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The SMC loader Scc2 promotes ncRNA biogenesis and translational fidelity in *Saccharomyces cerevisiae*

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Abstract

The Scc2-Scc4 complex is essential for loading the cohesin complex onto DNA. Cohesin generates cohesion between sister chromatids, which is critical for chromosome segregation. Scc2/NIPBL is mutated in patients with Cornelia de Lange syndrome, a multi-organ disease characterized by developmental defects in head, limb, cognition, heart, and the gastrointestinal tract. How mutations in Scc2 lead to developmental defects in patients is yet to be elucidated. One hypothesis is that the binding of Scc2/cohesin to different regions of the genome will affect transcription. In budding yeast, Scc2 has been shown to bind to RNA Pol III transcribed genes (tRNAs, and spliceosomal), as well as RNA Pol II-transcribed genes encoding small nuclear and nucleolar RNAs (snRNAs and snoRNAs) and ribosomal protein genes. Here, we report that Scc2 is important for gene expression in budding yeast. Scc2 and the transcriptional regulator Paf1 collaborate to promote the production of Box H/ACA snoRNAs which guide pseudouridylation of RNAs including ribosomal RNA. Mutation of Scc2 was associated with defects in the production of ribosomal RNA, ribosome biogenesis, and splicing. While the *scc2* mutant does not have a general defect in protein synthesis, it shows increased frameshifting and reduced cap-independent translation. These findings suggest Scc2 normally promotes a gene expression program that supports translational fidelity. We hypothesize that translational dysfunction may contribute to the human disorder Cornelia de Lange syndrome, which is caused by mutations in Scc2.

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Abbreviations

^{o}C	degrees centigrade
DNA	Deoxyribonucleic acid
DTT	Dithiothreitol
EDTA	Diaminoethanetetra-acetic acid
YPD	Yeast peptone-yeast extract-dextrose medium
PCR	Polymerase chain reaction
mM	millimolar
M	Molar
ml	milliliter
OD	optical density
Rpm	revolutions per minute
TCA	trichloroacetic acid
$MgCl_2$	magnesium chloride
KCl	Potassium chloride
B-Me	beta-Mercaptoethanol
dATP	deoxyadenosine triphosphate
dGTP	deoxyguanosine triphosphate
dCTP	deoxycytidine triphosphate
dTTP	deoxythymidine triphosphate
HCl	hydrochloric acid

GFP	green fluorescence protein
DEPC	diethyl dicarbonate
SB Buffer	Tris-HCl, Sorbitol Buffer
DRB	5, 6-Dichloro-1-*beta*-D-ribofuranosylbenzimidazole

CHAPTER 1

Introduction

I. Genome structure, function, and maintenance

The development of complex multicellular organisms involves numerous cell divisions, growth, and specialization to generate different cell types that contribute to the development of a high order body plan. This process requires duplication of a cell's genome and transfer of a copy to daughter cells. Duplication of the genome and its physical separation in eukaryotes is regulated in space and time. Accurate segregation of the genome requires the pairing of sister chromatids in a binary fashion from the moment they arise from the replication fork. This process of chromosome pairing is regulated by the multi-subunit protein complex called cohesin. Cohesin was originally identified in genetic screens for mutants that display premature separation of sister chromatids (Michaelis et al., 1997). Cohesin serves as the "biological glue" that ensures faithful chromosome segregation by holding sister chromatids together till their separation at the metaphase to anaphase transition. In this way, the integrity of the genome is maintained by ensuring proper transfer of genetic material from parent to daughter cells.

1. Cohesin structure

Cohesin consists of four core subunits: Smc1, Smc3, the α-kleisin protein Scc1/Mcd1/Rad21, and the HEAT repeat-containing protein Scc3 (Guacci et al., 1997; Michaelis et al., 1997; Uhlmann et al., 1999) (Figure 1). The complex forms a ring-like structure with an estimated diameter of 35 nm that entraps DNA (Figure 1) (Gruber et al., 2003; Haering et al., 2002). Smc1 and Smc3 belong to the structural maintenance of chromosome (SMC) family, each consisting of a folded coiled-coil domain flanked by a hinge domain that mediates SMC dimer formation and a head domain with ATPase activity. The N and C termini of the individual polypeptide chains of SMC proteins assemble into a globular ATPase "head" domain. The region at which the polypeptide chain folds back on itself is referred to as the "hinge" domain (Haering et al., 2002; Melby et al., 1998). The ATPase activity of the SMC subunits of cohesin is required for cohesin loading onto chromosomes (Arumugam et al., 2003; Weitzer et al., 2003). The SMC family also includes members of the condensin complex (Smc2 and Smc4) and the Smc5/6 complex. These complexes are evolutionarily conserved from yeast to human and contribute to the organization

of chromosomes. The kleisins (from the Greek word for closure), are a superfamily of proteins consisting of ScpAs, Scc1s/Rec8s, and a new sequence family, including the hypothetical *A. thaliana* NI_188295 (Schleiffer et al., 2003). There are four main classes of eukaryotic kleisins, α, β, γ, and δ (Schleiffer et al., 2003). Scc1/Rec8 subfamily is named α-kleisin because they share homology only between small domains at their very N- and C- termini (Schleiffer et al., 2003). The β-kleisins family which includes kle-2, is found in some eukaryotes, but absent in fungi. With the exception of *C. elegans*, γ-kleisins (also known as XCAP-H in *Xenopus* and Barren in *Drosophila*) are found in all eukaryotes. The δ-kleisins are related to budding yeast Ori2, which associate with the Smc5/6 complex (Nasmyth and Haering, 2005). The N-terminal domain of Rad21/Scc1/Mcd1 contains two α helices which forms a four-helix bundle with the coiled coil emerging from the ATP head of Smc3 to complete the cohesin ring structure (Gligoris et al., 2014). Scc3 binds to Rad21 of the cohesin ring structure.

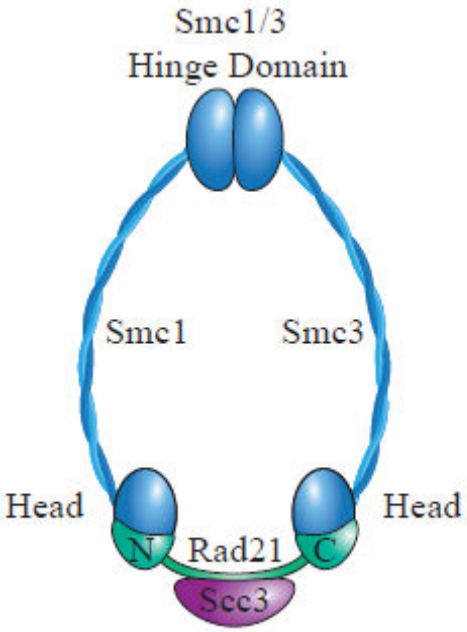


Figure 1. Cohesin subunits form a ring-like structure. Cohesin is composed of four subunits, Smc1, Smc3, Scc1/Rad21/Mcd1, and Scc3. Smc1 and Smc3 (blue) are long polypeptides that form a hinge domain at one end and an ATPase domain at the other end by folding back on themselves to form anti-parallel coiled-coils. The SMC heads are

connected by the α-kleisin Scc1/Rad21/Mcd1 (green), which interacts with the fourth subunit, Scc3 (pink). Illustration credit: Mark Miller for all cartoons.

2. Models for how DNA is held together by cohesin

A key function of cohesin is to hold sister chromatids together, especially at centromeres. This juxtaposing of sequences is termed cohesion. However, cohesin may also hold non-identical sequences in close proximity. In this capacity, cohesin may function in processes besides chromosome segregation. Cohesin may encircle both sister chromatids, or may associate with chromosomes in other ways. The embrace model predicts that the cohesin ring encircles two sister chromatids. In this way, two 10-nm chromatin fibers could be accommodated within a single ring to form chromatin in which DNA is wrapped into nucleosomes. This model proposes that the ATP hydrolysis of cohesin heads result in the dissociation and subsequent opening of the ring (Gruber et al., 2003). Another model, the "handcuff" model, predicts that a single cohesin ring encircles one sister chromatid while interacting with a second ring encircling the other sister chromatid, via the Rad21 and Scc3 subunits (Zhang et al., 2008). The snap model proposes that the single ring structure is not the active form, but rather, cohesin binds to chromatin and results in the tethering of sister chromatids which results from oligomerization Smc complexes (Huang et al., 2005). In any event, cohesin can hold two pieces of DNA in close proximity.

3. Cohesin regulation during the cell cycle

The deposition and removal of cohesin from chromosomes is regulated over the cell cycle. Many aspects of the cycle are evolutionarily conserved from yeast to human. Cohesin binds to specific sites on chromosomes called cohesin-associated regions (CARs) (Laloraya et al., 2000). Establishment of cohesion between sister chromatids during DNA replication is dependent on Eco1 (Figure 2) (Skibbens et al., 1999; Toth et al., 1999). Eco1 is an acetyltransferase that acetylates two lysine residues, 112 and 113 (K112, K113), within the head domain of Smc3. Acetylation of the lysine residues is required for cohesion establishment (Ivanov et al., 2002). While budding yeast have a single copy of the acetyltransferase, humans have two copies, ESCO1 and ESCO2 (Bellows et al., 2003), both of which are involved in sister chromatid cohesion and cell survival in response to radiation-induced DNA damage (Hou and Zou, 2005;

Ivanov et al., 2002; Vega et al., 2005). Once established, cohesion between sister chromatids is maintained by accessory proteins including Pds5 and Wapl (Kueng et al., 2006; Panizza et al., 2000) (Figure 2).

At the onset of mitosis, cohesin is removed from chromatin as a precursor to sister chromatid segregation. In budding yeast, full separation of sister chromatids occurs in one step at the metaphase-to-anaphase transition through site-specific cleavage of the α-kleisin Scc1 by Esp1/separase (Figure 2) (Uhlmann et al., 1999). In metazoans, cohesin removal occurs in two waves (Waizenegger et al., 2000). In vertebrates, while most cohesin along chromosome arms is removed through the action of PDS5, WAPL, PP2A, Aurora B, and phosphorylation of SA1/2 by Plk1 in prophase (Gandhi et al., 2006; Hauf et al., 2005; Kueng et al., 2006; Sumara et al., 2002); full separation of sister chromatids at the centromere region occurs at the metaphase-to-anaphase transition through site-specific cleavage of the α-kleisin Scc1 by Esp1/separase (Figure 2)(Uhlmann et al., 1999). The acetylation of Smc3 is removed by Hos1/HDAC8 to help recycle Smc3 for the next round of cohesin deposition (Beckouet et al., 2010; Borges et al., 2010; Xiong et al., 2010).

For meiotic cohesion, the Scc1/Mcd1 is substituted by the meiotic-specific ortholog Rec8 (Klein et al., 1999; Watanabe and Nurse, 1999). The process takes place in a step-wise manner during meiosis. During metaphase I to anaphase I of meiosis, cohesin at chromosome arms is removed. Centromeric and pericentromeric cohesins are protected until their degradation at the metaphase II to anaphase II transition by separase to enable separation of sisters. Thus cohesion of centromeres seems to be most critical for sister chromatid separation.

