to modulate condensin peaks and better understand the causes of the accumulation.

### 1.3.3. Condensin does not accumulate in WT cells at RNAPIII-transcribed genes in fission yeast

Scanning of condensin accumulation at three tRNA different loci (*SPCTRNAARG.10*; *SPCTRNATHR.10*; *SPBTRNATYR.04*) showed that condensin is not significantly accumulated in WT cells. This is surprising, as genome-wide data in fission yeast claim that condensin strongly accumulates at tRNA genes in mitosis (Nakazawa et al., 2015; Sutani et al., 2015). We do not have an

explanation yet for this incongruity, but we hypothesized that sequencing analysis do not consider the multiple copies of tRNA genes distributed on the genome. When reads are aligned to the genome, they could match to several copies of tRNA genes, which would result in artificial condensin peaks.

Together our data suggest that Sen1 modulates RNAPIII transcription, changes the topological state of chromatin, and thereby impacts condensin. To follow our investigation we asked how is RNAPIII transcription affected in the absence of Sen1. We addressed this question in Chapter 2.

## Chapter 2: Sen1 is required for robust RNAPIII transcription termination

### 2.1. Introduction

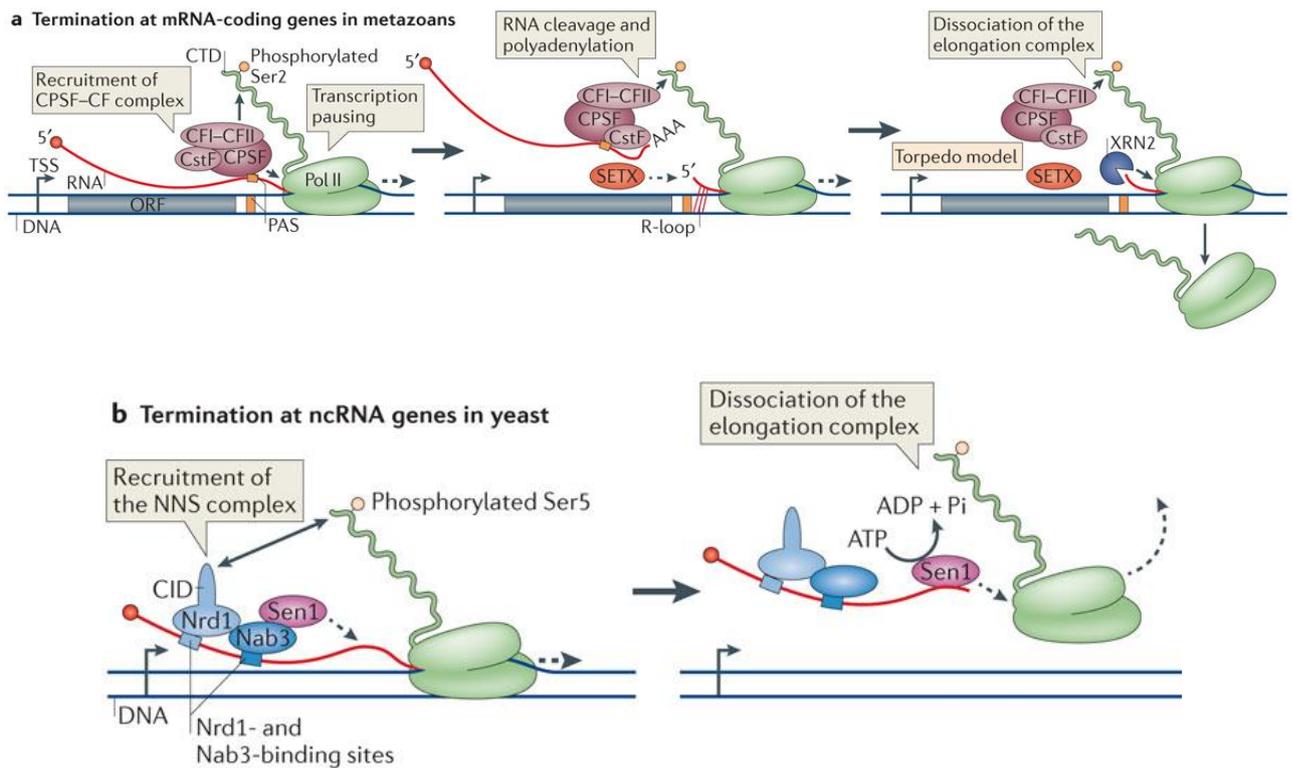
We have shown in Chapter 1 that Sen1, a DNA/RNA helicase that interacts with RNAPIII in *S. pombe*, regulates in *-cis* the accumulation of condensin in the vicinity of RNAPIII-transcribed genes. In addition, we presented evidence that the loading of RNAPIII on its target genes is required for lack of Sen1 to impact the accumulation of condensin around RNAPIII-transcribed genes. Our hypothesis is that Sen1 affects condensin positioning at RNAPIII-transcribed genes by controlling the RNAPIII transcription cycle. In the following, we asked how Sen1 impacts RNAPIII transcription.

In the following introduction, I will explain briefly the role of Sen1 in RNAPII transcription termination in budding yeast and human cells. As Sen1 binds to the RNAPIII in fission yeast, I will introduce current models of RNAPIII transcription termination and the arguments suggesting that Sen1 could participate in this process.

#### 2.1.1. Function of Senataxin/Sen1 in RNAPII transcription termination in human and in *S. cerevisiae*

Transcription consists of three main steps: initiation, elongation, and termination. Transcription initiation occurs when the RNA polymerase is loaded on the template at gene promoters. During elongation, the polymerase runs along the template strand and creates an RNA copy. When it reaches the termination site, the transcript is cleaved and released and the RNAP falls off the template. Transcription termination is a crucial step: proper transcription termination is essential for the release of RNAP from its template, it is important to avoid interference with the transcription of downstream genes, and also to ensure that a pool of RNAPs is available for reinitiation of transcription (reviewed in Richard and Manley, 2009).

At protein-coding genes, transcription of a poly(A) site at the 3' end of genes is followed by pausing of RNAPII transcription and endoribonucleolytic cleavage of the nascent transcript by the cleavage and polyadenylation factor (CPF in yeast, CPSF [cleavage and polyadenylation specific factor] in metazoans) machinery (Figure 22A). After the release of the mRNA, RNAPII continues to transcribe. Efficient release of RNAPII requires the 5'–3' exoribonuclease Xrn2 that degrades the nascent transcript from its 5' end. When this molecular torpedo catches up with RNAPII, then conformational shockwaves are transmitted into its active site, which releases RNAPII from the DNA



**Figure 22: Transcription termination of RNAPII-transcribed genes. (A)** Transcription termination at protein-coding genes is triggered by cleavage and polyadenylation specificity factor (CPSF), cleavage stimulatory factor (CstF), cleavage factor I (CFI) and CFII, which contain homologues of components of the yeast cleavage and polyadenylation factor (CPF) complex. Multiple interactions underlie the recruitment of the termination complex and the triggering of termination: CPSF directly binds to the body of the polymerase; CstF and CFI-CFII bind to the Ser2-phosphorylated form of the RNA polymerase II (Pol II) carboxy-terminal domain (CTD); and specific motifs (including the polyadenylation signal (PAS)) are recognized in the 3' untranslated region of the nascent RNA by CPSF and CstF. The RNA is cleaved and polyadenylated at the 3' end. Transcription pausing is thought to be required for termination. Senataxin (SETX), has been suggested to participate in termination of some mRNA genes, possibly by resolving R-loops to allow the entry of the 5'–3' exonuclease XRN2, the homologue of Rat1. Degradation of the 3' end fragment of the nascent transcript is thought to subsequently elicit transcription termination (the torpedo model). **(B)** During termination at non-coding RNA (ncRNA) genes, the Nrd1–Nab3–Sen1 (NNS) complex is recruited to the elongation complex through the recognition of specific motifs on the nascent RNA by Nrd1 and Nab3, and the interaction of the Nrd1 CID with the Ser5-phosphorylated form of the CTD. The RNA and DNA helicase Sen1 is then loaded onto the RNA, where it uses the energy of ATP hydrolysis to 'catch up' with Pol II and elicit termination. Adapted from Porrua & Libri, 2015.

template (Figure 22A) (Porrúa and Libri, 2015; Proudfoot, 2016). It was shown in HeLa cells that RNAi-mediated knockdown of Senataxin causes an increase in readthrough RNA and RNAPII density downstream of the poly(A) site, specifically at G-rich pause elements (G-rich sequences immediately downstream of the poly(A) signal), implying the involvement of Senataxin in transcriptional termination (Skourti-Stathaki et al., 2011). As the function of yeast Sen1 in transcription has been associated to the resolution of R-loops (Mischo et al., 2011), the authors tested whether the function of human Senataxin was linked to R-loops removal. R-loops were assessed by DNA immunoprecipitation (DIP) using the S9.6 antibody which recognizes RNA/DNA hybrids. The authors showed that R-loops form at these specific G-rich termination elements, and that upon depletion of Senataxin, R-loops increase downstream of the poly(A) signal. However, in this experiment, they do not demonstrate that the hybrids detected by DIP are RNase H-sensitive. In addition, the authors show that overexpression of RNase H1 (an enzyme that disassembles R-loops) induces transcriptional readthrough. These data seem contradictory since stabilization (by Senataxin depletion) and removal (by overexpression of RNase H1) of R-loops generate both readthrough transcription. The authors conclude that R-loops form over pause elements, but once they are formed their removal by Senataxin is required for efficient transcriptional termination. A model is proposed in which the formation of R-loops at transcriptional pause regions causes RNAPII pausing downstream of the poly(A) site prior to termination, these R-loops have to be subsequently resolved by Senataxin to release the nascent RNA and so allow its Xrn2-mediated degradation, which ultimately results in efficient RNAPII transcriptional termination (Figure 22A) (Skourti-Stathaki et al., 2011).

In budding yeast, short non-coding RNA genes, on the other hand, employ a poly(A)-independent pathway. The Nrd1–Nab3–Sen1 (NNS) complex is involved in the poly(A)-independent transcription termination of short non-coding genes including snoRNAs, snRNAs, and cryptic unstable transcripts (Figure 22B). Within the NNS complex, nuclear pre-mRNA downregulation (Nrd) 1 and nuclear polyadenylated RNA-binding (Nab) 3 proteins form a heterodimer, which binds specific sequences on the RNA, while Sen1 is proposed to provide the helicase activity needed to destabilize RNAPII and allow its dissociation from the DNA template (Figure 22B) (Carroll et al., 2007; Porrúa and Libri, 2015; Steinmetz et al., 2001). As Senataxin in human cells, it has been proposed that removal of R-loops by budding yeast Sen1, underlies its role in transcription termination. Several studies show that the *sen1-1* mutant (helicase domain mutant) accumulate R-loops 3' of specific genes (Grzechnik et al., 2015; Mischo et al., 2011) and genome-wide (Chan et al., 2014). The same mutant shows defects in transcription termination (Grzechnik et al., 2015; Ursic et al., 1997), suggesting that the function of Sen1 in transcription termination may be associated with the resolution of R-loops. However, current data do not firmly demonstrate this link. *In vitro* studies using a highly purified system (transcription-termination assay containing only purified RNAPII and Sen1) suggest that

budding yeast Sen1 interacts with the nascent RNA, may translocate along the transcript and induces RNAPII transcription termination (Porrúa and Libri, 2013). Additionally, the authors show that Sen1 uses its ATPase activity to dissociate the elongation complex. Interestingly, RNase H treatment in the *in vitro* reaction did not affect the efficiency of Sen1-mediated termination, suggesting that in this termination assay R-loops are not required for transcription termination (Porrúa and Libri, 2013).

Together these evidences strongly indicate that human Senataxin and budding yeast Sen1 are implicated in RNAPII transcription termination at a subset of RNAPII-transcribed genes. However, whether R-loops removal is involved in Sen1/Senataxin-mediated termination is not clear.

### 2.1.2. Function of Sen1 in RNAPIII transcription termination in *S. pombe*

Two Senataxin helicases exist in *S. pombe* Sen1 and Dbl8. Fission yeast Sen1 was shown to translocate 5' to 3' and unwind both DNA and RNA duplexes and also RNA/DNA hybrids *in vitro* (Kim et al., 1999). Regarding a role in RNAPII transcription, neither Sen1 nor Dbl8 does interact with RNAPII in *S. pombe* (Rivosecchi et al., 2019), and deletion of neither Sen1 nor Dbl8 results in readthrough transcription at mRNA and snoRNA genes (Larochelle et al., 2018). These findings are consistent with the observation that Seb1 (Nrd1 homolog), Nab3, Sen1, and Dbl8 do not to form a stable complex in fission yeast (Larochelle et al., 2018; Legros et al., 2014; Lemay et al., 2016). Together, these observations tend to rule out the possibility that Sen1 could play a role in RNAPII transcription.

Previous published data from our group revealed an unexpected interaction of Sen1 with RNAPIII. In addition, Sen1 mainly localizes at RNAPIII-transcribed genes (Legros et al., 2014). These observations opened a new possibility in which Sen1 could act on RNAPIII transcription. Consistent with this, the team also previously showed that, in the concomitant absence of Swd22 and Sen1, the amount of RNAPIII increased on chromatin specifically on its target genes, suggesting that Sen1 might indeed modulate RNAPIII transcription *in-cis* (Legros et al., 2014).

Considering that Sen1 has been implicated in transcription termination in other model systems, albeit at another class of genes (RNAPII genes), we speculated that Sen1 could be involved in transcription termination of RNAPIII-transcribed genes in fission yeast.

In fission yeast, RNAPIII transcribes tRNA genes, 5S rRNA genes, the U6 small nucleolar RNA (*snu6*) and the 7SL component of signal recognition particle (*srp7*), as mentioned in Chapter 1. It is claimed that RNAPIII transcription termination occurs efficiently on a simple oligo(dT) tract (a minimum of 5T in fission yeast) (Figure 15) (Hamada et al., 2000) independent of other *cis*-elements or *trans*-factors, at least *in vitro* (Arimbasseri and Maraia, 2015; Mishra and Maraia, 2018). Of the 17 RNAPIII subunits, C37/53/C11 have been shown to be required for termination (Arimbasseri and

Maraia, 2015; Mishra and Maraia, 2018). We challenged this autonomous model of RNAPIII transcription termination, speculating that Sen1 could participate in this process, at least *in vivo*.

The team already showed that R-loops form at tRNA genes in fission yeast (Hartono et al., 2018; Legros et al., 2014). We tested, therefore, whether the processing of R-loops might be implicated in a role of Sen1 in RNAPIII transcription termination.

We recently published in *EMBO Journal* compelling results demonstrating that Sen1 is required for robust RNAPIII transcription termination, independently of a putative role of Sen1 in R-loop disassembly. The conclusions of this work are presented in the next subsection (2.2.1), and the publication is attached at the end of the thesis.

In addition to the results that we published in *EMBO Journal*, I will include in this section unpublished results that reinforce our conclusions. In particular, we explored whether the ATPase activity of Sen1 is required for its function in RNAPIII termination. We also engineered two additional mutants that display readthrough RNAPIII transcripts and I will discuss how these additional mutants contribute to a better understanding of Sen1-mediated RNAPIII transcription termination.

## 2.2. Results

### 2.2.1. Publication: Senataxin homologue Sen1 is required for efficient termination of RNA polymerase III transcription

Using a wide variety of assays (ChIP-seq, ChIP-qPCR, a transcription termination colour assay, RT-qPCR and northern blot), we were able to show that RNAPIII accumulates downstream of RNAPIII-transcribed genes and produces exosome-sensitive readthrough transcripts in the absence of Sen1. These observations were consistent with a function of Sen1 in transcription termination. Interestingly our data show that this function of Sen1 is not shared with its homologue Dbl8.

We showed that the insertion of a strong terminator could overcome the need for Sen1 in RNAPIII transcription termination, suggesting that Sen1 acts in complement to the intrinsic RNAPIII transcription termination mechanisms.

Overexpression of RnhA to remove R-loops in the absence of Sen1 did not alter either RNAPIII accumulation or the formation of read-through transcripts, suggesting that Sen1 is required for RNAPIII termination in an R-loop-independent manner.

Our results show that Sen1 is the first known RNAPIII cofactor implicated in transcription termination, contrary to what is currently claimed, and we propose that the ancestral function of Sen1 is to destabilize elongating RNAP.