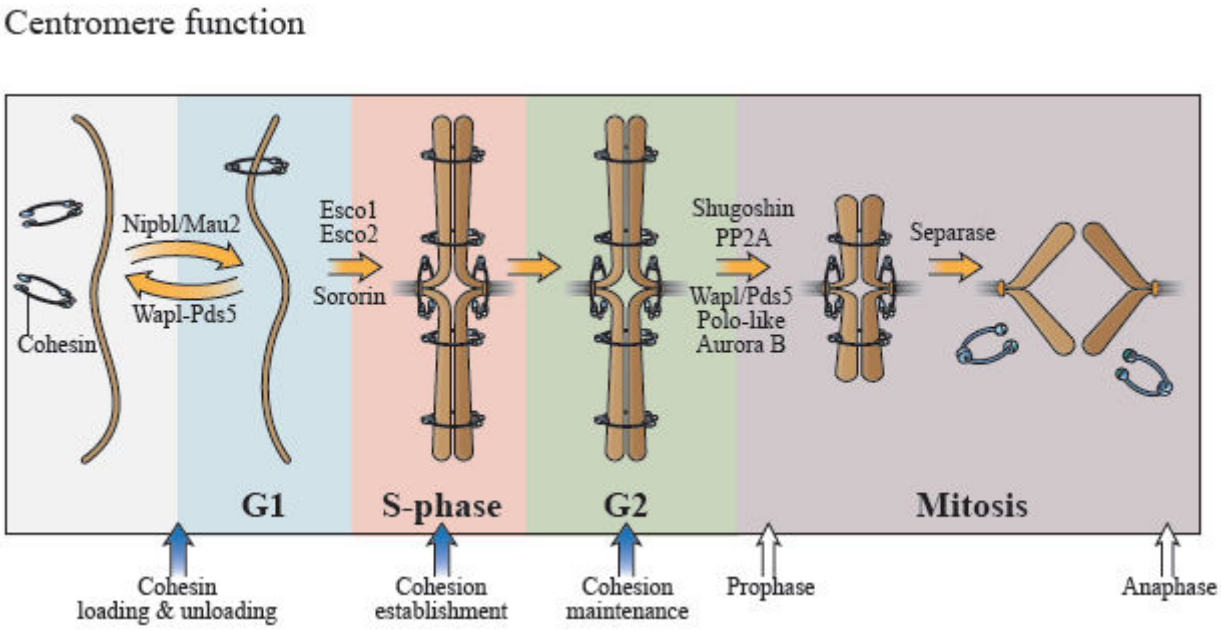


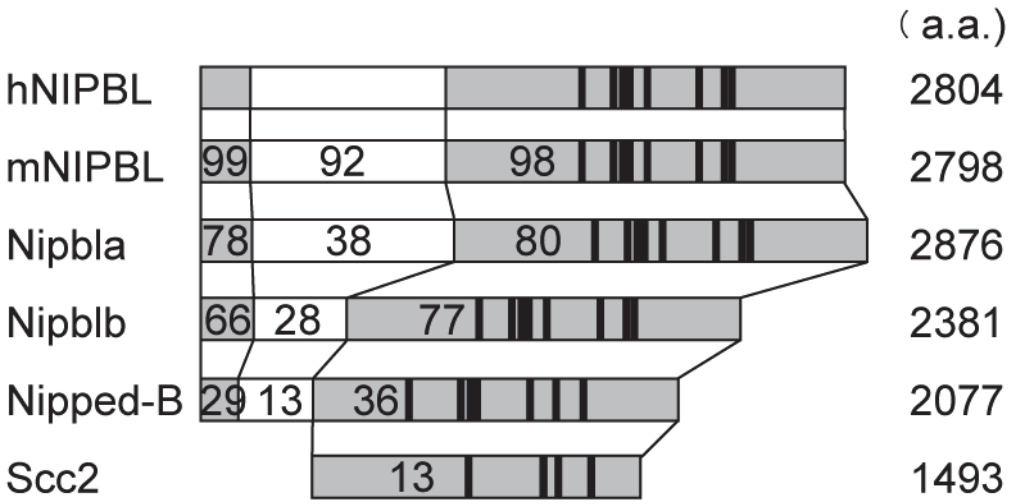
Figure 2. Schematic diagram showing cohesin regulation throughout the cell cycle. Cohesin (blue) is loaded onto chromosomes in telophase/G1 phase by the Scc2-Scc4 heterodimer and requires the opening of the hinge domain of Smc1 and Smc3 for DNA entry. Cohesion establishment at S phase is facilitated by Eco1-dependent acetylation of the Smc3 head domain, making cohesin refractory to removal from chromatin by Wpl1/Rad61. Cohesion is then maintained by other proteins such as Wapl, Pds5, and Sororin. Cohesin can be removed in prophase in a separase independent manner from chromosome arms. This removal depends on Plk1 and Wapl/Pds5. Pericentromeric cohesion is protected by shugoshin and PP2A. At the onset of mitosis, pericentromeric cohesion is destroyed by proteolytic cleavage of Scc1 by separase and recycled for the next cell cycle. Recycling of the Smc3 subunit requires deacetylation by Hos1/Hdac8.

4. The Scc2-Scc4 cohesin loading complex

The association of cohesin with chromosomes is critical for its function. The process of chromosome association is facilitated by the Scc2-Scc4 loading complex, in a reaction that depends on ATP hydrolysis by Smc1/3. (Ciosk et al., 2000; Michaelis et al., 1997). In the absence of Scc2 or Scc4, the association of cohesin with chromosomes and cohesion is impaired. Loss of Scc2 function however does not affect cohesin assembly, suggesting that the loading complex functions specifically to load cohesin onto chromosomes. In addition, the Scc2-Scc4

loading complex is dispensable for cohesion establishment during S phase and G2/M (Ciosk et al., 2000; Lengronne et al., 2006), further supporting the specific role of the cohesin loading complex in loading cohesin onto chromosomes.

Scc2 was originally identified in a genetic screen for the identification of chromosomal proteins involved in sister chromatid cohesion in yeast (Michaelis et al., 1997). Scc2 is known as Nipped-B and NIPBL in flies and humans respectively. In contrast, Scc4, the partner of Scc2, is known as Mau2 in both flies and humans. Scc2 is evolutionarily conserved from yeast to human (Figure 3). Scc2 is a large protein with well conserved N- and C-terminal domains with a less-conserved central domain. Most of the conservation is largely confined to a segment of approximately 1500 amino acids spanning most of the human NIPBL C-terminal domain. Scc2 has conserved multiple HEAT repeats within the C-terminal domain for protein-protein interactions, a glutamine-rich region at the N-terminal domain, a bipartite nuclear localization signal, and an HP1-interacting motif. At least two different isoforms of NIPBL exist in humans; a small isoform (2697 amino acids and a calculated molecular weight of 304 kDa), and a large isoform (2804 amino acids with a molecular weight of 316 kDa) (Jahnke et al., 2008). Human and budding yeast Scc2 do not align very well. However, the function of Scc2 is evolutionarily conserved from yeast to humans.



Muto et al, 2010

Figure 3. Domain structures of Scc2. Numbers indicate amino acid identity to human NIPBL, as determined by Clustal W analysis (http://clustalw.ddbj.nig.ac.jp/top-j.html). a.a = amino acids. The vertical black bars indicate the HEAT repeats.

5. Cohesin entry and exit gate

DNA entry into cohesin's ring during G1-phase is thought to require transient dissociation of the Smc1 and Smc3 hinge domains, which act as DNA entry point (Gruber et al., 2006). The process of DNA entry into the cohesin ring through the hinge domain has been proposed to be governed by the Scc2-Scc4 loading complex (Nasmyth, 2011). During S-phase, acetylation of Smc3 (K112 and K113) by Eco1 prevents dissociation of the Smc3/α-kleisin interface. DNA exit from the ring at the Smc3 head-Scc1 junction is regulated by Wapl, Scc3, the Smc3 nucleotide-binding domain, and Pds5 (Chan et al., 2012; Nasmyth, 2011).

6. Localisation of cohesin and its loading factor

The chromosomal pattern of cohesin binding has been mapped in *S. cerevisiae* by chromatin immunoprecipitation. Cohesin associates preferentially with centromeres and along chromosome arms with a preference for AT-rich sequences, although no consensus sequence has been identified (Blat and Kleckner, 1999). Cohesin association with centromeres requires functional kinetochores (Laloraya et al., 2000) and in *S. pombe* requires Swi6, a member of the heterochromatin HP1 protein family, for recruitment at centromeres (Bernard et al., 2001). Cohesin is enriched in intergenic regions between convergent RNA polymerase II-transcribed genes in budding yeast. Cohesin positions along chromosomes do not correlate with those of the loading complex Scc2-Scc4. Scc2-Scc4 binding along chromosomes coincided with strong transcription and does colocalize with cohesin (D'Ambrosio et al., 2008; Lengronne et al., 2004). In addition, the observation in SMC loader mutant *scc2-4*, where cohesin can be found at Scc2-Scc4 chromosome sites (Lengronne et al., 2004), supports the hypothesis that the permanent location of cohesin is different from its loading site (Ocampo-Hafalla and Uhlmann, 2011). Furthermore, mutations that compromise the disengagement of the nucleotide binding domains

of Smc1 and Smc3 result in the accumulation of cohesin at Scc2-Scc4 binding sites (Hu et al., 2011). The cohesin ring, therefore, appears to be loaded at the binding sites of Scc2-Scc4 complex and then relocates, possibly in response to RNA polymerase II activity.

In fission yeast, however, whilst the cohesin loader (Mis4-Ssl3) is found between convergent transcribed regions distinct from the loading factor at certain places, at other regions, Mis4-Ssl1 and cohesin colocalize. The association between cohesin and the loading factor has been suggested to be caused by (1) a stronger interaction between Mis4-Ssl3 and cohesin (2) the existence of different sub-pools of cohesin in fission yeast (Ocampo-Hafalla and Uhlmann, 2011).

II. Functional roles of cohesin and its accessory proteins

Apart from the canonical role of cohesin in chromosome separation, recent developments in the field have shown the role of cohesin in cell division-independent processes such as DNA double strand break repair, chromosome condensation, and gene regulation. These roles are discussed below.

1. Double strand break (DSB) repair

Damage to DNA can be caused by internal metabolic reactions and replication stress, or from external factors such as radiation and chemotherapeutics. Damage to DNA can manifest in different forms: DNA-protein cross-links, base lesions, intra- and inter-strand cross-links, and single- and double-strand breaks (DSBs) (Lindahl, 1993). Some DNA damages, such as oxidative damage to DNA bases, can be repaired quickly. Much less frequent are DNA DSBs, which are breaks in the phosphate backbone of the two complimentary DNA strands (reviewed in (Mehta and Haber, 2014)), and are the most toxic of these lesions. These lesions must be repaired in order to maintain genome integrity. Defects in DNA DSB repair are associated with human diseases and aging. Damage to DNA triggers cellular responses that involve slowing down cell cycle progression, transcription, and recruitment of repair-related proteins. The DNA damage response is regulated by many kinases. Some of these kinases include Atm and Atr, which are recruited earliest. Subsequently, additional kinases like Chk1 and Rad53 are recruited. These kinases target DNA repair proteins such as Mre11p, a subunit of the MRX (Mre11/Rad50/Xrs2) complex (reviewed in (D'Amours and Jackson, 2002)). An important

structural component of chromosomes that is important for DSB repair is cohesin (Figure 4A). (Birkenbihl and Subramani, 1992; Sjogren and Nasmyth, 2001). Cohesin binds DNA adjacent to DSBs and helps facilitate repair by homologous recombination (Sjogren and Nasmyth, 2001; Unal et al., 2004). The association of cohesin with DSBs is cell cycle regulated and requires the loading complex Scc2-Scc4 (Unal et al., 2004). Mutations in cohesin subunits result in reduced efficiency of post-replicative DSB repair (Sjogren and Nasmyth, 2001; Unal et al., 2004) and compromise the viability of cells in response to γ- irradiation (Birkenbihl and Subramani, 1992).

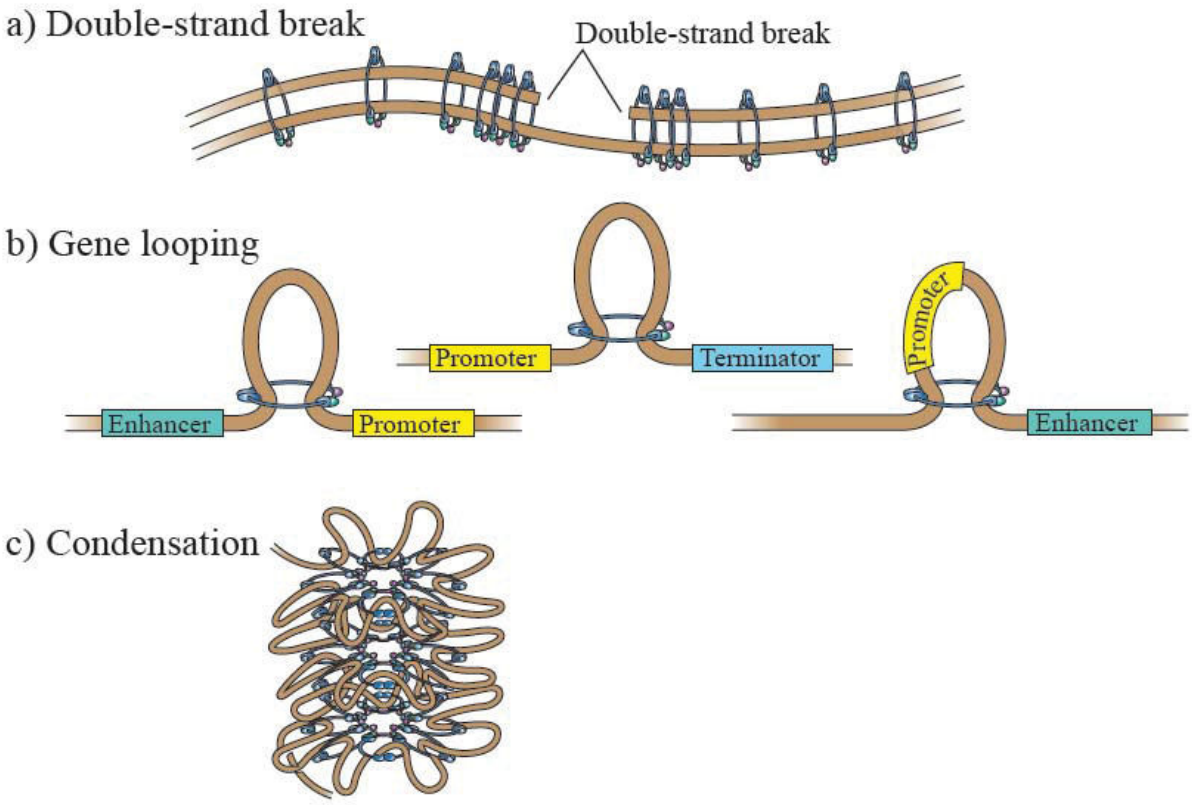


Figure 4. Schematic diagram depicting functions of cohesin at non-centromeric sites. (A) DSB repair is facilitated by cohesin binding to the break site as well as genome-wide. (B) Cohesin regulates gene expression by gene looping to promote long range 1) promoter-enhancer communication 2) promoter-terminator interaction (middle), or 3) insulation. These types of events may contribute to chromatin organization within topological domains. (C). Cohesin promotes chromosome condensation.

2. Chromosome condensation

One of the dramatic events that occurs on chromosomes during mitosis is the conversion of an amorphous mass of interphase chromatin into rod-shaped chromosomes, a process termed chromosome condensation. Chromosome condensation ensures both compaction of chromosome arms and disentanglement of chromosomes from the cleavage furrow (Koshland and Strunnikov, 1996). This process of chromosome condensation is regulated by cohesin in addition to condensin (Figure 4C). Mcd1 was the first cohesin subunit identified to have a role in chromosome condensation in addition to its role in sister chromatid cohesion (Guacci et al., 1997). Fluorescent in situ hybridization (FISH) experiments at the rDNA showed that in the absence of Mcd1 chromosome de-condensation in the form of a disorganized rDNA was detected. Additional studies in budding yeast and other model organisms showed that other members of the cohesin complex, such as Eco1, Wapl, and Smc3, are important for chromosome condensation (Guacci and Koshland, 2012; Lopez-Serra et al., 2013; Skibbens et al., 1999). In addition, two cohesin mutations, *scc2-D730V* and *eco1-W216G,* were shown to compromise chromosome condensation in budding yeast (Gard et al., 2009). These observations support the role of cohesin in promoting chromosome condensation (Figure 4C). A recent study in mouse embryonic stem cells and budding yeast also showed that the SMC loading factor Scc2 was also important for loading condensin onto chromosomes (D'Ambrosio et al., 2008; Dowen et al., 2013). The SMC loader therefore, appears to load condensin onto chromosomes which then promotes chromosome condensation. It is important to note however that, condensin loading depends on cohesin and the cohesin loader, so it is not clear if cohesin directly mediates condensation or if the effect is indirect.

3. Gene regulation

Control of gene expression in space and time plays a very important role in the development of embryos into complex organisms. The regulation of genes is made possible by many regulatory factors, cohesin amongst them. Genetic studies in flies originally identified a role for cohesin in gene regulation (Figure 4B) (Rollins et al., 1999). Subsequent studies in yeast and flies showed that a moderate reduction in cohesin affected chromosome condensation, gene expression, and

development without affecting chromosome segregation (Heidinger-Pauli et al., 2010; Lindgren et al., 2014; Schaaf et al., 2009). Cohesin regulates gene expression independent of chromosome segregation, as shown in non-dividing *Drosophila melanogaster* neurons and mouse thymocytes, where changes in cohesin activity altered gene expression (Pauli et al., 2008; Schuldiner et al., 2008). Based on these studies, it seems likely that changes in gene expression underlie the developmental defects associated with mutations in cohesin and its associated proteins (discussed below).

Cohesin association with chromatin appears to be relatively sequence independent. Cohesin binds between convergently transcribed genes in budding yeast (Glynn et al., 2004). Cohesin binds to highly transcribed genes in higher order organisms. In *Drosophila melanogaster* cells, cohesin is enriched at highly transcribed genes together with RNA Polymerase II (Misulovin et al., 2008). However, recent studies suggest that cohesin loading occurs at sites of active transcription in all organisms, whilst the differences between organisms may be due to cohesin redistribution after loading onto chromosomes. Various models of how cohesin regulates gene expression have been proposed and are discussed below.

3.1 Cohesin may promote boundary-element formation

Mutations in cohesin subunits were shown to cause the loss of function of boundary element surrounding the HMR silent-mating-type loci, indicating a role in gene regulation. Binding of cohesin to this locus help restrict the spread of gene silencing information (Donze et al., 1999). Loss of Smc1 and Smc3 in budding yeast disrupts boundary function. Interestingly, Bell and coworkers showed that cohesin blocks not only silent chromatin spreading but also de novo establishment of silencing (Lau et al., 2002). These observations suggest that cohesin plays a functional role in delineation boundary formation of chromosomes.

3.2 Cohesin may facilitate DNA looping

Transcription is controlled by regulatory regions. These regulatory regions are bound by DNA-binding proteins and control both the assembly and activity of the basal transcriptional machinery (Merkenschlager and Odom, 2013). Defects in the association of cohesin and Scc2/NIPBL with these highly transcribed regions could affect the transcription of genes critical for development. In eukaryotes such as yeast, the transcription of genes is controlled by

regulatory elements close to transcription start sites. Metazoans, however, have evolved long distance regulatory elements that are critical for development and gene expression (Merkenschlager and Odom, 2013). The interaction of DNA sequences can be mediated by cohesin, in some cases in conjunction with additional factors. In other instances, the interactions can activate transcription, for instance a looping event that brings a promoter near an enhancer or a terminator (Figure 4B); and in other cases they can be repressive, for instance an interaction could insulate a promoter from an enhancer (Figure 4B. extreme right).

One factor that binds to such regulatory elements is the sequence-specific DNA binding protein CTCF. The ability of CTCF to connect with other regulatory regions is facilitated by cohesin, polycomb, and the nuclear lamina (Galande et al., 2007; Morey and Helin, 2010; van Steensel, 2011). Depending on the cell type, 50-80% of CTCF binding sites genome-wide are also bound by cohesin, and disruption of cohesin results in defects in CTCF-mediated intra-chromosomal interactions (Hadjur et al., 2009). CTCF functions as a transcriptional activator through binding to mammalian c-MYC promoters (Filippova et al., 1996) as well as an insulator when placed between enhancers and promoters of genes such as the imprinted *IGF2/H19* locus (Beygo et al., 2013). Cohesin binding is thought to be critical for long-range chromatin interactions both between CTCF insulator elements and for gene activation (Filippova et al., 1996; Parelho et al., 2008). However, the association between cohesin and CTCF appears to be vertebrate-specific, since CTCF is not conserved in budding yeast and does not co-localize with cohesin in *Drosophila melanogaster*. These types of long-range interactions could help shape the genome by supplying organization within larger topological domains (Ong and Corces, 2014).

Another fraction of cohesin physically interacts with and co-localizes with the Mediator complex, which binds the enhancers and promoters of active genes. About half of the cohesin loading complex associates with Mediator sites, further implicating cohesin in transcription. The Mediator complex facilitates interactions between the basal transcriptional machinery and enhancer-bound transcriptional activators (Conaway and Conaway, 2013; Ebmeier and Taatjes, 2010; Kagey et al., 2010). In mouse embryonic stem cells, cohesin co-localizes with Mediator at extragenic enhancers and drives the expression of key pluripotency genes (Kagey et al., 2010). In addition, NIPBL and the Mediator complex cooperate to regulate the expression of genes critical

for limb development (Muto et al., 2014). In budding yeast, mutation in cohesin subunit Scc3 also affects transcription via interaction with the Mediator complex (Cena et al., 2013).