I performed all the experiments shown in the publication except:

- 1) the CHIP-seq experiments that were performed by the team of François Bachand at Sherbrooke University (Figure 1A-E; Figure 4A-D; Appendix Figure S2; Appendix Figure S4);
- 2) the mass spectrometry analysis of the binding partners of Sen1 and Dbl8 that was previously performed by Amélie Malapert (Appendix Table S1);
- 3) the CHIP-qPCR analysis of Rpc37 and Sen1 in the *arg10-TATAless* mutant that was performed by Camille Teste (Figure 1G);
- 4) the co-immunoprecipitation experiment (Co-IP) to demonstrate that Flag-tagged Dbl8 interacts with the GFP-tagged RNAP1 subunit Rpa43 that was performed by Amélie Malapert (Figure EV1);
- 5) the CHIP-qPCR analysis of GFP-tagged Sen1 in *dbl8Δ* strains that was performed by Amélie Malapert (Appendix Figure S3);
- 6) for the analysis by Northern blot and 3' RACE of read-through transcription at *SPACTRNAPRO.02* (Figure 5DE), I received significant help from Emiliano Ricci.

The publication is available at the following link:

<https://doi.org/10.15252/emj.2019101955>

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DOI 10.15252/emj.2019101955

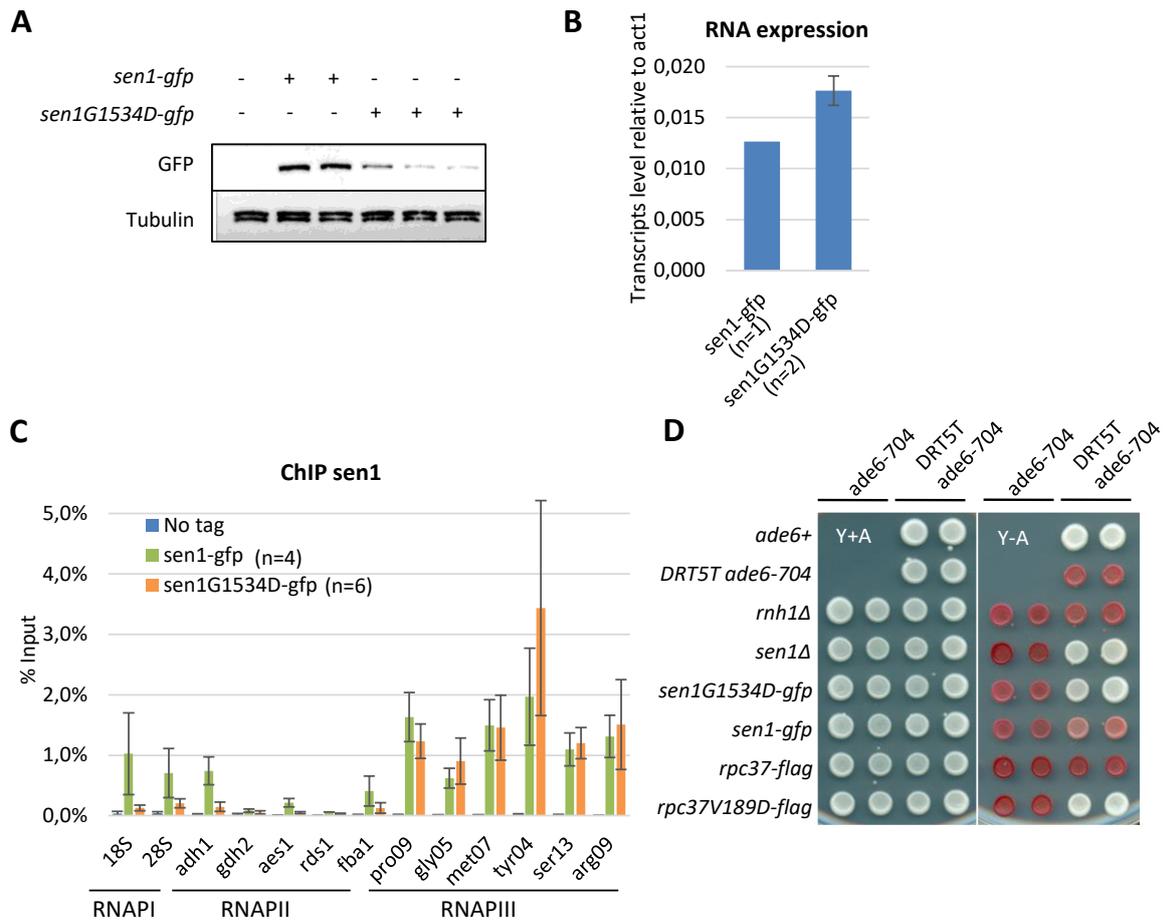
### 2.2.2. The helicase activity of Sen1 is required for efficient RNAPIII transcription termination

The C-terminal of Sen1 contains an ATP-dependent helicase domain (Kim et al., 1999). We asked whether the helicase domain is required for transcription termination at RNAPIII-transcribed genes. As ATP hydrolysis is essential for the helicase activity, we performed a site-directed mutagenesis at a conserved glycine in the ATPase domain that abolishes the ATPase activity. This mutation was shown to induce transcription termination defects at specific RNAPII transcribed genes *in vivo* in budding yeast (*sen1-1*) (Grzechnik et al., 2015; Ursic et al., 1997) and *in vitro* (Porrúa and Libri, 2013). Sequence alignment showed that G1747 of *S. cerevisiae* corresponds to G1534 of *S. pombe*. Site-directed PCR mutagenesis was used to produce the *sen1-G1534D* mutant. The mutant protein was integrated in the yeast genome at the endogenous locus under the control of the endogenous promoter and fused to a GFP epitope tag. In these strains, no wild-type version of the protein is expressed. Western blot analysis showed that the overall levels of the mutant protein were reduced compared to the wild type protein (Figure 23A). Steady-state RNA levels were measured by RT-qPCR and no significant changes were detected between *sen1-G1534D-gfp* and *sen1-gfp* (Figure 23B). We conclude from these results that the mutation interferes slightly with the stability of the protein.

To evaluate the impact of the mutation on the association of *sen1-G1534D* with chromatin, we used ChIP to monitor the distribution of Sen1 along chromosomes. These experiments indicated that the enrichment of the mutant protein at RNAPIII-transcribed genes is very similar to the wild-type protein (Figure 23C). Taken together, these data showed that the ATPase mutant of Sen1 is less stable but localizes at RNAPIII-transcribed genes with the same efficiency as the wild-type protein.

To evaluate the impact of the helicase activity of Sen1 on transcription termination at RNAPIII-transcribed genes, we used a genetic assay that translates a transcription termination defect at the synthetic tRNA DRT5T dimeric construct into a change of colour of yeast colonies from red to white (see diagram of the DRT5T construct in Figure 5A of the publication) (Iben et al., 2011). A transcription termination defect allows the synthesis of a suppressor tRNA that suppresses the accumulation of a red pigment caused by the *ade6-704* mutation, resulting in white colonies in adenine-poor medium. On an adenine-poor medium, the *sen1-G1534D* mutant showed white colonies like the deletion of Sen1 (data shown also in the publication), indicative of transcription termination defects (Figure 23D). Note that the tagged version of Sen1 (*sen1-gfp*) already confers a small change of colour, reflecting some degree of defective transcription termination (Figure 23D). These observations suggest that Sen1 utilizes its helicase activity to facilitate transcription termination at synthetic RNAPIII-transcribed genes.

To assess with this method whether stabilization of R-loops could interfere with transcription termination at RNAPIII-transcribed genes, we analyzed the effect of deleting Rnh1 (*rnh1Δ*) on the dimeric construct. Cells remained red on an adenine-poor medium in the absence of Rnh1,



**Figure 23: The helicase activity of Sen1 is required for transcription termination at synthetic RNAPIII genes.** (A) Western Blot analysis of the stability of *sen1-G1534D-gfp*. Tubulin is used as loading control. This experiment has been carried out twice. (B) Expression levels of the indicated strains analysed by RT-qPCR. The values are normalized to the expression levels of the house-keeping gene *act1*. One biological replicate was analysed for *sen1-gfp* and 2 for *sen1-G1534D-gfp*. (C) ChIP-qPCR analysis of Sen1 at different loci. (mean  $\pm$  standard deviation from 4 (*sen1-gfp*) and 6 (*sen1-G1534D-gfp*) biological replicates). (D) Transcription termination assay of the indicated strains in the presence of the DRT5T dimeric construct. (see diagram of the DRT5T construct in Figure 5A of the publication). Two different strain of each genotype are plated. All strains were grown in a medium rich in adenine as control. RNAPI: RNA polymerase I-transcribed genes. RNAPII: RNA polymerase II-transcribed genes. RNAPIII: RNA polymerase III-transcribed genes.

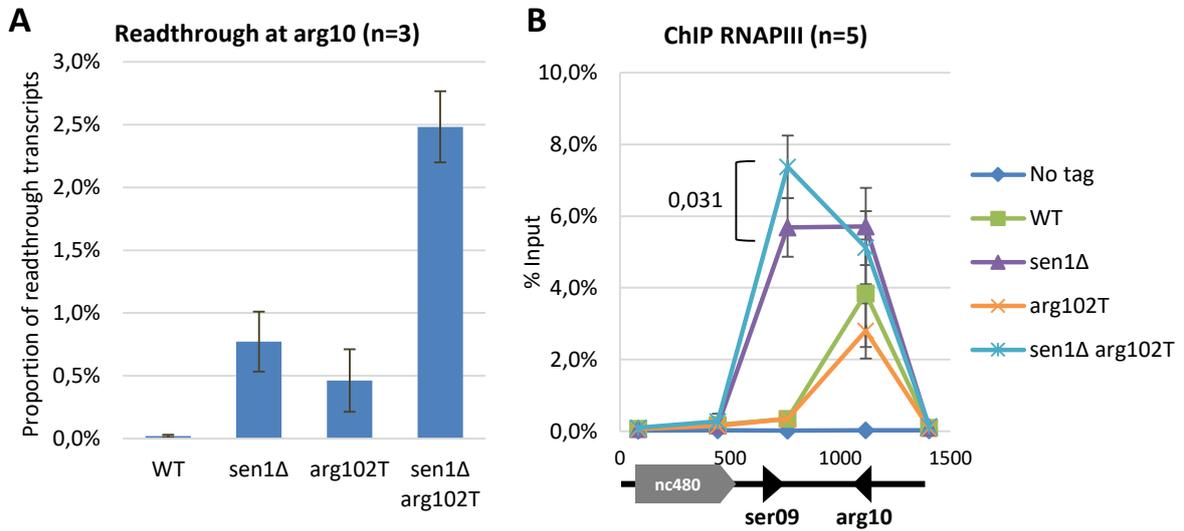
suggesting that R-Loop stabilization at RNAPIII-transcribed genes does not result in a transcription termination defect (Figure 23D). This suggests that it is not by unwinding R-loops that Sen1 participates in transcription termination of RNAPIII transcripts and that the helicase activity of Sen1 must have another substrate at RNAPIII-transcribed genes.

### 2.2.3. Sen1 dissociates RNAPIII molecules that override the primary terminator

Our published data did not indicate whether Sen1 only facilitates the release of RNAPIII at the primary terminator sequence or whether Sen1 could more generally destabilize elongating RNAPIII molecules that failed to terminate at the primary terminator. If Sen1 acts primarily at the canonical termination sequence, the level of read-through transcripts produced after inactivating this terminator sequence should not be affected further by lack of Sen1. To test this possibility, we inactivated the terminator sequence of *SPCTRNAARG.10* by reducing the stretch of 5 thymine residues to a terminator-inactivating 2 thymine residues (*arg10-2T* mutant) as described previously (Hamada et al., 2000) and monitored both the accumulation of RNAPIII past the terminator and the production of readthrough transcripts. Weakening the terminator resulted in accumulation of readthrough tRNA arg10 in a lower level compared to *sen1Δ* (Figure 24A) and surprisingly, RNAPIII did not accumulate at arg10 in the *arg10-2T* mutant (Figure 24B). Strikingly, the combination of *sen1Δ* and *arg10-2T* resulted in a synergistic accumulation of readthrough transcripts (Figure 24A) and an even greater accumulation of RNAPIII downstream of the terminator (Figure 24B). These observations established that Sen1 facilitates the release of RNAPIII, whether or not it is paused at the primary transcription termination signal. A corollary to these observations is that the accumulation of RNAPIII that occurs on tRNA genes in the absence of Sen1 does not result from the pile-up of RNAPIII molecules stalled at the primary terminator.

### 2.2.4. Investigating the transcription termination defects in the RNAPIII mutant *rpc37-V189D*

As a positive control for transcription termination defects, we generated a mutant of the C37 subunit of RNAPIII (Rpc37). We mutagenized the valine residue at position 189 into an aspartate residue (*rpc37-V189D*) by PCR-based site-directed mutagenesis. Overexpression of this mutant was shown to interfere with transcription termination in a dominant-negative manner (Rijal and Maraia, 2013). In the experiments described in the later publication, the wild type copy of the gene is expressed together with the mutant copy. We decided to introduce the mutation at the endogenous locus to obtain cells that express only the mutated copy of the gene. Western Blot analysis revealed that the mutant protein is as stable as the wild-type protein (Figure 25A). We analysed the impact of the mutation *rpc37-V189D* on transcription termination at synthetic RNAPIII-transcribed genes using



**Figure 24: The function of Sen1 is not restricted to the primary terminator. (A)** Strand specific RT-qPCR was used to quantify read-through transcripts at *SPCTRNAARG.10* in the weak terminator mutant (mean  $\pm$  standard deviation from 3 biological replicates). **(B)** ChIP-qPCR analysis of Rpc37 around *SPCTRNAARG.10* gene in the weak terminator mutant (mean  $\pm$  standard deviation from 5 biological replicates).

the genetic assay. *rpc37-V189D* mutant cells grew white on an adenine-poor medium (Figure 23D), suggesting that this mutation was able to induce a transcription termination defect at synthetic RNAPIII-transcribed genes (this result is already shown in the publication). We found a synthetic lethal interaction between *rpc37-V189D* and *sen1Δ* (see Figure 5B of the publication), consistent with a role of Sen1 in transcription termination of RNAPIII-transcribed genes. Using strand specific RT-qPCR, we observed that readthrough transcripts accumulate in *rpc37V189D* cells (Figure 25B). ChIP-qPCR of the Rpc37 subunit of RNAPIII showed, however, no accumulation of RNAPIII at tRNA genes (Figure 25C). These results were surprising and suggests that the RNAPIII termination mutant and the Sen1 mutant do not behave completely the same way.

As proposed above for the *arg10-2T* mutant, we speculate that in *rpc37V189D* cells, Sen1 dissociates the RNAPIII molecules that overrode the terminator.