Another type of DNA loop that may be mediated by cohesin is the promoter-terminator loop (Figure 4B, middle). ChIP experiments in human cells identified cohesin enrichment at transcription start sites and terminators in metagene analysis (Liu and Krantz, 2009). These looping events could potentially allow for the recycling of RNA polymerase, resulting in highly efficient transcription re-initiation and polymerase recycling. These types of loops have been proposed to form at the ribosomal DNA genes (Mayan and Aragon, 2010), the most highly transcribed locus in most cells. Cohesin binding sites are positioned such that they could mediate looping within the rDNA. In budding yeast, this proposed looped structure formed less efficiently in yeast bearing a mutation in the cohesin acetyltransferase Eco1, and production of rRNA was decreased, suggesting that the efficient formation of these loops depends on cohesin (Harris et al., 2014). Mutations in SMC complexes also result in aberrant nucleolar morphology and ribosome biogenesis (Bose et al., 2012; Xu et al., 2014b), implying a critical role for cohesin in the formation and function of the nucleolus. Since the nucleolus serves as an anchor point for chromosome organization, this finding has implications for both the architecture and expression of the genome.

3.3 Cohesin may regulate transcription initiation, elongation, and termination

Eukaryotic transcription is divided into 3 main steps: initiation, elongation, and termination. Each of these processes is regulated at multiple levels. Studies in several different model organisms have implicated cohesin in transcription initiation, elongation, and termination. In budding yeast, the cohesin loading complex and condensin associate with promoters of RNA polymerase II-transcribed genes that encode small nuclear RNAs, small nucleolar RNAs, ribosomal proteins, as well as with RNA polymerase III-transcribed genes encoding tRNAs and spliceosomal RNAs (D'Ambrosio et al., 2008). Studies in *Drosophila melanogaster* cells have shown that cohesin and Nipped-B/Nipbl colocalize at the transcription start sites of active genes (Misulovin et al., 2008), although less colocalization between these two proteins is observed in mouse cells (Kagey et al., 2010). When compared with RNA Polymerase II binding, NIPBL preferentially binds 100-200 nucleotides upstream of RNA Polymerase II. NIPBL knockdown

causes reduced transcription of these genes, suggesting a role for NIPBL in transcription initiation (Zuin et al., 2014).

Recent studies suggest cohesin also regulates transcription elongation. In *Drosophila melanogaster* cells, cohesin was shown to bind to genes with low levels of histone H3 lysine 36 trimethylation (H3K36me3), a chromatin mark associated with transcription elongation (Fay et al., 2011). Nipped-B and cohesin bind to long transcribed genes such as *cut*, *Abd-B*, *EcR* genes, and others. Many of these genes had paused RNA Polymerase II just downstream of transcription start sites and GAGA factor (GAF) binding sites (Fay et al., 2011). Cohesin, along with other pausing factors such as NELF, was proposed to play a role in the transition of RNA Polymerase II from the paused form to the elongating form. Furthermore, analysis of ChIP-seq data in mouse ESCs also showed that NIPBL binds to the TSS and coding regions of active genes, possibly to release paused RNA Polymerase II into productive elongation (Dowen et al., 2013). These findings raise the idea that Nipbl could play a functional role in transcription activation and elongation.

Cohesin may also promote transcription termination in eukaryotes. In *S. pombe*, cohesin promotes mRNA 3′-end formation and transcriptional termination between convergent gene transcripts (Gullerova and Proudfoot, 2008). Taken together, these studies suggest that cohesin might be playing an important role in all three stages of transcription.

3.2 Cohesin loader as a chromatin adaptor

Accurate genome transcription requires coordination between chromatin and the cellular machineries needed for these processes. The assembly of promoter DNA into nucleosomes prevents transcription initiation, while depletion of nucleosomes promotes gene activation (Han and Grunstein, 1988). Relief from repression is achieved by removing nucleosomes either by the occurrence of nucleosome free regions (NFRs) at transcription start sites (TSS) of TATA-less promoters (~ 80% of promoters in budding yeast) or by remodeling chromatin at genes with TATA-containing promoters (Boeger et al., 2003; Yuan et al., 2005). NFRs are important for transcription initiation since they serve as a “landing pad” for key transcription promoting factors for access to nucleosomal DNA. One mechanism of NFR formation is through the recruitment of chromatin remodeling factors such as the SNF/SWI2 superfamily (Vignali et al., 2000), ISWI, or

the CHD family, and through posttranslational modifiers of histone proteins, such as deacetylases (HDACs), methyltransferases (HMTases), and acetyltransferases (HATs) (Huang et al., 2004; Jenuwein and Allis, 2001). These remodelers have been directly linked to the removal of nucleosomes (Hartley and Madhani, 2009; Lorch et al., 2006). One of the factors identified that helps maintain NFRs is the cohesin loader. The SMC loader Scc2 binds to nucleosome-free regions in budding yeast (Lopez-Serra et al., 2014). Studies in model organisms from yeast to humans have implicated the cohesin loading complex in helping maintain nucleosome free regions (Hakimi et al., 2002; Huang et al., 2004; Lopez-Serra et al., 2014). Cohesin may therefore load at NFRs and relocate along chromosome arms. Because SMC complexes evolved in bacteria, which do not have nucleosomes, the loading complex may have evolved to assist SMC complexes loading onto DNA in the presence of nucleosomes.

Since NFR formation is important for gene expression, mutations that compromise this process have deleterious effects. The RSC (remodels the structure of chromatin) remodeling complex, a member of the SNF/SWI2 superfamily, may act upstream of genes to facilitate the recruitment of the cohesin loading complex Scc2-Scc4 (Huang et al., 2004; Lopez-Serra et al., 2014). Mutations in RSC remodeling complex subunits cause Coffin-Siris syndrome (CSS), a genetic disorder that results in developmental delay in patients (Fryns, 1986). CSS is caused by heterozygous mutations in *ARID1A, ARID1B, SMARCA4, SMARCB1*, or *SMARCE1*. Characteristic features of patients with CSS include aplasia or hypoplasia of the distal phalanx, moderate to severe developmental and cognitive delay, facial abnormalities, short stature, ophthalmologic abnormalities, microcephaly, brain malformations, and hearing loss. In humans, the ISWI (SNF2h)-containing chromatin remodeling complex interacts with the cohesin subunit RAD21 (Hakimi et al., 2002). The cohesin loader NIPBL was also reported to mediate the recruitment of HDAC1 and HDAC3 to chromatin (Jahnke et al., 2008). The Scc2-Scc4 complex may help to maintain nucleosome-free regions to promote cohesin loading as well as transcription. In this way, the cohesin loading complex might be serving as a "chromatin adaptor" for cohesin and for transcription-promoting complexes.

III. Cohesin and human diseases

Mutations in cohesin and its accessory proteins result in a broad spectrum of diseases termed "cohesinopathies." Roberts syndrome (RBS) and Cornelia de Lange syndrome (CdLS) patients

have a constellation of phenotypes including craniofacial, limb, heart, and gastrointestinal defects; poor growth; developmental delay; and intellectual disability (Skibbens et al., 2013). Both RBS and CdLS arise from mutations in cohesin-associated genes. Altered gene expression is clearly an important part of the etiology of these diseases and contributes to the developmental defects observed. Emerging evidence suggests cohesin and NIPBL may promote gene expression programs that support translation, making it interesting to consider translational defects as part of the etiology of these syndromes (Gerton, 2012).

1. Mutations in *ESCO2* cause Roberts syndrome (RBS)

RBS, an autosomal recessive disorder, is caused by homozygous or compound heterozygous mutations in Eco1/*ESCO2*, a member of the acetyltransferase family (Vega et al., 2005). *ESCO2* mutations cause loss of acetyltransferase activity in patients (Gordillo et al., 2008). *ESCO1* mutations have not yet been reported in humans, probably because the mutations would be lethal since this may function as the major cohesin acetyltransferase. Affected individuals display a wide variety of malformations including craniofacial deformities, hypoplastic nasal alae, cleft lip and palate, and reduction in digit number, bone length, or bone formation in both arms and legs (Van Den Berg and Francke, 1993). RBS newborns have a high mortality rate (Gordillo et al., 1993). Loss of *ESCO2* activity in mice is lethal and leads to termination of pre- and post-implantation stage embryos (Whelan et al., 2012a; Whelan et al., 2012b). In RBS cells, cytological observations include aneuploidy, micronuclei, and heterochromatic repulsion. Loss of *ESCO2* in early mitosis also results in changes in the chromosomal location of cohesin and its protector Sgo1 (Whelan et al., 2012b). The heterochromatic repulsions observed in human RBS cells are located at the pericentric domains and nucleolar organizing regions (NOR or rDNA) (Xu et al., 2014a), suggesting a defect in cohesion at these regions. Studies in various model organisms such as yeast, zebrafish, and human show that *ESCO2* mutation affects nucleolar organization, rRNA production, ribosome biogenesis, and protein translation (Bose et al., 2012; Harris et al., 2014; Xu et al., 2013). RBS cell lines show increased apoptosis with elevated p53 and reduced cell proliferation (Bose et al., 2012). Some fraction of the phenotypes associated with RBS could be the result of poor cell proliferation contributing to abnormal development during embryogenesis.

2. Mutations in *NIPBL* and cohesin cause Cornelia de Lange syndrome (CdLS)

CdLS, also known as Brachmann-de Lange syndrome, is the most common of the "cohesinopathies;" occurring in approximately 1 in 10,000 live births. CdLS is clinically heterogeneous and affects multiple aspects of development. Patients with CdLS have distinct phenotypic characteristics which vary from mild intellectual disability to severe developmental and intellectual impairment (Dorsett and Krantz, 2009). Affected individuals have craniofacial deformities, upper limb extremity defects, hirsutism, gastroesophageal dysfunction, and growth and neuro-developmental delay (Liu and Krantz, 2009; Skibbens et al., 2013). More than half (~65%) of CdLS cases arise from mutations in the *NIPBL* gene and are dominantly inherited (Krantz et al., 2004; Tonkin et al., 2004). Examination of the phenotypic and genotypic correlation of patients shows that severe clinical features arise from deletions or truncations in *NIPBL* (Dorsett and Krantz, 2009). The fact that *NIPBL* mutations accounted for only a little more than half of CdLS cases prompted investigators to look for mutations in other genes with related functions. Subsequent genetic screens in large cohorts of CdLS patients without *NIPBL* mutations identified 5% missense or small in-frame deletions in *SMC1A* to be causative (Borck et al., 2007; Deardorff et al., 2007; Musio et al., 2006). Mutations in *SMC3* were also identified (Deardorff et al., 2007). Since then, mutations in other cohesin associated genes (*RAD21, HDAC8*) have been identified as giving rise to CdLS or related disorders (Deardorff et al., 2012a; Deardorff et al., 2012b).

Even though mutations in cohesin and cohesin-associated proteins cause these two syndromes, the underlying etiology for RBS and CdLS could be different (Skibbens et al., 2013). In fact, mutations in different cohesin genes cause clinically distinct subtypes of CdLS. In contrast to RBS cells, CdLS patient cells do not exhibit defects in chromosome segregation, but primarily exhibit gene dysregulation. Most of the differential gene expression changes observed in cells derived from patients or mouse models for CdLS are modest at best (lower than 2 fold), suggesting that either these small changes in gene expression result in the developmental features or additional factors contribute to the etiology. Studies in *SMC1A* and *SMC3* mutated CdLS cell lines using a proteomic approach revealed dysregulation of proteins important for metabolism, cytoskeleton organization, and RNA processing, amongst others (Gimigliano et al., 2012). Defects in ribosomal RNA production and protein translation defects in RBS and CdLS model

organisms suggest that in addition to changes in gene expression, translational defects could contribute to "cohesinopathies."

3. Mutations in *DDX11* cause Warsaw Breakage syndrome

A third cohesinopathy - the Warsaw Breakage syndrome - has been described only recently. Biallelic mutation in the XPD helicase family member, *DDX11* (ortholog of yeast Chl1), important for sister chromatid cohesion in yeast and vertebrates, has been implicated (van der Lelij et al., 2010). Defective *DDX11* is associated with features of Fanconi anemia (drug-induced chromosomal breakage) and Roberts syndrome (chromosome segregation defects). Individuals with Warsaw Breakage syndrome have severe microcephaly, pre- and postnatal growth defects, and abnormal skin pigmentation.

4. Mutations in cohesin cause cancer

Recent studies have linked mutations in cohesin to cancer. Mutations in Smc1/3, Rad21, and SA2 have been implicated in acute myeloid leukemia (AML) (Welch et al., 2012). Mutations in cohesin were recently shown in a study to account for 13% of AML. In general, AMLs do not have abnormal karyotypes, raising the possibility that cohesin mutations may be affecting transcriptional and translational changes that lead to the disease (Kitamura et al., 2014; Thota et al., 2014). SMC1A is overexpressed in gliomas. Knockdown of SMC1A inhibits growth and leads to G2/M arrest in human glioma cells (Ma et al., 2013). RAD21 was also observed to be overexpressed in undifferentiated cancers such as breast, lung, bladder, brain, and ovarian (Rhodes et al., 2004). Interestingly, somatic mutations in the SMC loader *NIPBL* have also been implicated in colorectal cancers which exhibit chromosome instability (Barber et al., 2008). Furthermore, a whole range of tumor types have been shown to harbor deletions or inactivating mutations of *STAG2*. Inactivating *STAG2* caused sister chromatid cohesion defects and aneuploidy (Solomon et al., 2011). How the abnormal expression of cohesin subunit proteins cause cancer is not well understood. Therefore, understanding how transcriptional and translational defects contribute to RBS and CdLS may enable us understand how mutations in the SMC loader Scc2 and cohesin cause cancer.

IV. The Paf1 complex mediates transcriptional processes

In eukaryotic cells, DNA is wrapped around histones to form nucleosomes, which are the basic unit of chromatin. The nucleosomes is an octamer of core histones (two each of H2A, H2B, H3, and H4) wrapped around a 147 base pair of DNA (reviewed in (Hansen, 2002)). DNA compaction is provided by chromatin, which adds a layer of complexity to the regulation of processes which require access to DNA. In vitro studies have shown that Pol II-dependent transcription on chromatin is inhibited at both initiation and elongation (Izban and Luse, 1992; Knezetic and Luse, 1986). Progress in transcription by RNA polymerase II requires the removal of histones. Several chromatin remodeling and modifying factors have been shown to stimulate transcription. One of such factors that lies in the intersection of chromatin modification pathways and transcription is the RNA polymerase II-associated factor (Paf1) complex ((Shi et al., 1996), reviewed in (Zhou et al., 2012)). In budding yeast, the Paf1 complex comprises of Paf1, Ctr9, Cdc73, Rtf1, and Leo1. Human PAF1 complex has an additional subunit called WDR61 (also known as SKI8) (Kim et al., 2010).

The Paf1 complex associates with RNA polymerase II, and contributes to various aspects of RNA polymerase II elongation (Krogan et al., 2002; Wade et al., 1996). First, Paf1 has been shown to interact with RNA polymerase II and the coding regions of actively transcribed genes (Krogan et al., 2002; Mueller and Jaehning, 2002; Simic et al., 2003). Secondly, Paf1 complex members have strong genetic interactions with transcription elongation factors such as Spt4-Spt5, the histone chaperone yFACT (yeast Facilitates chromatin transcription), and proteins that control the phosphorylation of RNA polymerase II (Krogan et al., 2002; Mueller and Jaehning, 2002; Squazzo et al., 2002). Thirdly, Paf1 complex mutants are hypersensitive to base analogs such as 6-azauracil (6AU) (Krogan et al., 2002; Riles et al., 2004; Squazzo et al., 2002). Finally, Paf1 was also shown to be important for 3′-end formation of snoRNAs in budding yeast. Interestingly, mutations in members of the Paf1 complex was shown to cause the accumulation of snoRNA transcripts at their 3′-ends (Sheldon et al., 2005). In addition, Paf1 has been implicated in other cellular processes including, H2B ubiquitylation and methylation of downstream H3K4 and H3K74 in both yeast (Ng et al., 2003; Wood et al., 2003) and in human (Kim et al., 2009). In human PAF1 complex is involved in several cellular processes including

cell survival and embryonic development (Wang et al., 2008) as well as serving as candidate oncogenes and tumor suppressors (Chaudhary et al., 2007).

V. Ribosome can have regulatory capacity

Decades of research have shown that the development of single cells into complex organisms is regulated at transcriptional, posttranscriptional, translational, and posttranslational levels. Translation in eukaryotes is an intricate and essential process which requires various factors, chief amongst them being ribosomes. Ribosomes are large ribonucleoprotein (RNP) particles that convert mRNAs into proteins in the cytoplasm. Eukaryotic ribosomes consist of four ribosomal RNAs (25S, 18S, 5S and 5.8s rRNA) bound by about 75 ribosomal proteins that are assembled into large and small ribosomal subunits (60S and 40S). The ribosomal RNAs are transcribed from the ribosomal DNA located in a specialized nuclear compartment, called the nucleolus, by RNA polymerase I and III. Complete synthesis of ribosomes requires ribosomal proteins and additional processing factors which are important for maturation of the RNAs, transport of the immature ribosomal subunits, stabilization of ribosome structure, and regulation of mRNA translation.

Mutations that impair proper ribosome biogenesis in various model organisms cause developmental defects. Haploinsufficiency in ribosomal proteins gives rise to “minute” flies (Marygold et al., 2007). “Minute” flies are small and have short and thin bristles. The small size likely results from overall defects in translation, while the bristle phenotype probably occurs because bristle production places exceptional demands on translation. Developmental delay and bristle phenotypes are similarly observed in Drosophila *bobbed* mutants, which have fewer rDNA repeats. Together, these fly mutants demonstrate the essential nature of producing large amounts of both ribosomal proteins and RNAs. In a remarkable demonstration of the regulatory potential of ribosomal proteins, loss of function of *Rpl38* in the mouse is associated with a specific developmental defect, namely loss of axial skeletal patterning that results from a lack of translation of a specific subset of homeobox *Hox* mRNAs (Kondrashov et al., 2011). This study demonstrates the potential of the ribosome for a regulatory role in development.

One important group of ncRNAs for ribosome biogenesis are the small nucleolar RNAs (snoRNAs). SnoRNAs are divided into two groups, Box C/D and Box H/ACA. Box C/D snoRNAs along with protein subunits, Nop1p, Nop56p, Nop58p, and Snu13 guide 2′-O-methylation of rRNAs. Box H/ACA snoRNAs along with a different group of protein subunits guide pseudouridylation of ribosomal RNAs. Loss of these modifications individually has no apparent effect on cell growth. However, deletion of 2-3 modifications in the A- and P- sites of ribosomes cause growth defect, reduced amino acid incorporation rates, and significant reduction in modification of ribosomal subunits (Liang et al., 2009). Pseudouridines (Ψ) are the most common single nucleotide modification found in functional RNAs and are conserved from bacteria to humans (Ofengand, 2002). In budding yeast, ribosomal RNAs contain 44 Ψ residues (Liang et al., 2009), and tend to cluster in highly conserved and functionally important regions of ribosomal RNAs. Recently, mRNAs have been recognized to contain pseuodouridines (Carlile et al., 2014; Decatur and Fournier, 2003; Jack et al., 2011; Schwartz et al., 2014). These modifications have been shown to be important for RNA folding, interactions, and stability (Ofengand, 2002).

Cbf5p, the yeast homolog of mammalian dyskerin (DKC1), is the catalytic component of the larger ribonucleoprotein complex (H/ACA RNP) that along with the guiding H/ACA snoRNAs converts uridines to pseuodouridines in mRNAs and noncoding RNAs including rRNAs and telomerase RNA. H/ACA RNPs contain additional highly conserved proteins; Nop10p, Nhp2p, and Gar1. After processing in the nucleolus, ribosomes are assembled into 40S and 60S subunits and exported into the cytoplasm to partake in translation. In mammals, mutations in the *DKC1* gene have been linked to X-linked Dyskeratosis congenita (DKC). DKC is characterized by nail dystrophy, reticular skin pigmentation, oral leukoplakia, bone marrow failure, increased susceptibility to cancer, and skin abnormalities (Alter et al., 2009; Dokal, 2000). DKC can also be caused by mutations in other genes important for telomere biology such as *TERC*, *TERT*, *TINF2*, *NHP2*, and *NOP10*. Hypomorphic *DKC1* mutant mice have reduced pseudouridylation in their ribosomal RNAs, and their cells show impaired translational fidelity, including poor translation of mRNAs with an internal ribosome entry site (Bellodi et al., 2010; Jack et al., 2011). Whether these changes in translational fidelity contribute to the etiology of DKC is still an open question.

VI. Budding yeast as a model to study how cohesin regulates gene expression

Various model organisms for understanding molecular and cellular processes exist, and range from prokaryotic bacterium, *E. coli*, to single and multicellular eukaryotic organisms. The unicellular bakers' yeast *Saccharomyces cerevisiae* has proven to be a good model organism for studying molecular and cellular processes in eukaryotes. Budding yeast has a good number of special attributes that make it an ideal experimental system for studying biological processes. First, the entire genome sequence of yeast, comprised of 6000 functional genes, has been fully sequenced and the corresponding databases are generally accessible. The human genome, on the other hand, is comprised of 25000 genes, making it a more complicated organism (Lee et al., 2007). Secondly, budding yeast has short cell division cycles and can be maintained as haploids. It is also amenable to modifications such as mutations, deletions, and gene marking. Thirdly, many of the cellular processes, such as cell division and DNA replication, are conserved in higher eukaryotes such as humans, mouse, and flies. In addition, about 30% of known genes involved in human diseases have yeast ortholog and exhibit links to human diseases and therefore may provide a clue about the biological functions of these genes (reviewed in (Mager and Winderickx, 2005). Finally, budding yeast is a good genetic tool because it is amenable to genetic manipulation such as gene deletions, overexpression, and tagging. The high degree of gene conservation between yeast and humans, coupled with the ease of manipulation, makes budding yeast an ideal model for our studies.