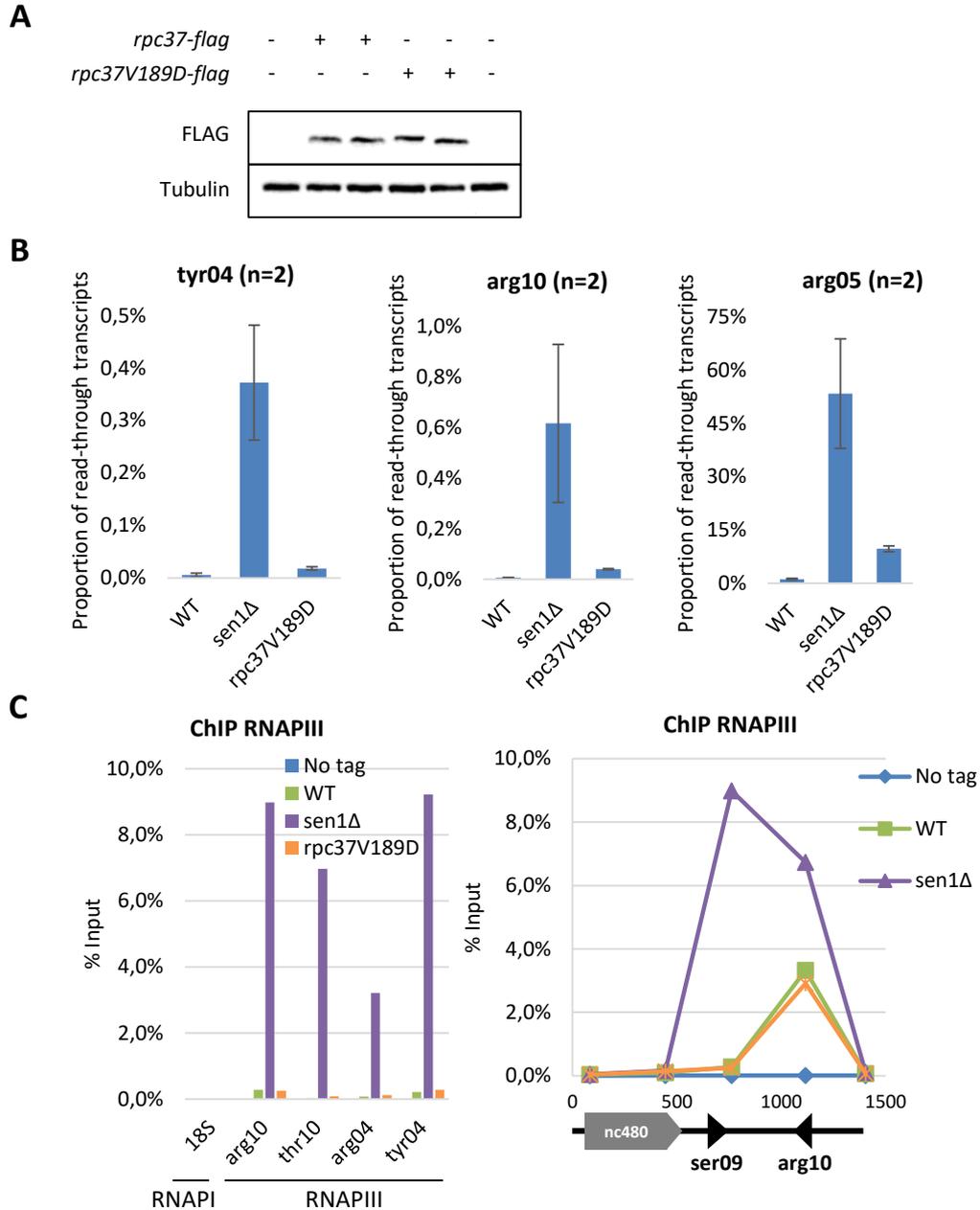
## 2.3. Discussion

We investigated how Sen1 could impact RNAPIII transcription and we demonstrated that Sen1 but not Dbl8, is required for robust RNAPIII transcription termination in an R-loop independent manner, and that the helicase activity of Sen1 is essential for this function.

### 2.3.1. The role of Sen1 in transcription termination is conserved

Sen1 in budding yeast and Senataxin in human cells are involved in transcription termination of RNAPII (Porrua and Libri, 2013; Skourti-Stathaki et al., 2011; Steinmetz et al., 2001; Ursic et al., 1997). Here, we showed that the role of Sen1 in transcription termination is conserved in fission yeast but at RNAPIII. It is intriguing that RNAP require the action of Sen1/Senataxin for efficient termination at only a subset of genes. In human cells, Senataxin acts to destabilize RNAPII paused specifically at genes containing G-rich sequences downstream of the poly(A) signal (Skourti-Stathaki et al., 2011). In budding yeast the NNS complex terminates transcription of short non-coding RNAs, such as snRNAs, snoRNAs and cryptic unstable transcripts (Porrua and Libri, 2013; Steinmetz et al., 2006; Ursic et al., 1997). In fission yeast, the requirement of Sen1 for RNAPIII transcription termination seems to vary between tRNA genes. Weak terminators ( $\leq 5T$ ) need Sen1 for efficient RNAPIII termination, while strong terminators can overcome the requirement of Sen1 for RNAPIII transcription termination. This is well illustrated at *SPATRNAPRO.02* (Figure 5D of the publication). However, an extensive genome-wide analysis should be done to confirm the correlation between the strength of the terminator and the defect in termination.

The implication of R-loops in transcription termination is not conserved between the three organisms. In human cells, R-loops first form at G-rich pause elements to recruit Senataxin which



**Figure 25: Inducing tRNA readthrough by mutating the Rpc37 subunit of RNAPIII do not result in RNAPIII accumulation.** (A) Western Blot analysis of the stability of *rpc37V189D-flag*. Tubulin is used as loading control. (B) Strand specific RT-qPCR of three tRNAs in the indicated strains (mean± standard deviation from 2 biological replicates). (C) ChIP-qPCR analysis of Rpc37 at different loci and scanning of the *ser09-arg10* locus. (1 biological replicate). RNAPI: RNA polymerase I-transcribed genes. RNAPIII: RNA polymerase III-transcribed genes.

then removes the hybrids to promote transcription termination (Skourti-Stathaki et al., 2011). In budding yeast *in vivo* data suggest that R-loop disassembly is involved in transcription termination (Mischo et al., 2011), but *in vitro* data opposes this model (Porrúa and Libri, 2013). Our results show that in fission yeast, RNAPIII transcription termination mediated by Sen1 is independent of R-loops. In conclusion, Senataxin homologues have a conserved function in destabilizing RNAP at termination regions, at a subset of genes, and its substrate might differ between organisms.

### 2.3.2. Does Sen1 act on both RNAPIII termination and RNAPIII elongation?

Our data show that in the absence of Sen1, RNAPIII accumulates in the body of tRNA genes and downstream of terminators. However, this enhanced amount of RNAPIII is not accompanied by increased tRNA but instead our data suggest that there could be reduced tRNA transcripts, suggesting that lack of Sen1 impairs RNAPIII transcription and that RNAPIII are maybe paused at tRNA genes. As discussed in the publication, these results could reflect reduced recycling due to the termination defect or an independent elongation defect. The observation that the super terminator mutant at *arg10* (*arg10-23T*) suppresses the accumulation of RNAPIII downstream of the terminator but not in the body of *arg10* in the absence of Sen1 (Figure 6B of the publication), is consistent with an elongation defect. The *thr10-20T* mutant behaves differently (Figure EV4B of the publication), as it is not clear whether in the presence of the strong terminator, RNAPIII is reduced over *thr10* in the absence of Sen1. I should repeat the experiment to improve the data and their statistics.

Sen1 mutants seem to present two deficiencies, in termination and in elongation, however, the molecular basis for these defects is still unclear.

The two different termination mutants, *rpc37V189D* and *arg10-2T* act in different ways: the RNAPIII mutant is not able to recognize the oligo(dT) terminator, and the *arg10-2T* lacks the oligo(dT) signal that pauses RNAPIII (Arimbasseri and Maraia, 2015). Both show very interesting phenotypes. By RT-qPCR we detected readthrough transcripts accumulation, however RNAPIII did not accumulate in these mutants, contrary to what we observe in the *sen1Δ*. One possible explanation for the lack of RNAPIII accumulation downstream of *rpc37V189D* and *arg10-2T* is that in these strains Sen1 acts to detach those RNAPIII that readthrough the terminator. This implies that Sen1 is able to remove RNAPIII from sequences downstream of the terminator, suggesting that Sen1 affects RNAPIII independently of the terminator sequence.

Another argument in favour of the existence of elongation defects in the Sen1 mutant, is the RNAPIII distribution observed in the double *sen1Δ arg10-2T* mutant. If RNAPIII accumulated at the body of *arg10* came from RNAPIII stalled at the terminator of *arg10* (as a result of termination defects), weakening the terminator in the absence of Sen1 should displace the entire RNAPIII peak

downstream of the 2T weak terminator. However, RNAPIII still accumulates at the body of *arg10* in the double mutant, suggesting that RNAPIII accumulated at *arg10* do not come from stalled RNAPIII at the terminator. This is consistent with an elongation defect in the absence of Sen1, independent of the termination defect.

A counter argument for a role of Sen1 in RNAPIII termination and elongation is that kinetic models of RNAPIII termination suggests that elongation rate is inversely related to termination efficiency. This means that RNAPIII mutants with readthrough phenotypes present increased elongation rates (Rijal and Maraia, 2016). The idea is that RNAPIII elongation slows down at the terminator, which helps the C37 subunit to recognize T residues and promote termination (Rijal and Maraia, 2016). The phenotypes we observe in *sen1Δ* are consistent with termination and elongation defects, which is contrary to current models. If RNAPIII elongation is reduced, this should facilitate RNAPIII transcription termination. We still do not have an explanation for this contradiction. We could speculate that if the function of Sen1 is to induce a conformational change in RNAPIII to promote its detachment from the template, even if elongation is reduced and the terminator is recognized by C37, RNAPIII will remain strongly bound to DNA in the absence of Sen1.

To better characterize the elongation and termination defects, we should perform CRAC (UV crosslinking and analysis of cDNA) or NET-seq (Native elongating transcript sequencing) of RNAPIII in the Sen1 mutant. This would map the location of active RNAPIII at the nucleotide resolution, relative to nascent transcription. We could, therefore, obtain information about transcription rates and the exact position of RNAPIII accumulated in *sen1Δ*. CRAC of RNAPIII was already performed in budding yeast (Turowski et al., 2016). Two peaks of RNAPIII were observed at 5' and 3' of tRNA genes, where the A box and B box are located. The authors suggested that TFIIC could constitute a transient barrier for RNAPIII (Turowski et al., 2016). Similarly, in the absence of Sen1, RNAPIII accumulation in the body of tRNA genes could be specifically localized at TFIIC-binding sites. If it is the case, Sen1 would be important for RNAPIII to overcome TFIIC bound to tRNA genes. Another possibility is to find RNAPIII accumulated at terminators or downstream of terminators in the absence of Sen1. Based on our results, we expect to observe RNAPIII accumulated both at terminators and downstream of terminators when Sen1 is missing.

On the other hand, CRAC of Sen1 would establish exactly where Sen1 binds on the tRNA and this would give a better idea of the mechanism of Sen1-mediated RNAPIII termination. For example, at *snu6*, we observe the accumulation of Sen1 at 5' and 3' of the gene (Figure 1B of the publication). These two peaks could represent the accumulation of Sen1 at A and B boxes, likely mediated by RNAPIII.

To definitively demonstrate that Sen1 possess two independent functions in RNAPIII termination and elongation one would have to identify mutants of Sen1 that separate termination and elongation functions.

### 2.3.3. What is the mechanism of Sen1-mediated RNAPIII transcription termination?

Our data do not allow us to understand in details the mechanism of Sen1-mediated RNAPIII transcription termination. The observation that weakening the terminator in the absence of Sen1 (*sen1Δ arg10-2T* mutant) results in increased RNAPIII downstream of the terminator and enhanced readthrough transcripts, suggests that RNAPIII does not accumulate at the primary terminator in the absence of Sen1. It is more likely that Sen1 could displace RNAPIII elongation complexes independently of the terminator, for example those that already read through the terminator. This result is consistent with the model proposed in the publication for the role of Sen1 in RNAPIII transcription, based on the release and catch-up mechanism described for the Mfd translocase in *E. Coli* (Le et al., 2018). Sen1 could push RNAPIII molecules that are weakly paused at tRNA and thereby facilitate transcription elongation, or release RNAPIII molecules that are stalled at canonical terminator sequences to promote transcription termination.

In Chapter 1 I showed that deletion of Sen1 introduces transcription-driven changes in condensin distribution at RNAPIII-transcribed genes. Based on our results we postulated that Sen1 could regulate condensin by modulating RNAPIII transcription. The results of Chapter 2 demonstrate that Sen1 is a cofactor of RNAPIII required for robust transcription termination. The next step is to test whether the transcription termination defects observed in the absence of Sen1 could underlie the accumulation of condensin at RNAPIII-transcribed genes. It is worth noting that all the data presented in this chapter were obtained from cycling cells and not from mitotic cells. As a result, in order to link the function of Sen1 in transcription termination with the regulation of condensin accumulation, we should verify that Sen1 acts on RNAPIII transcription termination in mitosis, when condensin binds to chromatin.

Could transcription termination defects explain the mitotic accumulation of condensin in the absence of Sen1? This is addressed in Chapter 3.



## Chapter 3: The control of RNAPIII transcription termination by Sen1 determines condensin positioning in the vicinity of RNAPIII-transcribed genes

### 3.1. Introduction

In Chapter 1 we demonstrated that Sen1 regulates the positioning of condensin at RNAPIII-transcribed genes. ChIP-qPCR scanning of three different tRNA gene loci (*SPCTRNAARG.10*; *SPCTRNATHR.10*; *SPBTRNATYR.04*) show that condensin is predominantly enriched at 3' of tRNA genes (Figure 20C and 21A) in the absence of Sen1. In Chapter 2 we have established that Sen1 is required for efficient termination of RNAPIII transcription. Consistent with this, we detected an accumulation of RNAPIII 3' of tRNA genes when Sen1 is missing. These results show an overlap between RNAPIII and condensin accumulation downstream of tRNA gene terminators in *sen1Δ*, suggesting that defective transcription termination could underlie condensin accumulation. In this last chapter we tested whether transcription termination defects at RNAPIII-transcribed genes could be responsible for the accumulation of condensin in the absence of Sen1. As mentioned at the end of Chapter 2, it is important to first confirm that Sen1 does indeed control RNAPIII transcription termination in mitosis also, at the time when condensin accumulates on chromosomes.

#### 3.1.1. Transcription as a positioning factor for cohesin

In yeast (Lengronne et al., 2004; Mizuguchi et al., 2014) and in mammalian cells (Busslinger et al., 2017) cohesin is enriched at sites of convergent transcription. *In vitro* experiments showed that active RNAPII could push cohesin along DNA (Davidson et al., 2016). An interesting study shows that the NS1 protein from influenza A produces a global inhibition of transcription termination that causes readthrough transcription (Heinz et al., 2018). In these readthrough regions, elongating RNAPII disrupts chromatin interactions by inducing cohesin displacement from CTCF sites. In this study RNAPII that readthrough terminators induce cohesin clearance rather than translocation downstream of genes. Furthermore, they show that in general, elongating RNAPII is able to displace cohesin (Heinz et al., 2018).

From these evidences we can conclude that RNAPII can either push cohesin or clear cohesin from chromatin, as it has also been observed in *S. cerevisiae* (Bausch et al., 2007).

### 3.1.2. Transcription interferes with translocation of bacterial SMC

Bacteria emerged as an interesting model to address transcription-SMC interactions. Bacterial SMC activity is easy to study since it possesses a unique loading site and translocates unidirectionally. In *B. subtilis* and *C. crescentus*, ParB loads SMC onto the chromosome at the *ori*-proximal *parS* sites. Loaded SMC then translocates from *parS* to distal parts of the chromosome aligning progressively the two chromosome arms from *ori* to *ter* (Tran et al., 2017; Wang et al., 2017). It has been demonstrated that head-on transcription slows down condensin translocation (Tran et al., 2017; Wang et al., 2017). These observations point out the importance of the relative orientation between gene transcription and SMC translocation for SMC loop extrusion.

### 3.1.3. Which feature of RNAPIII transcription controlled by Sen1 could impact condensin?

Our results reveal that Sen1 modulates RNAPIII transcription in several ways. We showed that it is required for transcription termination and that it might also impinge on transcription elongation. In the absence of Sen1, we observed the following phenotypes: reduced overall RNAPIII transcription, accumulation of readthrough tRNA transcripts and accumulation of RNAPIII over and 3' of tRNA genes. Is there a link between any of these phenotypes and the accumulation of condensin at RNAPIII-transcribed genes? The first possibility is that reduced RNAPIII elongation rates in the absence of Sen1 might facilitate the function of condensin. This hypothesis would be in agreement with the model proposed by L. Aragon claiming that condensin cannot access chromatin until transcription is significantly reduced. In this model, Cdc14-dependent inhibition of RNAPI allows condensin loading at rDNA (Clemente-Blanco et al., 2009; Wang et al., 2006), and Cdc14-dependent inhibition of RNAPII promotes condensin loading at subtelomeric repetitive regions (Clemente-Blanco et al., 2011). We think that reduced RNAPIII elongation in the absence of Sen1 is unlikely to explain the enhanced accumulation of condensin at RNAPIII-transcribed genes, since impairing the TFIIC-dependent loading of RNAPIII onto tRNA genes in *sen1Δ* cells resulted in a reduction rather than an increase in the accumulation of condensin (Figure 20D), as might have been predicted from the Aragon model. We therefore ruled out the possibility that reduced nascent transcription in the absence of Sen1 could explain the accumulation of condensin. To test whether readthrough tRNAs and/or RNAPIII accumulation could be responsible for condensin accumulation in the absence of Sen1, we made use of the tRNA gene terminator mutants showed in chapter 2: *arg10-2T*, *arg10-23T* and *thr10-20T*.

## 3.2. Results

### 3.2.1. RNAPIII accumulates on its target genes in the absence of Sen1 in mitosis

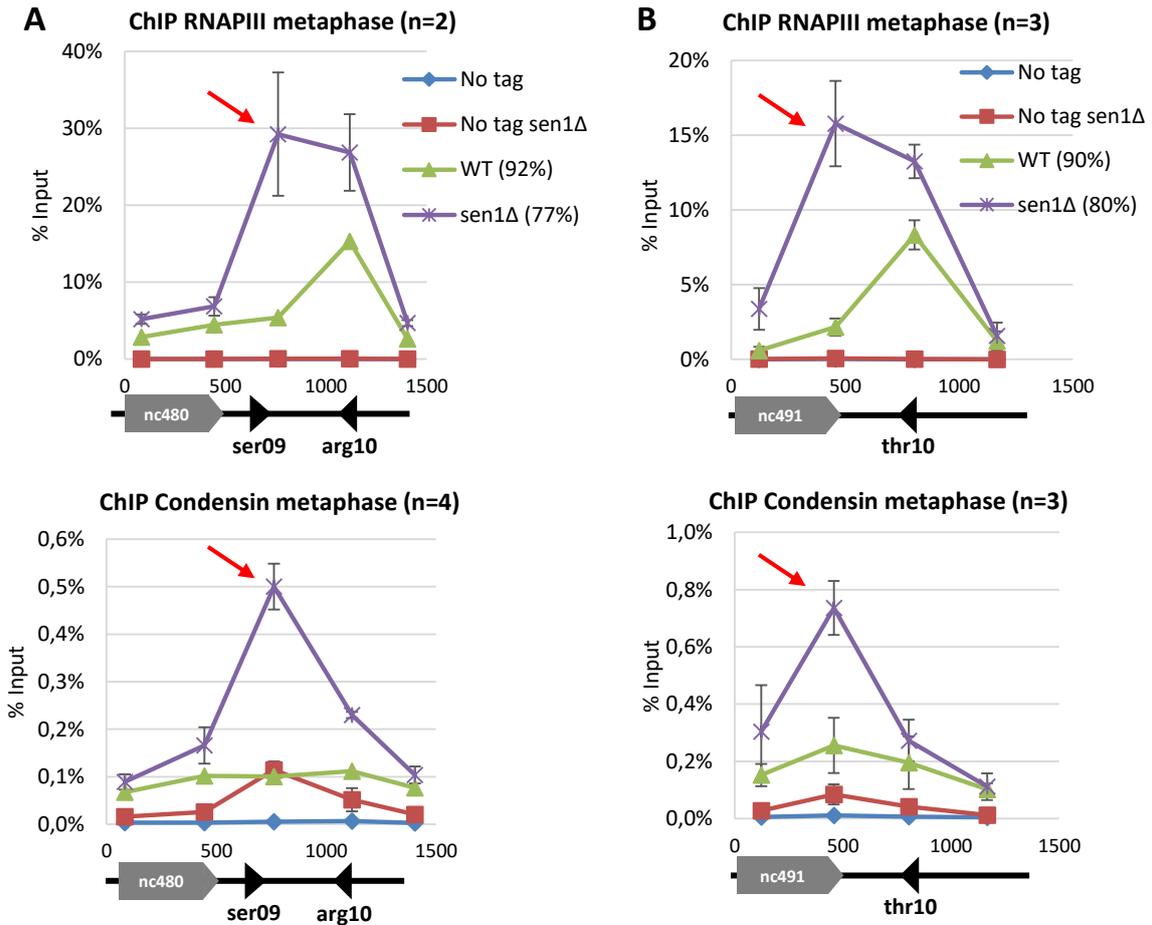
In Chapter 2, we demonstrated that Sen1 is required for RNAPIII transcription termination. All these experiments were performed on cycling cells (a population of cycling cells in fission yeast is composed of around 80% of cells in G2, 10% of cells in S-phase and 10% of mitotic cells). We showed that RNAPIII accumulates over and downstream of tRNA genes when Sen1 is missing (Figure 4 - publication). Here we tested whether RNAPIII also occupies a wider domain around tRNA genes in the absence of Sen1 in cells synchronized in metaphase.

We analysed Rpc37 occupancy in cells synchronized in metaphase using the *cdc2as-M17* system in the presence or absence of Sen1. Using ChIP-qPCR, we detected an accumulation of RNAPIII at tRNA genes and 3' of tRNA genes in the absence of Sen1, at two different loci (the same tested in cycling cells; see Figure 4E of the publication) (Figure 26AB), consistent with transcription termination defects. Note however that the experiments were not carried out in a way that would allow us to determine whether RNAPIII accumulates to the same extent in cycling and in mitotic cells when Sen1 is missing. Such a comparison is not possible because the sonication protocols (fragmentation of chromatin) that we used were different between experiments. Differences in power and time of sonication impacts the resolution (size of DNA fragments) and the stability of the protein-DNA binding, thus affecting enrichment percentages.

These results suggest that Sen1 is required for RNAPIII transcription termination in G2 and in mitosis. We therefore asked whether RNAPIII accumulation in mitosis when Sen1 is missing could explain the accumulation of condensin at tRNA genes in mitosis.

### 3.2.2. The accumulation site of condensin coincides with the peak of the RNAPIII domain in *sen1Δ*

An interesting observation we made is that condensin tends to accumulate where the accumulation of RNAPIII is maximum (Figure 26AB). This overlap between RNAPIII and condensin downstream of tRNA gene terminators suggest that transcription termination defects might be responsible for condensin accumulation in the absence of Sen1. We already showed in Chapter 2 that RNAPIII at 3' of tRNA genes correspond to those RNAPIII that readthrough the terminator in the absence of Sen1. We demonstrated that introducing strong terminators at *arg10* (*arg10-23T*) and *thr10* (*thr10-20T*) was sufficient to suppress the accumulation of RNAPIII downstream of the



**Figure 26: Condensin peak correlates with the 3' flank of the RNAPIII domain in the absence of Sen1.** ChIP-qPCR analysis of Rpc37 and Cnd2 in cells synchronized in metaphase using the *cdc2as-M17* system, in the presence or absence of Sen1. **(A)** Scanning of the *ser09-arg10* locus. ChIP-qPCR Rpc37: 1 biological replicate (No tag and WT) and mean  $\pm$  standard deviation from 2 biological replicates (No tag *sen1Δ* and *sen1Δ*). ChIP-qPCR Cnd2: 1 biological replicate (No tag and WT) and mean  $\pm$  standard deviation from 4 biological replicates (No tag *sen1Δ* and *sen1Δ*). **(B)** Scanning of the *thr10* locus. Mean  $\pm$  standard deviation from 2 biological replicates (No tag and WT) and 3 biological replicates (No tag *sen1Δ* and *sen1Δ*). Mitotic indexes are expressed in percentage between brackets. Results shown Figure 27 and 28 come from the same experiments, they were split to differentiate the messages.

terminator in the absence of Sen1 and readthrough transcripts (Figure 6 of the publication). Note that these experiments were performed in cycling cells.

We asked therefore whether RNAPIII transcription termination defects could underlie the accumulation of condensin in the absence of Sen1.

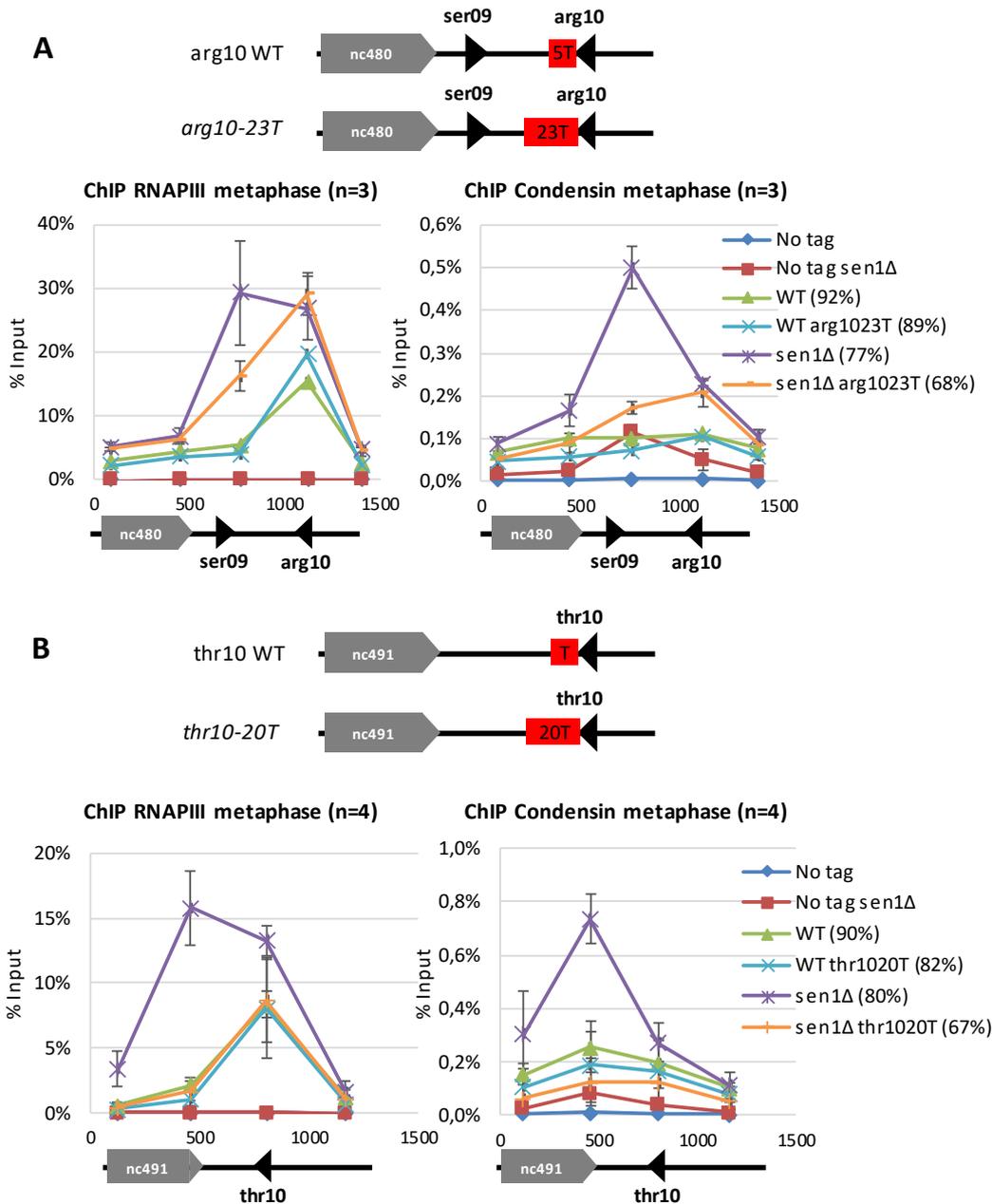
### 3.2.3. RNAPIII that readthrough the primary terminator in the absence of Sen1 are responsible for positioning condensin 3' of RNAPIII-transcribed genes

Based on our results, we hypothesized that in the absence of Sen1 RNAPIII that override tRNA gene terminators and accumulate downstream of terminators determine the positioning of condensin 3' of tRNA genes. We used the super terminator mutants (*arg10-23T* and *thr10-20T*) to test whether suppressing the termination defect in the absence of Sen1 could be sufficient to suppress also the accumulation of condensin.

We introduced the *arg10-23T* and *thr10-20T* mutations in tagged Rpc37-FLAG and Cnd2-GFP *sen1Δ* cells. We synchronized cells in metaphase using the *cdc2as-M17* method. The presence of strong terminators at *arg10* and *thr10* suppressed the accumulation of RNAPIII downstream of terminators in mitosis (Figure 27AB). At *arg10*, the reduction of the size of the RNAPIII peak in *arg10-23T sen1Δ* cells was sufficient to abolish the accumulation of condensin (Figure 27A). At *thr10*, the complete suppression of RNAPIII accumulation also abolished the accumulation of condensin in *thr10-20T sen1Δ* cells (Figure 27B). Compare violet (*sen1Δ*) and orange (*arg10-23T sen1Δ* or *thr10-20T sen1Δ*) lines. Graphs shown in Figures 26 and 27 come from the same experiment. I split the data in two figures to simplify the presentation of the different observations.

These results show that we managed to alter the positioning of condensin (accumulation or not) simply by changing the size of the domain occupied by RNAPIII. These results confirm that it is not the rate of transcription or the fact that RNAPIII transcribes a longer tRNA that explains the accumulation of condensin in the vicinity of RNAPIII-transcribed genes in the absence of Sen1. Instead, our results indicate that the size of the region occupied by RNAPIII is a key determinant in the positioning of condensin in this mutant. Finally, these observations confirm the conclusions that we had inferred from our results in Chapter 1 that lack of Sen1 directly impacts the positioning of condensin by impacting RNAPIII transcription.

It is interesting to note that at *arg10*, RNAPIII still accumulates over the *arg10* transcription unit in the presence of the strong terminator when Sen1 is missing (see Discussion of the publication). However, our results indicate that this accumulation of RNAPIII over a smaller distance is not sufficient to impact the positioning of condensin. This could be explained by two different hypotheses: 1) only RNAPIII molecules that traverse the *arg10* terminator acquire a feature that



**Figure 27: Strengthening the terminator in the absence of Sen1 suppress the termination defect and the accumulation of condensin.** (A) Scheme showing the *arg10-23T* mutant in which the primary terminator 5T was replaced by 23T. ChIP-qPCR of Rpc37 and Cnd2 in cells tagged for both proteins, synchronized in metaphase using the *cdc2as-M17* system. ChIP-qPCR Rpc37: 1 biological replicate (No tag, WT and WT *arg10-23T*) and mean  $\pm$  standard deviation from 2 biological replicates (No tag *sen1Δ*, *sen1Δ* and *sen1Δ arg10-23T*). ChIP-qPCR Cnd2: 1 biological replicate (No tag, WT and WT *arg10-23T*), mean  $\pm$  standard deviation from 4 biological replicates (No tag *sen1Δ* and *sen1Δ*) and 3 biological replicates (*sen1Δ arg10-23T*). (B) Diagram showing the *thr10-20T* mutant in which the primary terminator (T-rich region) was replaced by consecutive 20T. ChIP-qPCR of Rpc37 and Cnd2 in cells tagged for both proteins, synchronized in metaphase using the *cdc2as-M17* system. ChIP-qPCR Rpc37: mean  $\pm$  standard deviation from 2 biological replicate (No tag, WT and WT *thr10-20T*), 3 biological replicates (No tag *sen1Δ* and *sen1Δ*) and 4 biological replicates (*sen1Δ thr10-20T*). ChIP-qPCR Cnd2: mean  $\pm$  standard deviation from 2 biological replicate (No tag, WT and WT *thr10-20T*), 3 biological replicates (No tag *sen1Δ* and *sen1Δ*) and 4 biological replicates (*sen1Δ thr10-20T*). Mitotic indexes are expressed in percentage between brackets. Results shown Figure 27 and 28 come from the same experiments, they were split to differentiate the messages.