VII. Aim and scope of this study

Tremendous work has been done over the last decade to decipher how mutations in Scc2/cohesin cause human diseases. One hypothesis is that Scc2/cohesin regulates key developmental steps. However, how cohesin and its associated proteins directly mediate transcriptional processes is still not clear. In this context, we analyzed the functional role of the SMC loader Scc2 in the regulation of transcriptional and translational processes in budding yeast.

CHAPTER 2

Scc2 promote ncRNA biogenesis and translational fidelity

I. Abstract

The Scc2-Scc4 complex is essential for loading the cohesin complex onto DNA. Cohesin has important roles in chromosome segregation, DSB repair, and chromosome condensation. Here, we report that Scc2 is important for gene expression in budding yeast. Scc2 and the transcriptional regulator Paf1 collaborate to promote the production of Box H/ACA snoRNAs, which guide pseudouridylation of RNAs including ribosomal RNA. Mutation of Scc2 was associated with defects in the production of ribosomal RNA, ribosome biogenesis and splicing. While the *scc2* mutant does not have a general defect in protein synthesis, it shows increased frameshifting and reduced cap-independent translation. These findings suggest Scc2 normally promotes a gene expression program that supports translational fidelity. We hypothesize that translational dysfunction may contribute to the human disorder Cornelia de Lange syndrome, which is caused by mutations in Scc2.

II. Introduction

Cohesion between sister chromatids generates the force that holds sister chromatids together until the onset of anaphase (Michaelis et al., 1997). Cohesion is generated by cohesin, an evolutionarily conserved multi-subunit protein complex consisting of four core subunits: Smc1, Smc3, the α-kleisin subunit Mcd1/Scc1/Rad21, and the HEAT repeat-containing protein Scc3/SA1 or SA2. The complex forms a ring-like structure that entraps DNA (Gruber et al., 2003; Haering et al., 2002). Smc1 and Smc3 belong to the structural maintenance of chromosome (SMC) ATPase superfamily (Michaelis et al., 1997; Strunnikov et al., 1993). This family also includes subunits of the condensin and Smc5/6 complexes. Cohesin and condensin loading are facilitated by Scc2-Scc4 (Ciosk et al., 2000; D'Ambrosio et al., 2008; Dowen et al., 2013). In addition to its role in chromosome segregation, cohesin promotes DNA damage repair (Unal et al., 2004) and regulates gene expression (Horsfield et al., 2007; Rollins et al., 2004).

It has been proposed that the human ortholog of Scc2, NIPBL, could regulate the expression of its target genes (Misulovin et al., 2008). In budding yeast, Scc2 binds to Pol II transcribed genes encoding ribosomal protein genes, small nuclear and nucleolar RNA genes (snRNA and

snoRNAs), Pol III transcribed genes encoding tRNAs and other noncoding RNAs (D'Ambrosio et al., 2008), and pericentric domains. These same regions are bound by condensin (D'Ambrosio et al., 2008). Defects in the association of Scc2 with these highly transcribed regions could potentially affect the expression of these genes. For instance, a recent report suggested Scc2 may help maintain a nucleosome-free region (Lopez-Serra et al., 2014), which could potentially promote both SMC complex loading and transcription. Since Scc2 targets contribute to translation, a decrease in their expression may negatively affect translation.

Mutations in Scc2 (*NIPBL*, mammalian ortholog) and cohesin subunits result in a developmental syndrome known as Cornelia de Lange syndrome (CdLS). The causative mutations are spread throughout the *NIPBL* gene, resulting in a partial loss of function (Mannini et al., 2013). How mutations in Scc2 cause developmental defects remains largely unknown. Examination of cells from affected CdLS probands suggests differential gene expression might cause the developmental defects observed in CdLS patients, rather than chromosome missegregation (Kaur et al., 2005; Liu et al., 2009). Recent work using zebrafish to model CdLS is consistent with the idea that partial loss of function of Nipbl negatively affects translation (Xu et al., 2014b), but the molecular mechanisms are unclear.

In this study, we examined how the cohesin loader Scc2 regulates gene expression in budding yeast. We utilized a temperature-sensitive partial loss of function mutation in Scc2 (*scc2-4*) that has previously been shown to (1) lack cohesin and condensin association with chromosomes at 37^{o}C (Ciosk et al., 2000; D'Ambrosio et al., 2008), (2) delay DSB repair (Unal et al., 2004), and (3) disrupt nucleolar morphology at 30^{o}C (D'Ambrosio et al., 2008). We found that the mutant protein is expressed at normal levels and displays a normal binding profile at centromeres, but has reduced association with genic regions including ribosomal DNA (rDNA), tDNAs, snoDNAs, and ribosomal protein genes. To examine the biological processes and RNAs regulated by Scc2, we performed RNA sequencing at permissive temperature. Consistent with a previous report (Lindgren et al., 2014), we observed differential expression of hundreds of genes. Gene expression signatures suggested both ribosome biogenesis and mitochondrial function would be impacted in the mutant and functional analysis confirms they are both negatively affected. One group of down-regulated genes was the H/ACA snoRNAs, which guide the site specific pseudouridylaton of rRNAs, tRNAs, mRNAs, and noncoding RNAs (Carlile et al., 2014;

Ofengand, 2002; Schwartz et al., 2014). The production of H/ACA snoRNAs appears to be facilitated by Scc2-dependent recruitment of the RNA Polymerase II-associated factor (PAF1) complex. The Scc2 mutant showed defective rRNA production and modification with a mild reduction in global protein synthesis. In addition, translational fidelity was reduced as shown by decreased internal ribosomal entry site (IRES) usage, increased frameshifting, and decreased resistance to translational inhibitors. Our results in budding yeast strongly suggest that the Scc2 regulated gene expression program promotes translational fidelity.

III. Results

1. The *scc2-4* mutant protein has reduced binding to genic regions

Scc2 is a large gene with several domains (Fig. 5A). Human and budding yeast Scc2 do not align very well. However, the function of Scc2 is evolutionarily conserved from yeast to humans. A protein-binding motif for Scc4 and an evolutionarily conserved HEAT repeats are located at the N- and C- terminus respectively. By chemical mutagenesis, a temperature sensitive Scc2 mutant was isolated (E534K), named *scc2-4* (Michaelis et al., 1997). The mutated amino acid is evolutionarily conserved between yeast to human (Fig. 5B). The E534K mutation falls in a central region of the protein with unknown function. At 30^{o}C, the *scc2-4* mutant grows more slowly than WT (Fig. 5C), while at 35^{o}C it does not grow at all (data not shown). Western blot analysis showed that the level of the mutant protein is similar to WT (Fig. 5D).

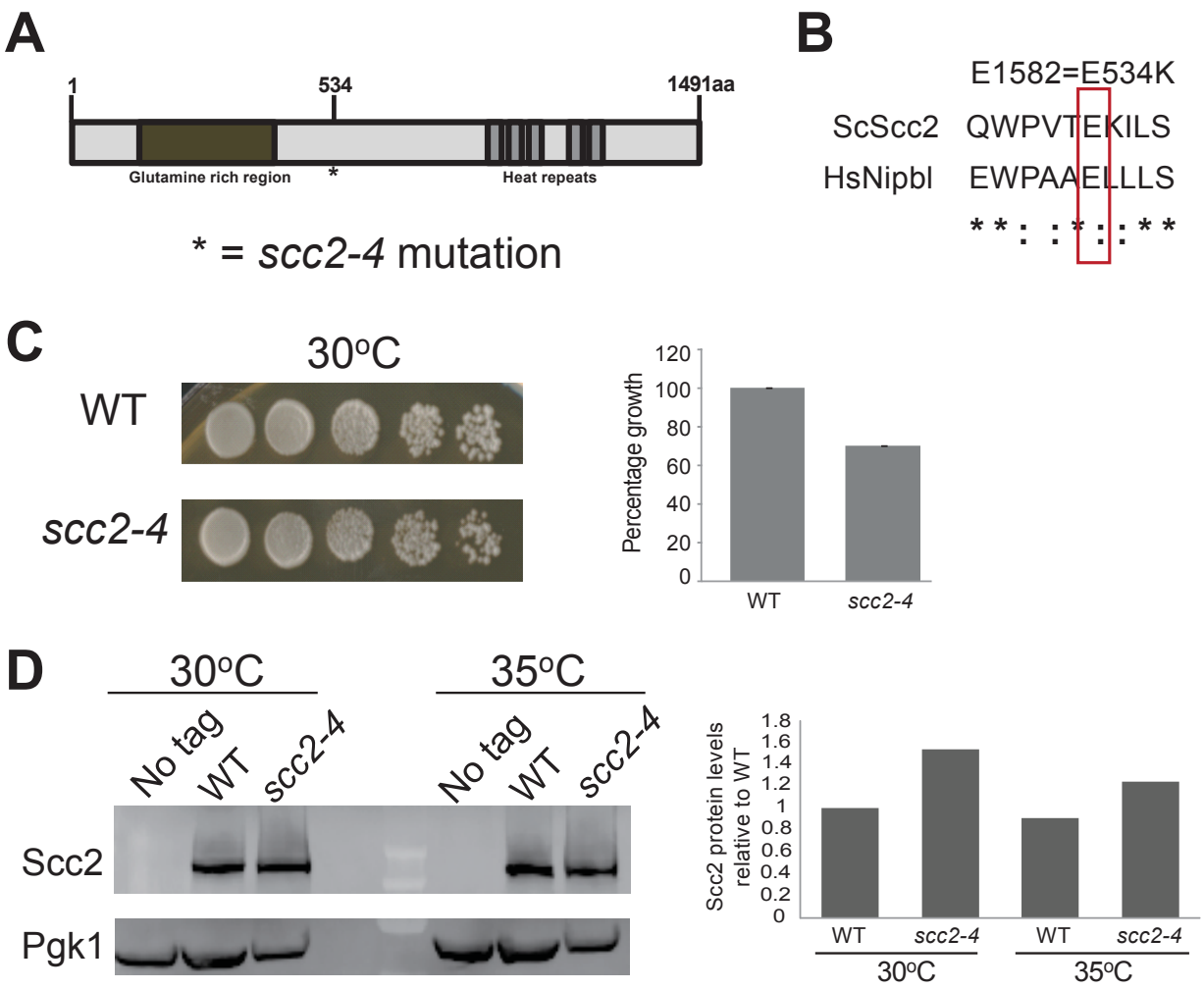


Figure 5. ***scc2-4*** **is a partial loss of function mutation. (A) Scc2 has a highly conserved N terminus with a glutamine-rich region and a conserved C terminal domain with HEAT repeats. (B) The amino acid mutated in** ***scc2-4*** **is conserved from yeast to human (E534). (C) Ten-fold dilution spot assays (left) and growth curves from which the maximal growth rate was calculated (right) of WT and the** ***scc2-4*** **mutant show the mutant has a slower growth rate at 30°C. Growth curves were measured using a TECAN machine. Error bars indicate the standard deviation from three independent experiments. $p < 0.05$. (D) The levels of Scc2-Myc were measured by western blotting (left) and quantified (right). Pgk1 serves as a loading control. The E534K mutation does not reduce the amount of Scc2 protein at permissive (30°C) or non-permissive temperature (35°C) for 3 hr.**

In order to understand where the mutant Scc2 protein associates with the genome, we performed ChIP-seq. Strains were cultured in YPD+CSM (complete supplement mixture) at 30^{o}C until mid-log phase, fixed in formaldehyde for 2 hrs, and chromatin was harvested for ChIP-seq. The basic pattern of enrichment for the WT Scc2 protein was similar to previous reports (D'Ambrosio et al., 2008; Hu et al., 2011). Enrichment for the WT and *scc2-4* mutant proteins at different regions of the genome was further characterized using metagene plots (Figure 6). On the y-axis is the mean count per million (cpm) and on the x-axis is the base pair of genes. No apparent difference in Scc2 enrichment at the centromere was observed for Scc2^{E534K} when compared to the WT (Figure 6A). In contrast, we observed reduced binding of Scc2^{E534K} to rDNA, snoDNAs (Box H/ACA and Bo C/D), tDNAs, and the small and large ribosomal protein genes (Figure 6). Thus, the mutation appears to compromise genic association without compromising centromere association.