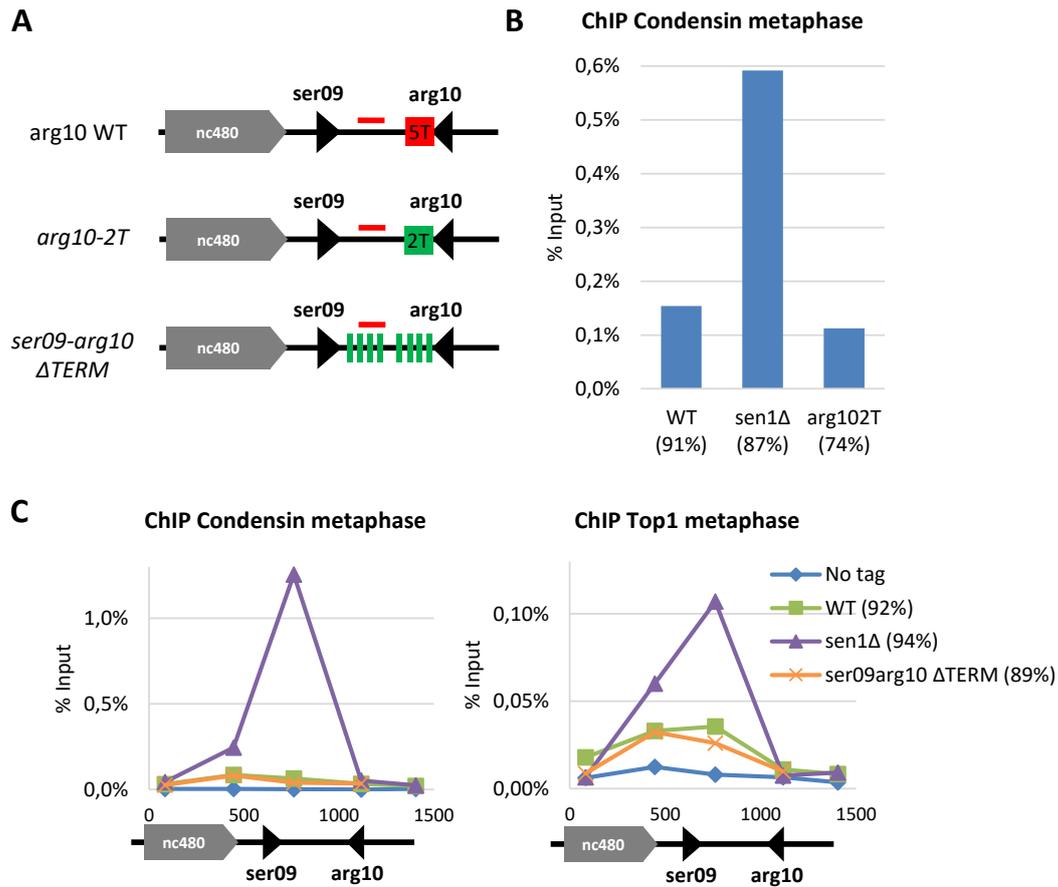
allow them to impact condensin, or 2) a small peak of RNAPIII accumulated over *arg10* is not sufficient to impact condensin. I rather favour the latter, based on my results and current models of condensin blockage by large domains of DNA-bound proteins. At *thr10* it is not clear whether in the presence of the strong terminator RNAPIII still accumulates over *thr10* in the absence of Sen1. I should repeat the experiment to improve the data and their statistics. In the Discussion of this chapter, I will discuss in greater depth the impact of the size of the domain occupied by RNAPIII on the accumulation of condensin.

### 3.2.4. Readthrough transcription at RNAPIII-transcribed genes is not sufficient to impact condensin

We asked whether termination readthrough is sufficient to create the accumulation of condensin.

We recreated RNAPIII readthrough transcription by another mean to test whether it was sufficient to trigger the accumulation of condensin. We used the mutant of the tRNA gene *arg10* already presented in Chapter 2, named *arg10-2T*, in which the primary terminator (5T) has been mutated into a weak terminator (2T) (Figure 28). We showed previously that this mutant accumulates readthrough transcripts, in interphase at least (Figure 24A). However, we showed that RNAPIII does not accumulate either on the gene or downstream in this mutant (Figure 24A). If the production of readthrough transcripts in the absence of Sen1 explains the accumulation of condensin at a subset of tRNA genes, re-creating readthrough transcription with the *arg10-2T* mutant should generate an accumulation of condensin. Contrary to this hypothesis, the levels of condensin observed by ChIP-qPCR in mitotic cells remained unchanged in the *arg10-2T* mutant (Figure 28B). This suggests that RNAPIII readthrough transcripts are not sufficient to trigger the accumulation of condensin.

At the time we engineered the *arg10-2T* mutant, we expected all transcribing RNAPIII readthrough the weak 2T terminator. However, we obtained a modest accumulation of readthrough tRNA transcripts. To explain this situation, we thought at that time that RNAPIII downstream of the 2T terminator could encounter other alternative terminators that may promote its detachment from chromatin, resulting in low levels of readthrough transcripts. For example, 16 bp downstream of the primary terminator of *arg10* there is a T-rich sequence (TTTATTT) that could likely promote termination of RNAPIII that already bypassed the 2T terminator. We therefore decided to mutate all putative sequences that could potentially act as secondary terminators for RNAPIII initiating at both *arg10* and *ser09*. In total we mutated the primary terminators of *ser09* and *arg10* genes as well as 3



**Figure 28: Readthrough transcription at tRNA genes is not sufficient to impact condensin binding. (A)** Diagram showing the two terminator mutants of the tRNA gene *arg10*. In the *arg10-2T* mutant, the primary 5T (TTTTT) terminator (red box) of *arg10* was replaced by 2T (TT) (green box). In the *ser09-arg10ΔTERM* strain the primary 5T terminators and the alternative terminators of *ser09* and *arg10* (8 in total shown as green bars) have been mutated. The red bar designs the location of the qPCR site tested in (b). **(B)** ChIP-qPCR analysis of Cnd2 at *arg10* in the indicated strains (1 biological replicate). **(C)** ChIP-qPCR analysis of Cnd2 and Top1 in cells tagged for both proteins in the indicated strains. Scanning of the *ser09-arg10* locus (1 biological replicate). ChIP-qPCR experiments in (a) and (b) have been performed in metaphase cells synchronized using the *cdc28-M17* system. Mitotic indexes are expressed in percentage between brackets.

alternative terminators on both strands (i.e 8 putative terminators in total) to generate the *ser09-arg10ΔTERM* mutant. We synchronized cells in mitosis and assessed condensin and Top1 binding in this mutant using CHIP-qPCR but observed no change in the amount of condensin or Top1 in the vicinity of the *arg10-ser09* locus (Figure 28C). Because condensin was not impacted, we did not pursue further the analysis of the *ser09arg10ΔTERM* mutant and as a result we do not know whether the accumulation of readthrough transcripts or RNAPIII was indeed greater in this mutant compared to the *arg10-2T* mutant. This result suggests that the production of readthrough transcripts at RNAPIII-transcribed genes does not explain in itself the accumulation of condensin observed in the absence of Sen1.

Based on our results shown in Chapter 2, we now think that in both mutants (*arg10-2T* and *ser09arg10ΔTERM*), Sen1 is destabilizing the RNAPIII molecules that readthrough weak terminators and promote their termination and detachment from the template. Consistent with this, RNAPIII does not accumulate in the *arg10-2T* mutant (Figure 24B).

### 3.2.5. Cohesin accumulates at RNAPIII-transcribed genes in the absence of Sen1

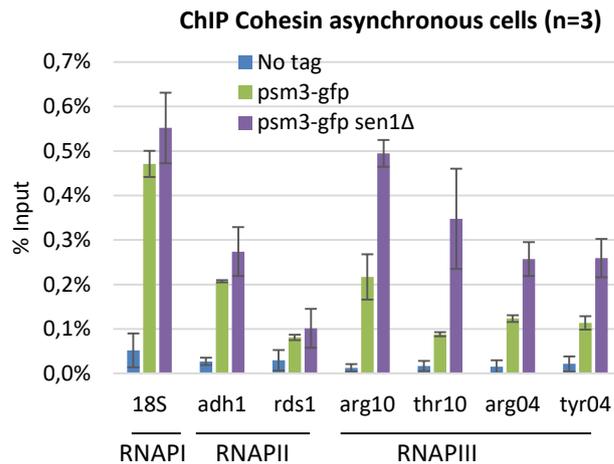
Together our results suggest that Sen1, by modulating the quality of RNAPIII transcription termination can regulate the positioning of condensin in the vicinity of RNAPIII-transcribed genes.

We assessed whether the function of Sen1 in RNAPIII transcription impacts specifically condensin or whether it could also regulate the positioning of the other SMC complex cohesin. We assessed the binding of cohesin in the absence of Sen1 in asynchronous cells and we observed a specific enrichment of the subunit Psm3/Smc3 at RNAPIII-transcribed genes (Figure 29). This suggest that Sen1 could also regulate cohesin binding and its effect is not specific to condensin.

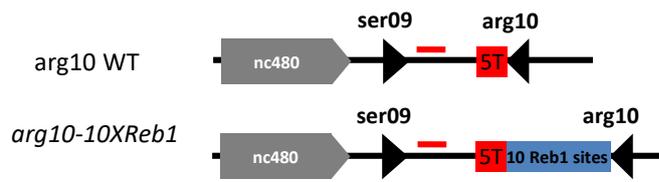
### 3.2.6. Introducing 10 Reb1 sites downstream of a tRNA gene is not sufficient to impact condensin

We reasoned that if RNAPIII accumulated 3' of tRNA genes in the absence of Sen1 is sufficient to regulate the positioning of condensin, then re-creating the RNAPIII accumulation by another mean should also impact condensin.

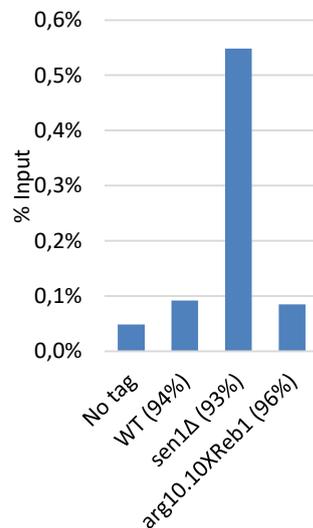
Reb1 is a DNA binding protein that was shown to act as a roadblock for RNAPI and RNAPII to trigger termination (Colin et al., 2014; Zhao et al., 1997). *In vitro* RNAPII accumulates at a Reb1 site in a Reb1-dependent manner (Colin et al., 2014). NET-seq of RNAPII in *S. Cerevisiae* revealed that RNAPII pauses in a Reb1-dependent manner at natural termination sites containing a unique Reb1 site (Colin et al., 2014). We wondered whether Reb1 could also pause RNAPIII in fission yeast. To test this, we introduced 10 Reb1-binding sites between *arg10* and its 5T terminator (Figure 30). The Reb1



**Figure 29: The SMC cohesin accumulates in the absence of Sen1 at RNAPIII transcribed genes.** ChIP-qPCR analysis of Psm3 (Smc3 subunit of cohesin) in the presence or absence of Sen1 at the indicated loci in cycling cells (mean  $\pm$  standard deviation from 3 biological replicates). RNAPI: RNA polymerase I-transcribed genes. RNAPII: RNA polymerase II-transcribed genes. RNAPIII: RNA polymerase III-transcribed genes.



**ChIP Condensin metaphase arg10**



**Figure 30: Introducing 10 Reb1 binding sites downstream of the tRNA gene arg10 does not impact condensin binding.** Scheme showing the locus ser09-arg10. In the strain *arg10-10XReb1*, 10 Reb1 binding sites (170 bp) have been inserted between the arg10 transcription unit and its terminator. The red bar indicates the qPCR primer. ChIP-qPCR analysis of Cnd2 in cells synchronized using the *cdc2as-M17* system, in the indicated strains (1 biological replicate). Mitotic indexes are shown in brackets.

consensus binding sequence in fission yeast contains 17 nucleotides (GTGCATTACCCTTACCT) (Zhao et al., 1997). We named this strain *arg10-10XReb1*.

We measured condensin accumulation in metaphase cells in this strain but did not detect any change. There again, we did not pursue the analysis of this strain further and we did not check whether Reb1 actually binds to its binding sites or whether Reb1 binding is indeed sufficient to trigger the accumulation of RNAPIII. Several explanations could account for this negative result. It may be that Reb1 is hardly expressed in fission yeast, so Reb1-binding sites are maybe not fully occupied by Reb1. In this case we could overexpress Reb1 under a thiamine-inducible promoter. Another possibility is that RNAPIII is blocked by Reb1, and in turn, Sen1 acts to terminate RNAPIII transcription at these artificial pausing sites. In this case, we could try to delete Sen1 and test whether in the absence of Sen1, RNAPIII accumulation is further increased by Reb1 or whether the position of the domain occupied by RNAPIII changes. An alternative explanation is that RNAPIII pauses over *arg10* when it encounters Reb1, in this case the domain occupied by the RNAPIII would be around 100 bp (the size of the *arg10* transcription unit). When we scan RNAPIII occupancy at loci where condensin accumulates (*arg10*, *thr10*, *tyr4*) in *sen1Δ*, we observe that RNAPIII accumulation extends up to 350-400 bp. So, it might be that 100 bp of RNAPIII accumulation is not sufficient to create an accumulation of condensin. To improve this situation, we could move further downstream the Reb1 sites or increase the number of Reb1-binding sites.

### 3.3. Discussion

Understanding condensin distribution and positioning on chromosomes is key to decipher the chromosome organizing function of this motor protein. Increasing amount of evidence reveals a striking but poorly understood link between high transcription rates and condensin positioning.

Throughout this manuscript, we investigated how the RNAPIII factor Sen1 could regulate condensin positioning at RNAPIII-transcribed genes. In the first chapter we described how Sen1 regulates condensin binding in mitosis specifically at RNAPIII-transcribed genes. In the second chapter, we provided evidence that Sen1 is required for efficient RNAPIII transcription termination. Finally, we showed in this last chapter that strong terminator sequences compensate the transcription termination defects in the absence of Sen1 and this is sufficient to suppress the accumulation of condensin. Our results therefore demonstrate that Sen1 regulates the distribution of condensin around RNAPIII-transcribed genes by controlling RNAPIII transcription termination.

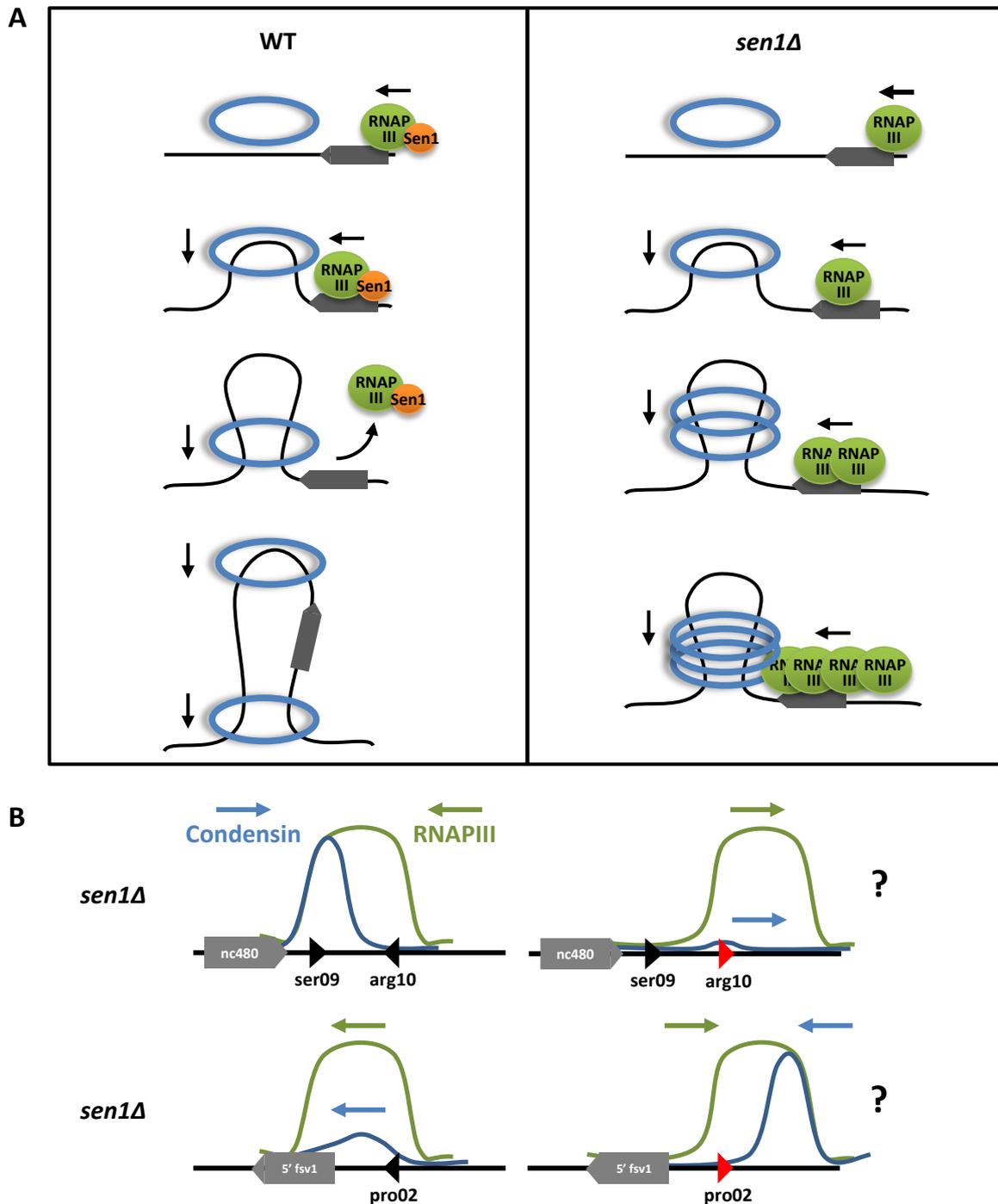
In this section I propose a model in which RNAPIII act as an obstacle for condensin translocation, provided that RNAPIII occupies a sufficiently large domain with a sufficiently high density. I will discuss current models that consider DNA-bound proteins as barriers for SMC translocation. Finally, I

will propose experiments in which we could use our tRNA genes as positioning device to address unsolved questions about the function and dynamics of condensin.