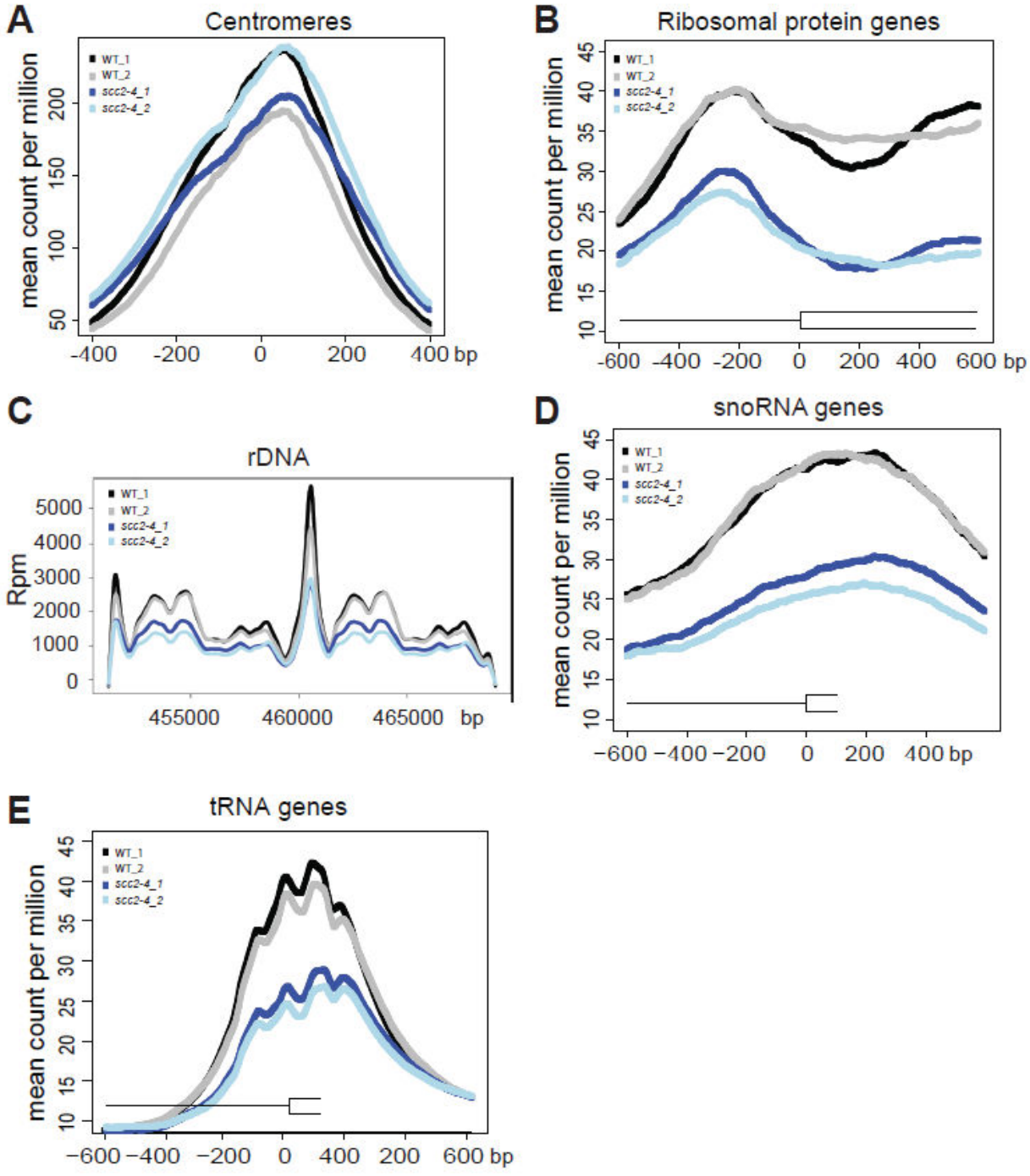


Figure 6. The *scc2-4* mutation compromises the association with genic regions at 30°C. WT and *scc2-4* mutant strains in YPD media were cultured to mid-log phase (~ OD_{600} = 0.5-0.8). Strains were cross-linked and chromatin extracted for ChIP. Metagene analysis was carried out for Scc2-Myc and $Scc2^{E534K}$-Myc for ChIP-seq data. Two biological replicates

for each are shown. (A) The *scc2-4* mutation does not affect the association of Scc2 with centromere regions. (b) The mutation reduces the association with ribosomal protein genes (132) (C) rDNA (D) snoDNAs (77) and (E) tDNAs (275).

2. Hundreds of genes are differentially expressed in the *scc2-4* mutant

To investigate the biological processes regulated by Scc2, RNA sequencing was performed for WT and the *scc2-4* mutant. Each strain was grown in triplicate, at 30^{o}C in YPD+CSM to log phase. The distribution of gene expression can be viewed in the MA plot, defined as a plot of log-intensity ratios (M-values) versus log-intensity averages (A-values) (Fig 7A). The $\log_2$ ratio of *scc2-4*/WT is shown on the y-axis and the geometric mean of the reads per kilobase of transcript per million reads mapped (RPKM) values on the x-axis. Using an adjusted *p-value* of 0.05 as criteria, there are 2644 differentially expressed genes, with 1285 up-regulated and 1359 down-regulated in *scc2-4* mutant. Further applying a fold change threshold of 1.5 (corresponding to an absolute $\log_2$ value of 0.6) returns 823 up-regulated and 760 down-regulated genes for GO analyses and general comparisons. This is many more genes than are bound by Scc2, suggesting that a significant fraction of the changes in gene expression are due to indirect effects. GO term analyses for upregulated genes show enrichment for genes involved in ribosome biogenesis and rRNA processing (Figure 7B). The up-regulation of ribosomal protein genes (RPs) and the processome in general suggests these messages are not rate limiting in the *scc2-4* mutant for ribosome biogenesis, although ribosome biogenesis does appear to be affected (see below). This up-regulation is in direct contrast to our previous analysis of gene expression in the cohesin acetyltransferase mutant (*eco1-W216G*), which shows down-regulation of these gene groups. In fact, the overall pattern of gene expression in the *scc2-4* mutant is inversely correlated with the acetyltransferase mutant (data not shown). GO term analysis for the downregulated genes shows enrichment for genes required for oxidative phosphorylation (Figure 7C).

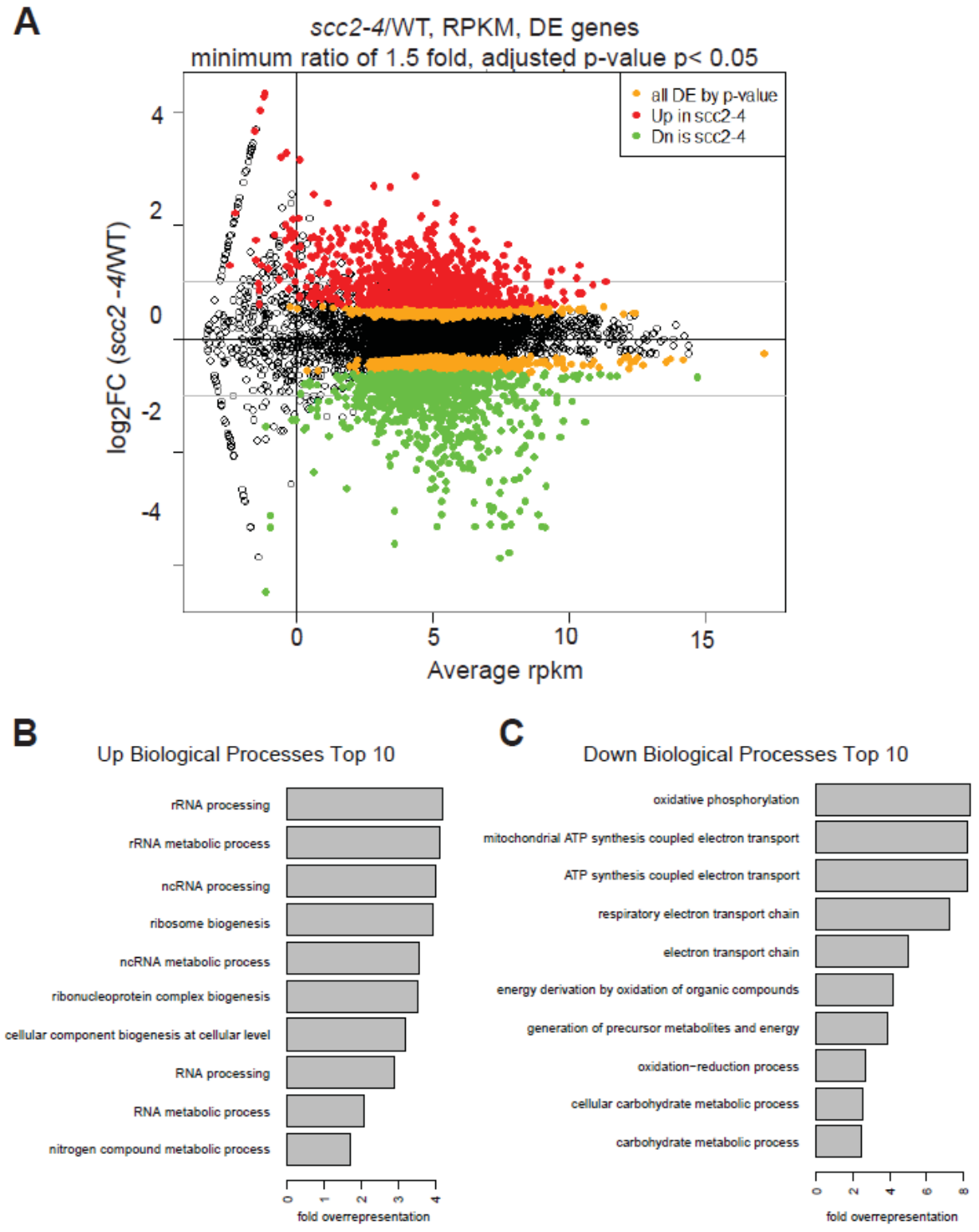

A
scc2-4/WT, RPKM, DE genes
minimum ratio of 1.5 fold, adjusted p-value p< 0.05
all DE by p-value
Up in scc2-4
Dn is scc2-4
log2FC (scc2 -4/WT)
Average rpkm
B
Up Biological Processes Top 10
rRNA processing
rRNA metabolic process
ncRNA processing
ribosome biogenesis
ncRNA metabolic process
ribonucleoprotein complex biogenesis
cellular component biogenesis at cellular level
RNA processing
RNA metabolic process
nitrogen compound metabolic process
fold overrepresentation
C
Down Biological Processes Top 10
oxidative phosphorylation
mitochondrial ATP synthesis coupled electron transport
ATP synthesis coupled electron transport
respiratory electron transport chain
electron transport chain
energy derivation by oxidation of organic compounds
generation of precursor metabolites and energy
oxidation−reduction process
cellular carbohydrate metabolic process
carbohydrate metabolic process
fold overrepresentation