### 3.3.1. Speculative model: High RNAPIII density over tRNA loci in the absence of Sen1 creates a barrier for condensin

We showed in this chapter that strengthening the RNAPIII terminator in the absence of Sen1 suppressed the accumulation of RNAPIII downstream of tRNA gene terminators and that this is sufficient to prevent the accumulation of condensin locally. This is a striking result that suggests that RNAPIII occupancy (i.e. the abundance and the length of the domain occupied by RNAPIII) on chromatin does determine the local positioning of condensin to some extent. We propose a model in which RNAPIII could constitute a steric barrier for condensin translocation (Figure 31A). This model predicts also that the translocation of condensin at a given locus is defined and predominantly unidirectional, as explained below.

To analyse whether RNAPIII accumulation could constitute a block for condensin, I would like to study why tRNA genes are not highly enriched condensin sites in WT cells but they become strong condensin sites in the absence of Sen1. As in WT cells we fail to detect significant condensin enrichments around tRNA genes (Figure 21A and Figure 26), we speculate that even if tRNA genes are highly transcribed, the abundance and the length of the region occupied by RNAPIII is not enough to create a condensin peak. The length of the tRNA gene *arg10* from the TATA box to the terminator comprises 120 bp; *thr10*, 133 bp and *tyr04*, 126 bp. RNAPIII density in ~130 bp in WT cells is therefore not enough to act as an obstacle for condensin. We can conclude that at least in our system, high rates of RNAPIII transcription are not sufficient to block condensin in the vicinity of tRNA genes. However, when Sen1 is deleted, RNAPIII occupancy increases and occupies a wider domain that is sufficient to explain the emergence of the condensin peak. In the absence of Sen1, the domain occupied by RNAPIII is around 350-400 bp in length (Figure 4E of the publication and Figure 26). We demonstrated at *arg10* and *thr10* that reducing the size of the RNAPIII domain is sufficient to abolish the accumulation of condensin. Our results are therefore consistent with the idea that the abundance of RNAPIII and the length of the domain it occupies in the absence of Sen1 determine whether or not RNAPIII can act as a barrier for condensin translocation (Figure 31A). However, it is important to note that these two parameters are not enough to explain condensin accumulation. We did indeed find examples of tRNA genes for which lack of Sen1 hardly impacted the accumulation of condensin, even if the domain occupied by RNAPIII was both dense and large (for example *pro02* see Figure 2A and 5D of the publication and 17A). This suggests that RNAPIII accumulation over large



**Figure 31: Speculative model for condensin blockage by RNAPIII accumulation in the absence of Sen1.** (a) In WT cells, Sen1 assists RNAPIII transcription termination, RNAPIII detaches from the template at the terminator, allowing condensin translocation through the RNAPIII transcription unit. In the absence of Sen1 (*sen1Δ*), RNAPIII fails to terminate and accumulates at tRNA genes and downstream, this dense and large RNAPIII accumulation impedes condensin translocation, that accumulates 3' of the RNAPIII transcription unit. Condensin is shown in light blue, the gray box represents an RNAPIII transcription unit, RNA polymerase III (RNAPIII) is shown in green and the helicase Sen1 in orange. (b) Proposed experiments to test the influence of directionality on the stalling of condensin by RNAPIII in *sen1Δ*. Schematic RNAPIII and condensin curves are represented at two different loci (*ser09-arg10* and *pro02*). The graphs on the left schematize RNAPIII and condensin accumulation observed in the absence of Sen1. On the right, *arg10* and *pro02* transcription units have been turned and we ask whether these inversion of gene orientation in one case suppress the accumulation of condensin (at *arg10*, gene transcription and condensin translocation would be codirectional) or create an accumulation of condensin (at *pro02*, gene transcription would encounter condensin translocation in a head-on conformation).

domains was not sufficient to impact the distribution of condensin and that additional features must contribute.

We speculate that the orientation of tRNA transcription relative to the direction of condensin translocation could be an important parameter to take into account. In particular, we speculate that if RNAPIII transcription is codirectional with condensin translocation, then condensin would be able to overtake the RNAPIII obstacle and reach the 3' of the tRNA gene. However, if the tRNA gene and the translocation of condensin are in a head-on conformation, RNAPIII blocked over and downstream of the tRNA gene are constantly hindering the advance of condensin through the tRNA transcription unit.

To sum up, we propose a model in which RNAPIII density, size of RNAPIII accumulation domain and transcription directionality could determine condensin positioning.

Other works have suggested that tight DNA-bound proteins could act as blocks for SMC sliding. In these cases (discussed individually below), condensin peaks would reflect sites where condensin accumulates after being blocked or slowed down by obstacles. In other words, condensin 'passively' accumulates as a side effect of translocation through crowded chromatin. This view opposes the models of Sutani (Sutani et al., 2015) and Yanagida (Nakazawa et al., 2019), which claim that transcription is detrimental for chromosome condensation, as it produces ssDNA or RNA that needs to be removed by condensin in order to faithfully achieve condensation. However, why ssDNA or chromatin-associated RNA would be harmful for chromosome condensation and segregation is not clear from these models. In these models, the peaks of condensin at highly-expressed genes would reflect sites that require condensin to correct a defect. Said differently, condensin would be 'actively' recruited to these sites. Our results agree with the obstacle model for condensin translocation.

In the following sections, I will discuss different cases where DNA-bound proteins act as blocks for SMC sliding. I will confront our model to these examples and finally propose experiments that could help to test our model and gain further insights into the mechanism of condensin action.

### 3.3.2. Nucleosomes as barriers for SMC loading

In yeast, nucleosomes have been shown to oppose the recruitment of both condensin and cohesin. It was shown that chromatin remodelling is required to generate a nucleosome-free region at RNAPII-transcribed genes to facilitate condensin and cohesin loading (Muñoz et al., 2019; Toselli-Mollereau et al., 2016). Similarly, our data are consistent with the idea that RNAPIII constitutes a barrier for condensin, and Sen1 is required for RNAPIII removal to facilitate the function of condensin. What is contrasting is that nucleosome occupancy and condensin enrichment are inversely correlated, but in our model RNAPIII and condensin accumulation are positively correlated.

An explanation for this divergence could be that in the case of nucleosomes acting as barriers, they impede the recruitment of SMC, whilst in our system RNAPIII would block SMC translocation, thereby hindering the function of SMC but not its loading. A way to test whether the translocation but not the loading of SMC is impacted in the *sen1Δ* mutant would be to generate a mutant of condensin that is able to load but not to translocate along chromatin. If this condensin mutant unable to translocate were not able to accumulate in front of the RNAPIII barrier in the absence of Sen1, then we could attribute the accumulation of condensin in the vicinity of RNAPIII to an impediment of translocation. If the condensin mutant unable to translocate still accumulates at RNAPIII-transcribed genes in the absence of Sen1 we could conclude that the accumulation of condensin at RNAPIII-transcribed genes is due to increased loading locally. In this case, we should revisit our model of RNAPIII as a barrier for condensin and think about RNAPIII accumulation as a situation in which chromatin is more accessible for condensin recruitment. In conclusion, in our model RNAPIII constitute a barrier for condensin, as nucleosomes for SMC, and Sen1 and chromatin remodellers are required to remove these barriers to facilitate the function of condensin.

### 3.3.3. CTCF as directional barriers to cohesin

The function of CTCF as a directional barrier for cohesin movement is the best described case in which a tightly DNA-bound protein is able to block SMC sliding (Busslinger et al., 2017). It has been proposed that cohesin acts by extruding loops of DNA until it encounters convergently-oriented CTCF sites (Rao et al., 2014). Interesting experiments showed that inverting or deleting CTCF-binding sites results in decrease cohesin binding and reduced DNA-looping interactions (de Wit et al., 2015; Guo et al., 2015). The CTCF binding site contains 20 bp (Kim et al., 2007).

In our model, we could test these aspects deduced from CTCF/cohesin interactions: directionality of the block and requirement for two convergent loci. To test whether directionality is important for RNAPIII to block condensin, we could reorient the tRNA gene *arg10* and place it codirectional to *ser09* (Figure 31B). In this strain, we would still expect to see an accumulation of RNAPIII due to defective termination in the absence of Sen1. However, if directionality of transcription is an important parameter to explain the accumulation of condensin, we would expect RNAPIII accumulation not to block condensin translocation or to just generate a mild slowing down of its progress, reflected by no condensin accumulation or a small peak (Figure 31B). Alternatively, we could take a tRNA gene that exhibits a strong RNAPIII transcription termination defect (such as *pro02*) and reverse its orientation to test whether it becomes able to block condensin translocation (Figure 31B).

Regarding the need for one or two RNAPIII-transcribed genes to block condensin in the absence of Sen1, we could imagine that condensin extrudes loops of DNA the same way than cohesin, i.e. two DNA strands slide through the condensin ring. In this case, translocation would stop when condensin encounters two tRNA genes, distantly separated, in which RNAPIII are densely accumulated. We could test this possibility by performing chromosome conformation capture techniques in the Sen1 mutant. We expect to detect increased frequency of interactions between tRNA gene loci that become strong condensin binding sites in the absence of Sen1. The ChIA-PET technique would be even more informative, since it would allow us to detect interactions between sites that are bound, in this case, by condensin. It would be important to reach enough resolution to detect changes in frequency interactions between tRNA genes. Using ChIA-PET, one could expect to observe that two tRNA genes in which condensin accumulates in the absence of Sen1 interact (in this case one could think on two strands of DNA sliding inside the condensin ring) or in the contrary one could observe that an isolated tRNA gene can block condensin, but it does not interact with another tRNA gene (in this case, one could imagine that a single DNA strand slides inside the condensin ring).

Our observation that cohesin accumulates in the absence of Sen1 at RNAPIII transcribed genes (Figure 12), suggest that RNAPIII could constitute a block for the sliding of SMC in general. Since CTCF is not present in *S. pombe*, cohesin accumulates predominantly at convergent genes (Lengronne et al., 2004; Mizuguchi et al., 2014). The fact that RNAPII could push cohesin to 3' end of genes supports the idea that RNAPIII could also constitute a steric block for cohesin. We could analyse whether cohesin accumulation is also correlated with RNAPIII accumulation in the Sen1 mutant. To do this, we could test cohesin enrichment in the mutants in which we modulate RNAPIII transcription: the *arg10 TATA-less* mutant in which Tbp1-dependent recruitment of RNAPIII is strongly reduced, the *sen1Δ sfc3-1* mutant in which TFCIII-dependent recruitment of RNAPIII is reduced, *sen1Δ arg10-23T* and *sen1Δ thr10-20T* in which RNAPIII accumulated downstream of terminators are suppressed.

#### 3.3.4. The telomere-binding factor Rap1 as a condensin stalling factor

An interesting work in budding yeast reveals a role for condensin in folding dicentric chromosomes, that contributes to favour break at telomere fusions upon cytokinesis (Guérin et al., 2019). Abnormal dicentric chromosomes form when telomeres fuse and it has been shown in budding yeast that they break at the fusion to restore a normal karyotype. This study shows that introducing arrays of at least 6 binding sites for the telomere-binding factor Rap1 within dicentrics is sufficient to create a breakage hotspot in a mechanism dependent on condensin. Under Smc2 depletion the breakage frequency at Rap1 sites diminishes, and chromosomes break instead in the middle part of the intercentromeric region (Guérin et al., 2019). The authors propose a model in

which condensin folds dicentric chromosomes and it is stalled at kinetochores on one extreme and at telomeres on the other. The tight Rap1-DNA interaction would create a roadblock for condensin at telomeres and promote the location of Rap1 covered telomere fusions at abscission sites. Interestingly, the authors show that inserting LacO arrays within a dicentric establishes also a breakage hotspot, suggesting that LacI like Rap1 is able to stall condensin. In order to better study the action of Rap1 as a roadblock for condensin, the authors manipulated Rap1 and LacO sites. An elegant experiment shows that a Rap1 bound array containing 16 binding sites (300 bp/100 nm) creates a breakage hotspot, however when 35 bp LacO arrays are inserted in between Rap1 sites, breakage does not occur. Strikingly, the expression of LacO-bound LacI transformed the same array into a strong breakage hotspot. These experiments suggest that a close spacing of tightly bound proteins is crucial to favour breakage, likely by blocking condensin. This model is very reminiscent of the model we proposed to explain the impact of RNAPIII transcription termination defects on the accumulation of condensin.

These experiments also demonstrated that the minimal size of the Rap1-bound array that would block condensin is 300 bp. This is in agreement with our estimated RNAPIII domain (between 350-400 bp) required to block condensin in the Sen1 mutant. On the other hand, in our experiment in which we added 10 Reb1 binding sites to try to block RNAPIII progression, one could imagine that Reb1 could directly block condensin. However, 10 Reb1 binding sites constitute 170 bp, which it might not be enough to create a steric hindrance for condensin. We could test whether inserting 20 Reb1 binding sites is able to directly block condensin.

Based on this work, many experiments could be done to test our model. For instance, inserting Rap1 or LacO sites at different distances downstream of tRNA genes and check whether they can directly stall condensin or to indirectly stall condensin by blocking RNAPIII. As proposed for Reb1-binding sites experiments, it may be necessary to perform these experiments in Sen1 deleted strains.

### 3.3.5. The permeable moving barrier model from bacterial SMC to explain RNAPIII/condensin interactions in the Sen1 mutant

Our results suggest that RNAPIII constitute a barrier for condensin (Figure 31A) and the current model that best fits with our observations is the recently proposed permeable moving barrier model in *B. subtilis* (Brandao et al., 2019). It suggests that by increasing the density of transcribing RNAP beyond a critical value, actively transcribed loci will become directionally impermeable to condensin translocation. Condensin translocation through a locus would depend on gene orientation, transcript length and RNAP density. Thus, while individual RNAP have little effect on the progress of condensin translocation, long, highly transcribed genes could significantly impede the extrusion process. Our

comparison between RNAPIII accumulation in WT and *sen1Δ* cells already agree with the bacterial model in that the amount and the size of the RNAPIII accumulation domain are determinant to generate an accumulation of condensin (see above section 3.3.1). In addition, in our tRNA genes model we showed that reducing RNAPIII loading with the *sfc3-1* mutant (Figure 4d) results in reduced condensin accumulation in the absence of Sen1. This is in good agreement with the moving barrier model, since reducing RNAPIII density reduces the impact on condensin. To properly confirm this, we should check in the *sen1Δ sfc3-1* mutant that RNAPIII still accumulates in the same length (around 350-400 bp) and that only the abundance is decreased. To test whether gene orientation is also important in our model, we previously proposed (see above section 3.3.3) to change the orientation of candidate tRNA genes and check the effect of the directionality on condensin accumulation in the *sen1Δ* mutant (Figure 31B). Finally, to test the influence of transcript length we could play with the strong terminator mutants. We could move further downstream the 23T terminator at *arg10* in the Sen1 mutant and check whether changing the length of the RNAPIII accumulation domain impacts condensin positioning. We could expect, based on our previous results (Figure 27AB), that a larger RNAPIII accumulation domain would create a stronger accumulation of condensin.

The authors of the study in bacterial SMC, developed a quantitative model in which they take into account the mentioned parameters and, interestingly, they consider that RNAP behave as permeable moving barriers (Brandao et al., 2019). This new parameter (permeability) helps to better fit their model to the experimental observations. Permeability means that translocating condensin can bypass transcribing RNAP. They speculate that condensin could overcome large steric barriers either by large opening of the ring or by transient disengagement of the topological embrace of DNA. Permeability of RNAP to loop extrusion also suggests that SMC should be able to effectively bypass other large steric barriers and that they may have different permeabilities. For instance, cohesin seems to be strongly blocked by CTCF. In the case of TADs formation by loop extrusion, the observations suggest that CTCF has very low permeability to cohesin sliding. In our tRNA genes model in the absence of Sen1, we could hypothesize that what determines the permeability of RNAPIII to condensin is the strength of RNAPIII-DNA binding. We could imagine two different situations: 1) in the absence of Sen1, defective elongation and termination creates an accumulation of RNAPIII that remain paused at the tRNA gene and hardly move 3' of the tRNA gene. In this case, RNAPIII abnormally and tightly bound to the DNA template could constitute a high impermeable barrier for condensin; 2) in the absence of Sen1 increased number of RNAPIII are bound to tRNA genes, but RNAPIII-DNA binding remains dynamic and RNAPIII progress through tRNA genes at a normal speed, representing a more permeable barrier for condensin. Our finding that tRNA levels are reduced in the absence of Sen1, even if we detect enhanced RNAPIII at tRNA genes (Figure 2-publication), favours the first scenario. To test the hypothesis that RNAPIII are paused at tRNA genes in *sen1Δ* (and could

represent a low permeable barrier for condensin), we tried to measure the strength of the RNAPIII-DNA interaction at the tRNA gene *arg10* in WT and *sen1Δ* cells, to try to see whether the strength of RNAPIII binding to *arg10* is increased in the absence of Sen1. I performed one experiment in which I immunoprecipitated Rpc37-Flag from non-crosslinked whole-cell protein extracts and quantified the amount of the tRNA gene bound by Rpc37 by qPCR. After immunoprecipitation, I washed the precipitated fraction with increasing salt concentration buffers to compare the salt resistance of the Rpc37-*arg10* binding in *sen1+* and *sen1Δ* cells. Unfortunately, this experiment did not work the first time due to high background levels and abnormal qPCR values and I haven't had time to repeat it. We should set up the right conditions for this experiment as it would be helpful to understand whether RNAPIII binds its DNA template more tightly in the Sen1 mutant and how this could affect condensin translocation.

In conclusion, our model of RNAPIII acting as a condensin barrier in *sen1Δ* is strongly supported by the permeable moving barrier model in *B. subtilis*. In this model RNAP density, transcript length and gene orientation could determine the blockage of condensin translocation. This model contributes to the debate about the link transcription-condensin. It favours a view in which the conserved pattern of condensin localization at highly expressed genes could result from a combination of recruiting factors that enrich condensin at specific sites, and the positioning of condensin by RNAP.

Across this discussion I proposed some experiments that could help us to test our speculative model in which RNAPIII accumulation in the absence of Sen1 behave as a barrier for condensin translocation (Figure 31A). I give here a detailed list of the experiments suggested throughout this discussion:

- 1) Generate a condensin mutant able to load but not to translocate to test whether RNAPIII accumulation in the absence of Sen1 facilitates *de novo* condensin recruitment or impacts condensin translocation.
- 2) Reverse tRNA gene orientations to test whether directionality is an important factor for the stalling of condensin by RNAPIII accumulation in the absence of Sen1. This experiment would also reveal whether condensin translocation is unidirectional or not at these sites.
- 3) Insert different binding sites for proteins (such as Reb1, Rap1 or LacI) around tRNA genes, that could potentially block RNAPIII or directly block condensin. Playing with different number of binding sites and different locations of insertion could give a better overview of the stalling of condensin by DNA-bound proteins.

- 4) Playing with the position of strong terminators in the Sen1 mutant to change the size of the RNAPIII accumulation domain and check the impact on condensin accumulation.
- 5) Measure the strength of the RNAPIII-tRNA genes binding in the Sen1 mutant to better describe the state of accumulated RNAPIII and better understand the role of RNAPIII accumulation in determining condensin positioning.
- 6) To perform ChIA-PET in the Sen1 mutant to test whether condensin accumulation at RNAPIII-transcribed genes reflects the link between distant tRNA genes or not. This experiment would help us also to gain insights into the loop extrusion mechanism of condensin.

### 3.3.6. Can we gain insights into condensin function from condensin positioning?

An issue of our tRNA genes model in *sen1Δ* cells is that we have no evidence that condensin accumulation in the absence of Sen1 could have a biological impact in these cells. However, we focused our research on a very concrete and significant observation that is the 'emergence' of condensin accumulation at specific sites. We demonstrated that by manipulating tRNA genes and RNAPIII transcription we gained insights into a transcription-dependent pathway of condensin positioning on chromosomes. Our model is indeed appropriate to study the link between gene transcription and condensin localization. Understanding how condensin is positioned on chromosomes by transcription contributes to better understand the chromosome-organizing activities of condensin.

The next step in my project would be to validate our model at RNAPII-transcribed genes. Does the control of RNAPII transcription termination impact condensin positioning? We created strains to deplete Seb1 (Nrd1), Dhp1 (Rat1/XRN2) and Rna14, to create a defect in RNAPII transcription termination and monitor condensin accumulation 3' of affected genes. The experiments are ongoing.

## Conclusion

How condensin complexes form highly compact and individualized chromosomes efficiently and rapidly in mitosis is a fascinating unsolved question. In the last years genome-wide experiments in different organisms showed a conserved link between gene transcription and condensin binding on chromosomes. Several works addressed this functional link between transcription and condensin, however, there is no current model that could explain how gene transcription contributes to position condensin on chromosomes.

We identified the helicase Sen1 as a transcription-associated factor that regulates condensin at RNAPIII-transcribed genes. During my PhD project I addressed two main questions: 1) What is the role of Sen1 in RNAPIII transcription? 2) How does Sen1 regulate condensin binding at RNAPIII-transcribed genes?

We conclude that: 1) Sen1 is required for RNAPIII transcription termination. Our observations challenged the current models of RNAPIII transcription termination and were published in the EMBO Journal; 2) Sen1 regulates condensin positioning at RNAPIII-transcribed genes by modulating the quality of RNAPIII transcription termination. Based on our results we proposed a model in which lack of Sen1 interferes with RNAPIII removal from the DNA template, resulting in a wide and dense accumulation of RNAPIII that blocks the translocation of condensin in mitosis, thereby creating condensin peaks 3' of RNAPIII-transcribed genes.



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## **Sen1-mediated RNAPIII transcription termination controls the positioning of condensin on mitotic chromosomes**

The condensin complex is a key driver of chromosome condensation in mitosis. The condensin-dependent assembly of highly compacted chromosomes is essential for the faithful transmission of the genome during cell division. Many independent studies have established that gene transcription impacts the association of condensin with chromosomes, but the molecular mechanisms involved are still unclear. This is especially true as a number of sometimes contradictory mechanisms have been proposed so far. Here, we show in *Schizosaccharomyces pombe* that condensin accumulates specifically in the vicinity of a subset of RNA polymerase III-transcribed genes in the absence of the conserved DNA/RNA helicase Sen1. We demonstrate that Sen1 is a cofactor of RNA polymerase III (RNAPIII) required for efficient transcription termination. These results are important because they fundamentally challenge the pre-existing view that RNAPIII terminates transcription autonomously. Strikingly, we show that the RNAPIII transcription termination defects are directly responsible for the accumulation of condensin in the absence of Sen1. This indicates that the quality control of transcription impacts the distribution of condensin on mitotic chromosomes. We propose that above a certain density threshold, the accumulation of RNAPIII constitutes a barrier for the translocation of condensin on chromosomes.

**Keywords** Condensin – RNA polymerase III – Senataxin – Transcription termination

## **L'hélicase Sen1 contrôle le positionnement de condensine sur les chromosomes en régulant la terminaison de la transcription par l'ARN polymérase III**

Le complexe condensine est le moteur de la condensation mitotique des chromosomes, un processus essentiel à la stabilité du génome au cours de la division cellulaire. De nombreuses données publiées indiquent qu'il existe des liens fonctionnels étroits entre le processus de transcription des gènes et le processus d'organisation des chromosomes par condensine. Ces données sont toutefois souvent contradictoires et aucun modèle ne fait actuellement consensus pour expliquer les liens entre transcription et condensine. Au cours de cette thèse, nous avons montré dans la levure *Schizosaccharomyces pombe* que condensine s'accumule spécifiquement à proximité des gènes transcrits par l'ARN Polymérase III en l'absence de l'hélicase à ADN/ARN Sen1. Nous avons utilisé ces observations pour mieux comprendre les liens entre transcription par l'ARN polymérase III et le positionnement de condensine. Nos données montrent que Sen1 est un cofacteur de l'ARN Polymérase III impliqué particulièrement dans la terminaison de la transcription. Ce résultat est important car il démontre que les modèles existants prédisant que l'ARN polymérase III termine de transcrire de façon autonome sont erronés. Nous avons ensuite démontré que les défauts de terminaison de l'ARN polymérase III observés en l'absence de Sen1 suffisent entièrement à expliquer l'accumulation de condensine en ces sites. Cette observation importante démontre que le contrôle de qualité de la transcription est directement impliqué dans le positionnement de condensine sur les chromosomes en mitose. Nos résultats nous permettent de proposer qu'une grande densité en ARN polymérase est un obstacle à la translocation de condensine sur les chromosomes.

**Mots-clés** Condensine – RNA polymérase III – Senataxine – Terminaison de la transcription

# **L'hélicase Sen1 contrôle le positionnement de condensine sur les chromosomes en régulant la terminaison de la transcription par l'ARN polymérase III**

## **Résumé**

Le complexe condensine est le moteur de la condensation mitotique des chromosomes, un processus essentiel à la stabilité du génome au cours de la division cellulaire. De nombreuses données publiées indiquent qu'il existe des liens fonctionnels étroits entre le processus de transcription des gènes et le processus d'organisation des chromosomes par condensine. Ces données sont toutefois souvent contradictoires et aucun modèle ne fait actuellement consensus pour expliquer les liens entre transcription et condensine. Au cours de cette thèse, nous avons montré chez la levure *Schizosaccharomyces pombe* qu'en l'absence de l'hélicase à ADN/ARN Sen1, condensine s'accumule spécifiquement à proximité des gènes transcrits par l'ARN Polymérase III. Nous avons utilisé ces observations pour mieux comprendre les liens entre transcription par l'ARN polymérase III et le positionnement de condensine. Nos données montrent que Sen1 est un cofacteur de l'ARN Polymérase III impliqué dans la terminaison de la transcription. Ce résultat est important car il démontre que les modèles existants qui affirment que l'ARN polymérase III termine de transcrire de façon autonome sont erronés. Nous avons ensuite démontré que les défauts de terminaison de l'ARN polymérase III observés en l'absence de Sen1 suffisent entièrement à expliquer l'accumulation de condensine en ces sites. Cette observation importante démontre que le contrôle de qualité de la transcription est directement impliqué dans le positionnement de condensine sur les chromosomes en mitose. Nos résultats nous permettent de proposer qu'au-delà d'un certain seuil, la densité en ARN polymérases est un obstacle à la translocation de condensine sur les chromosomes.

## **Introduction**

L'organisation du génome dans le noyau cellulaire influence de nombreuses fonctions liées au métabolisme de l'ADN, comme par exemple l'expression des gènes (Fraser and Bickmore, 2007). Les perturbations de l'architecture du génome peuvent conduire à des mutations, des réarrangements chromosomiques, de l'aneuploïdie et à l'apparition de cancers (Corces and Corces, 2016; Fudenberg et al., 2011). Il est donc essentiel de comprendre les mécanismes qui régulent l'organisation du génome au cours de la vie de la cellule.

Depuis une vingtaine d'années, notre compréhension de l'organisation du génome a été grandement facilitée par l'apparition de nouvelles techniques de microscopie comme l'hybridation *in situ* fluorescente (FISH pour 'Fluorescent In Situ Hybridization') et de nouvelles techniques moléculaires de cartographie de la conformation des chromosomes (3C pour 'Chromosome Conformation Capture'). Ces nouvelles techniques ont permis de décrire avec grande précision la réorganisation massive des fibres de chromatine dans le noyau au long du cycle cellulaire (pour revue voir Fraser et al., 2015).

En interphase, les chromosomes sont organisés à plusieurs niveaux. A l'échelle du mégabase (Mb), les chromosomes sont organisés en deux types principaux de domaines structuraux, appelés compartiments A et B (Nagano et al., 2017; Rao et al., 2014). Le compartiment A regroupe des domaines de chromatine accessible où la transcription est active, alors que le compartiment B est associé à une chromatine réprimée. A l'échelle plus locale, les chromosomes s'arrangent en domaines, au sein desquels les interactions sont plus fréquentes qu'avec les régions adjacentes. Ces domaines sont appelés TADs (pour 'Topologically Associated Domains') et ont été décrits dans plusieurs organismes (Dekker and Heard, 2015; Lieberman-Aiden et al., 2009). Aux échelles plus petites, entre 10-100 kilobases (kb), la chromatine forme des boucles qui rapprochent des régions distantes. L'une des fonctions les mieux caractérisées de ces boucles est de regrouper dans l'espace promoteurs et enhanceurs distants (Szalaj and Plewczynski, 2018). En conclusion, les boucles de chromatine constituent l'unité structurelle de l'organisation du génome en interphase. Ces boucles sont soumises à une régulation complexe contrôlant les processus cellulaires essentiels comme la transcription.

Au cours du cycle cellulaire et de la différenciation, les chromosomes subissent de profonds changements morphologiques. A l'entrée en mitose en particulier, les chromosomes sont rapidement et efficacement individualisés et compactés afin de permettre leur mobilisation par le fuseau de microtubules et leur transmission correcte aux deux cellules filles au cours de l'anaphase.

Lorsque les cellules progressent de l'interphase à la mitose, l'organisation en compartiments et en TADs est effacée pour être remplacée par une organisation en grandes boucles de chromatine de l'ordre de 1-10 Mb disposée en hélice autour d'un axe central (Gibcus et al., 2018; Naumova et al., 2013). Chez la levure à fission, il a également été montré que des interactions à courte portée (<100 kb) en interphase sont remplacées par des interactions à longue portée (~1 Mb) en mitose (Kakui et al., 2017; Tanizawa et al., 2017). Les chromosomes mitotiques présentent donc une organisation très uniforme dans laquelle de larges boucles de chromatine constituent l'unité structurelle.

Pour conclure, la boucle de chromatine est l'unité structurelle du chromosome, mais la nature et la taille de ces boucles de chromatine diffèrent significativement entre l'interphase et la mitose.

La réorganisation drastique de l'architecture du génome à chaque division cellulaire nécessite l'action de machineries dédiées. Des expériences biochimiques et génétiques ont conduit à la découverte des complexes SMC (pour 'Structural Maintenance of Chromosomes') en tant que composants majeurs des chromosomes. De nombreuses études ont démontré que le complexe SMC condensine est crucial à la formation des boucles de chromatine pendant la mitose, alors que le complexe SMC cohésine contrôle la formation de boucles de chromatine en interphase (Gibcus et al., 2018; Naumova et al., 2013; Rao et al., 2014, 2017).

Les SMC sont des complexes protéiques conservés depuis les bactéries jusqu'à l'Homme, qui jouent des rôles fondamentaux dans l'organisation, la réplication, la transmission et la préservation de l'intégrité structurale et fonctionnelle des génomes (Hirano, 2012). Ils sont caractérisés : 1) par leur structure en anneau capable d'encercler l'ADN, et 2) par leur activité ATPase (Uhlmann, 2016). Les complexes condensines sont essentiels à la condensation et à la ségrégation des chromosomes au cours de la mitose. L'inactivation de condensine entraîne un défaut de compaction et de résolution des chromosomes. En conséquence, les chromosomes restent emmêlés pendant leur séparation, ce qui conduit à la formation de pont chromatiniens en anaphase (Cuylen et al., 2013; Hirano and Mitchison, 1994; Hirano et al., 1997; Saka et al., 1994; Tada et al., 2011; Woodward et al., 2016). Les complexes cohésines ont été découverts pour leur rôle essentiel dans la cohésion des chromatides sœurs depuis la réplication et jusqu'au début de l'anaphase, afin d'assurer une correcte ségrégation des chromosomes (Michaelis et al., 1997). Des travaux plus récents ont par la suite attribué un rôle important aux cohésines dans l'organisation 3D du génome en interphase (Gibcus et al., 2018; Naumova et al., 2013; Rao et al., 2014, 2017).

Il a été proposé que cohésine et condensine auraient une activité d'extrusion de boucles de chromatine dépendant de l'ATP. Ces complexes seraient capables de capturer une petite boucle d'ADN et de l'élargir progressivement (Fudenberg et al., 2016; Ganji et al., 2018; Goloborodko et al., 2016; Rowley and Corces, 2018; Stigler et al., 2016; Terakawa et al., 2017). La question reste de savoir comment cohésine et condensine peuvent former des boucles de chromatine de tailles si différentes alors que ces complexes sont structurellement très similaires. Les modèles actuels proposent que ces complexes interagissent avec différentes sous-unités régulatrices qui pourraient expliquer leurs différences fonctionnelles (Abramo et al., 2019; Baxter et al., 2019).

Pendant ma thèse je me suis essentiellement intéressée au complexe condensine et à sa fonction dans la condensation des chromosomes mitotiques.

La liaison de condensine à la chromatine est essentielle pour accomplir ses fonctions dans la compaction des chromosomes mitotiques. Des mutants thermosensibles de condensine chez la levure à fission, dans lesquels l'association de condensine aux chromosomes est réduite, ne

parviennent pas à séparer correctement les chromosomes en anaphase (Nakazawa et al., 2015; Sutani et al., 2015; Tada et al., 2011). Il a été montré dans plusieurs organismes que condensine est particulièrement enrichie à proximité des gènes fortement transcrits, quelle que soit l'ARN polymérase impliquée (D'Ambrosio et al., 2008; Downen et al., 2013; Gruber and Errington, 2009; Kim et al., 2013, 2016; Kranz et al., 2013; Nakazawa et al., 2015; Sutani et al., 2015). Le fait que ce patron de localisation soit très conservé suggère qu'il existe un lien fort entre la transcription et le chargement et/ou le positionnement de condensine. Ce lien semble toutefois paradoxal, puisque chez les vertébrés au moins, la transcription est globalement éteinte en mitose (Gottesfeld and Forbes, 1997). Néanmoins, des études récentes suggèrent qu'il pourrait exister un niveau basal de transcription en mitose (Palozola et al., 2017). Comment la transcription participe au chargement/positionnement de condensine sur les chromosomes reste à éclaircir.

Au début de mon projet de thèse, la littérature était assez controversée sur ce sujet. Certaines publications décrivaient un rôle positif de la transcription dans la détermination du positionnement de condensine, alors que d'autres études affirmaient qu'au contraire la transcription est un obstacle au chargement de condensine. Par exemple, certaines études envisagent que la transcription génère une structure qui interfère avec l'organisation des chromosomes en mitose et que condensine est capable de repérer et d'éliminer cette structure (Nakazawa et al., 2019; Sutani et al., 2015). Cette fonction dédiée de condensine expliquerait qu'elle soit enrichie à proximité des gènes fortement transcrits. D'autres études au contraire ont montré que l'inhibition de la transcription est nécessaire à l'association de condensine avec les gènes codants pour les ARN ribosomiaux et avec les régions répétées des télomères (Clemente-Blanco et al., 2009, 2011; Wang et al., 2006).

Au cours de mon projet de thèse, j'ai cherché à mieux comprendre ce lien entre transcription et condensine. Avant mon arrivée, l'équipe avait utilisé des cribles génétiques chez *Schizosaccharomyces pombe* pour identifier des facteurs associés à la transcription qui impactent la fonction de condensine (Vanoosthuysse et al., 2014). Au cours de ma thèse, j'ai étudié en particulier un des régulateurs identifiés par ces cribles : l'hélicase à ADN/ARN Sen1, une enzyme très conservée. Mon équipe avait montré précédemment que la délétion de Sen1 supprimait partiellement les défauts de croissance d'un mutant de condensine (Legros et al., 2014), suggérant que Sen1 pourrait être un régulateur négatif de condensine.

Au cours de mes 4 années au laboratoire (M2 + Doctorat), j'ai caractérisé Sen1 comme un facteur qui empêche l'accumulation de condensine spécifiquement autour des gènes transcrits par l'ARN polymérase III (RNAPIII) (chapitre 1). J'ai démontré que Sen1 est un cofacteur de la RNAPIII qui est important pour la terminaison de la transcription (chapitre 2). Finalement, j'ai décrit comment la

fonction de Sen1 dans la terminaison de la transcription de la RNAPIII explique son rôle dans le positionnement de condensine autour des gènes transcrits par la RNAPIII (chapitre 3).

## Chapitre 1 : Le facteur Sen1 associé à l'ARN polymérase III régule l'accumulation de condensine à proximité des gènes transcrits par l'ARN polymérase III

Le premier objectif de mon projet était d'améliorer les résultats obtenus précédemment dans l'équipe. Des expériences d'immunoprécipitation de la chromatine (ChIP pour 'Chromatin Immunoprecipitation') avaient montré que condensine s'accumule à proximité des gènes transcrits par la RNAPIII en l'absence concomitante des protéines Sen1 et Swd22 (Legros et al., 2014). Sen1 est une hélicase à ADN/ARN et Swd22 est un composant non essentiel du complexe CPF (pour 'Cleavage and Polyadenylation Factor'). Ces expériences avaient toutefois été menées sur des cellules asynchrones alors qu'il aurait été préférable de synchroniser les cellules en mitose pour mieux suivre la localisation de condensine. Par ailleurs, des deux protéines Sen1 et Swd22, seule Sen1 s'associe à la RNAPIII (Legros et al., 2014), suggérant que Sen1 seule pourrait moduler directement l'accumulation de condensine à proximité des gènes transcrits par RNAPIII.

Dans ce premier chapitre, nous avons montré que la seule absence de Sen1 entraîne l'accumulation de condensine à proximité d'un sous-groupe de gènes transcrits par la RNAPIII en mitose. De façon intéressante, nous avons observé que condensin s'accumule en 3' et non sur les gènes codant pour les tRNAs (transcrits par l'ARN polymérase III). Nos résultats montrent que l'impact de Sen1 sur la distribution de condensine est spécifique puisque les délétions de l'hélicase Dbl8 ou de Pso2 (un interacteur de Sen1 précédemment identifié dans le laboratoire) n'ont pas d'impact sur la distribution de condensine. Des travaux chez la levure *S. cerevisiae* et chez l'homme ont montré que Tbp1 (pour 'TATA-binding protein') et TFIIC (pour 'Transcription Factor III C') interagissent physiquement avec condensine pour la recruter aux gènes transcrits par la RNAPIII (D'Ambrosio et al., 2008; Iwasaki et al., 2010, 2015; Yuen et al., 2017). Nous avons montré qu'en l'absence de Sen1, l'accumulation de condensine ne peut pas être expliquée par une accumulation de Tbp1 ou TFIIC. Cela montre que l'absence de Sen1 a un impact sur le positionnement de condensine par une voie qui n'a pas encore été caractérisée. Finalement, nous avons remplacé un gène tRNA (transcrit par la RNAPIII) par un gène transcrit par l'ARN polymérase II (RNAPII) (*ura4*). Dans ce mutant, l'association de la RNAPIII est fortement réduite à proximité d'*ura4* et il n'y a pas d'accumulation de condensine en l'absence de Sen1. Ceci suggère fortement que l'effet de Sen1 est spécifique des gènes transcrits par la RNAPIII et que Sen1 pourrait impacter le positionnement de

condensine *via* son interaction avec RNAPIII. En accord avec ce résultat, nous avons montré que réduire le chargement de RNAPIII sur les gènes tRNA (en utilisant un mutant de TFIIC) a pour conséquence de réduire l'accumulation de condensine en l'absence de Sen1. Ces résultats suggèrent que Sen1 régule l'accumulation de condensine aux gènes transcrits par la RNAPIII par un mécanisme dépendant de la transcription.

Par ailleurs, nous avons démontré que la topoisomérase 1 (Top1) s'accumule également autour des gènes transcrits par la RNAPIII en l'absence de Sen1, et que cette accumulation est corrélée avec l'accumulation de condensine. De façon importante, nos données montrent que Top1 s'accumule également lorsque les cellules sont en interphase, ce qui montre que l'accumulation de Top1 ne résulte pas de l'accumulation de condensine (qui n'est recrutée sur les chromosomes qu'en mitose). Par ailleurs, en l'absence de Sen1, l'inhibition de Top1 spécifiquement en mitose induit une accumulation accrue de condensine. Nos données nous permettent donc de proposer que l'absence de Sen1 impacte la transcription de la RNAPIII tout au long du cycle cellulaire, ce qui conduit à la formation d'une structure d'ADN qui est reconnue par Top1 et qui induit une accumulation de condensine à proximité des gènes transcrits par la RNAPIII en mitose.

Dans le chapitre 2, nous avons abordé la question du rôle de Sen1 dans la transcription par la RNAPIII. Ensuite, dans le chapitre 3, nous avons étudié comment Sen1 régule le positionnement de condensine à proximité des gènes transcrits par la RNAPIII en régulant leur transcription.

## Chapitre 2 : Sen1 est nécessaire à la terminaison de la transcription par l'ARN polymérase III

Nous avons cherché à comprendre comment Sen1 pourrait moduler la transcription par la RNAPIII. Chez la levure bourgeonnante et chez l'homme, Sen1 agit sur la terminaison de la transcription d'un sous-groupe de gènes transcrits par la RNAPII (Grzechnik et al., 2015; Mischo et al., 2011; Porrua and Libri, 2013; Skourti-Stathaki et al., 2011; Steinmetz et al., 2001; Ursic et al., 1997). De plus, il a été montré que Sen1 peut désassembler les hybrides ADN/ARN *in vitro* (Kim et al., 1999) et il a été proposé que cette activité de Sen1 pourrait être impliquée dans son rôle dans la terminaison des gènes transcrits par la RNAPII (Grzechnik et al., 2015; Mischo et al., 2011; Skourti-Stathaki et al., 2011). Etant donné que Sen1 se lie à la RNAPIII chez la levure à fission et que les gènes transcrits par la RNAPIII accumulent des hybrides ADN/ARN (Legros et al., 2014), nous avons cherché à savoir si Sen1 pourrait moduler la terminaison de la transcription par la RNAPIII via le désassemblage des hybrides ADN/ARN.

En utilisant un vaste répertoire méthodologique (ChIP-qPCR, ChIP-seq, tests génétiques, RT-qPCR et Northern blot), nous avons montré qu'en l'absence de Sen1, la RNAPIII s'accumule en 3' des séquences terminatrices de la transcription. Nous avons également détecté la production de transcrits allongés en 3' sensibles à l'exosome. Ces observations démontrent que Sen1 est impliqué dans la terminaison de la transcription des ARNs transcrits par la RNAPIII. Nos données montrent que cette fonction de Sen1 n'est pas partagée avec son homologue Dbl8. Grâce à un mutant de Sen1 dans le domaine ATPase, nous avons également montré que l'activité ATPase de Sen1 est nécessaire à sa fonction dans la terminaison de la transcription. En revanche, nos données révèlent que le désassemblage des hybrides ADN/ARN n'est pas impliqué dans cette fonction de Sen1 dans la terminaison la transcription par la RNAPIII. Pour finir, nous avons montré que l'insertion d'un terminateur fort était suffisante pour supprimer l'accumulation de RNAPIII en aval des séquences terminatrices et la production de transcrits étendus en 3' en l'absence de Sen1. Ces résultats suggèrent que Sen1 agit en complément du mécanisme intrinsèque de terminaison de la transcription par la RNAPIII.

Nos résultats identifient pour la première fois en Sen1 un cofacteur de la RNAPIII impliqué dans la terminaison de la transcription, et nous proposons que la fonction ancestrale de Sen1 est de déstabiliser des ARN polymérase en pause.

Ces résultats importants, qui ont été obtenus en partie en collaboration avec le laboratoire de François Bachand (Université de Sherbrooke, Canada) ont été publiés dans *EMBO Journal* (Rivosecchi et al., 2019).

### Chapitre 3 : Sen1 régule le positionnement de condensine à proximité des gènes transcrits par la RNAPIII en contrôlant la terminaison de la transcription de la RNAPIII

Dans le chapitre 1 nous avons montré que Sen1 régule le positionnement de condensine spécifiquement à proximité des gènes transcrits par la RNAPIII. Dans le chapitre 2 nous avons montré que Sen1 est un cofacteur important pour la terminaison de la transcription des gènes transcrits par la RNAPIII. Nous avons ensuite voulu déterminer si ces deux phénomènes étaient liés.

Nous avons d'abord confirmé que les défauts de terminaison de la transcription RNAPIII existaient également dans des cellules synchronisées en mitose en l'absence de Sen1. Ensuite, en manipulant les séquences des terminateurs primaires de deux gènes différents, nous avons démontré que les défauts de terminaison de la transcription de la RNAPIII sont directement

responsables de l'accumulation de condensine à proximité des gènes transcrits par RNAPIII en l'absence de Sen1.

En nous basant sur toutes nos données, nous proposons le modèle suivant : l'absence de Sen1 interfère avec l'élimination de la RNAPIII sur ses gènes cibles, ce qui entraîne une accumulation large et dense de RNAPIII. Au-delà d'un certain seuil, cette accumulation pourrait bloquer la translocation de condensine sur la chromatine, créant ainsi une accumulation de condensine en 3' des gènes transcrits par la RNAPIII. Ce modèle est en accord avec le modèle qui prédit que la transcription est une barrière directionnelle à la translocation des protéines SMC chez *B. subtilis* (Brandao et al., 2019). En particulier, nos données sont en parfaite adéquation avec l'idée que des paramètres comme la densité de l'ARN polymérase, la longueur et l'orientation des gènes influencent la nature de l'obstacle qu'offre la transcription à la translocation de condensine sur la chromatine. Ce modèle est en accord avec l'idée que la transcription mitotique pourrait interférer avec la fonction de condensine.

## Conclusion

Un des défis de la biologie cellulaire est de comprendre comment les complexes condensines assurent de façon extrêmement rapide et efficace la formation de chromosomes individualisés et compacts en début de mitose.

Au cours des dernières années, des études menées dans différents organismes ont montré un lien conservé entre la transcription des gènes et le positionnement de condensine sur les chromosomes. Mais les conclusions de ces études sont souvent contradictoires et aucun modèle crédible ne suffit à expliquer ce lien.

Nous avons identifié l'hélicase Sen1 comme un facteur associé à la RNAPIII qui empêche l'accumulation de condensine à proximité des gènes transcrits par la RNAPIII. Au cours de ma thèse, j'ai abordé deux questions principales: 1) Quel est le rôle de Sen1 dans la transcription de la RNAPIII ?; 2) Comment Sen1 régule le positionnement de condensine autour de gènes transcrits par la RNAPIII ?

Nous avons conclu que:

1) Sen1 est nécessaire à la terminaison de la transcription de la RNAPIII. Nos observations s'opposent aux modèles actuels de terminaison de la transcription par la RNAPIII, qui prédisent que RNAPIII termine de transcrire d'une façon autonome. Ces données ont été publiées dans *EMBO Journal*;

2) Sen1, en assurant la terminaison correcte de la transcription RNAPIII, empêche l'accumulation de condensine à proximité des gènes transcrits par la RNAPIII.

Ce travail suggère que la transcription régule le positionnement de condensine, mais que ce n'est pas le taux de transcription en tant que tel qui est le facteur le plus déterminant dans le positionnement de condensin. Nous proposons qu'au-delà d'un certain seuil, la taille et la densité du domaine occupé par RNAPIII représente un obstacle directionnel à la translocation de condensine sur la chromatine.