Figure 7. Hundreds of genes are differentially expressed in the *scc2-4* mutant compared to WT at 30^{o}C in YPD. (a) Gene expression values (mean of triplicate samples) are shown in MA plot. Differentially expressed genes (adjusted p-value <0.05) were colored in orange, and then colored red or green after a minimum fold change cut-off of 1.5 was applied. There are 2644 genes differentially expressed genes in the *scc2-4* mutant, with 1285 up-regulated and 1359 down-regulated. Applying a fold-change cutoff of 1.5 (corresponding to an absolute $\log_2$ value of 0.6) returns a more reasonable number of genes for GO analysis and general comparison. (b) GO term analysis for the up-regulated genes shows enrichment for genes important for RNA processing/metabolism and ribosome biogenesis. (c) GO term analysis for down-regulated genes shows enrichment for genes important for biological processes such as oxidative phosphorylation, electron transport chain, and carbohydrate metabolic processes.

We further explored specific aspects of the gene expression profile in the *scc2-4* mutant. The down-regulation of genes involved in oxidative phosphorylation suggested the mutant might be hypersensitive to a drug that inhibits mitochondrial function, such as chloramphenicol which blocks protein synthesis by the mitochondrial ribosome. Consistent with their respective gene expression profiles, the *scc2-4* mutant, but not the *eco1-W216G* mutant, showed very poor growth on plates with a sub-lethal dose of chloramphenicol (Figure 8). Further examination of the gene expression profile revealed the down-regulation of mitochondrial ribosomal proteins (data not shown) in mutant *scc2*. Down-regulation of oxidative phosphorylation was associated with reduced cytochrome c oxidase activity in the mutant *scc2* (Figure 8B). This observation suggests that Scc2 may be important for mitochondria function.

Another group recently published the gene expression profile of the *scc2-4* mutant (Lindgren et al., 2014). However, these experiments were conducted at non-permissive temperature whereas our experiments were all conducted under permissive conditions. The two data sets have some differences, as expected, but the gene expression profile from Lindgren et al. suggests mitochondrial function and translation would be negatively impacted in the *scc2-4* mutant, although this was not tested. However, they did show that the levels of cohesin binding were not

significantly affected in the mutant background, helping to rule out this effect as an explanation for the differential gene expression.

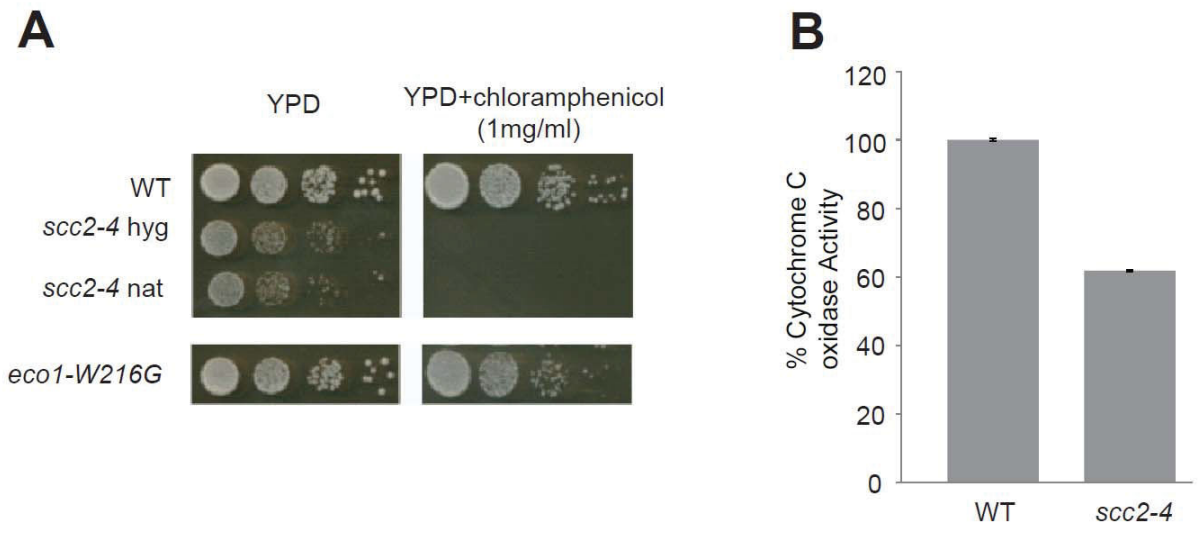


Figure 8. The *scc2-4* mutant strain has defective mitochondria function. (A) 10-fold serial dilutions of WT and the *scc2-4* mutant strain from overnight cultures were grown at 30°C on YPD or YPD with 1µg/ml chloramphenicol. Plates were observed after 2-3 days. (B) Cytochrome C oxidase activity was examined using manufacturer's protocol. The difference between WT and mutant *scc2* was significant at *p<0.001.* Error bars indicate the standard deviation from three independent experiments.

Scc2 binds to genes that encode RNA components of ribonucleoprotein complexes such as *NME1*, a component of RNAse MRP that modifies ribosomal RNA; *SCR1*, part of the signal recognition complex; and *SNR6*, the U6 component of the spliceosome. The mutant protein binds less well to these regions, correlating with significantly lower expression in the mutant ($p<0.05$). Given the reduction in U6, we decided to analyze splicing in the mutant compared to WT. By measuring splicing efficiency (computing the mean nucleotide coverage over every spliced junction divided by the mean nucleotide coverage for reads falling within the exonic splicing

unit), we were able to detect a modest but reproducible defect in splicing (Figure 9). This defect was not present in the *eco1-W216G* mutant (data not shown).

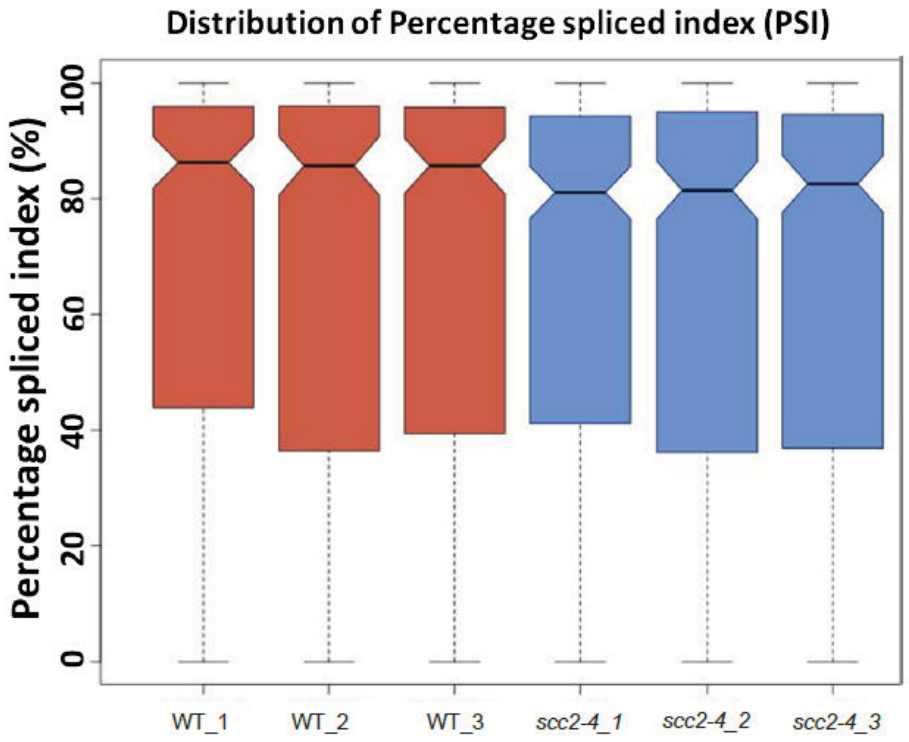


Figure 9. The *scc2-4* mutant strain shows reduced splicing. Analysis of percentage splicing index (calculated as described in material and methods) in WT and the *scc2-4* mutant as indicated in method section. Significant changes in percentage spliced out (PSO) were computed by performing ANOVA for each splicing unit. Standard error is indicated for n=3.

Analysis of the correlation between genome-wide enrichment of Scc2 with gene expression revealed interesting findings. While loss of binding of Scc2 correlated with lower gene expression at certain regions of the genome (e.g. Box H/ACA, Box C/D snoDNAs), there was increased expression at RP genes, and no correlation at other regions. Overall the gene expression pattern is likely the product of a combination of direct effects from changes in Scc2

binding and indirect effects. It is also possible that Scc2 may play different roles in gene expression at different groups of genes.

3. The *scc2-4* mutant has defects in rRNA production and modification

The reduced binding of Scc2 at the ribosomal DNA repeats (Figure 6C) combined with our previous work demonstrating that cohesion is required to form a nucleolus (Harris et al., 2014) and produce normal levels of ribosomal RNA (Bose et al., 2012) drove me to examine rRNA production and nucleolar morphology in the *scc2-4* mutant. rRNA synthesis was measured by pulse-labeling cells for 5 min with ^{3}H-uridine. This approach is a reliable method for estimating Pol I activity since most RNA synthesis during the exponential phase is Pol I derived. Incorporation of uridine into total RNA was reduced by approximately 3-fold in the *scc2-4* mutant (Figure 10A), consistent with the observed decrease in growth rate in the *scc2-4* mutant. rRNA production was further investigated by pulse-labeling cells with ^{3}H-methylmethionine for 5 min, chased with excess cold methionine for 5 min and extracting RNA. Incorporation of ^{3}H-labeled methyl groups was quantified in 25S and 18 rRNAs (Figure 10B). rRNA is methylated co-transcriptionally in yeast, making this approach a reliable method for quantifying the production of the methylated forms of the 25S and 18S rRNAs (Zhang et al., 2009). By this method, we also observed a 3.6-fold reduction in the production of 25S rRNA (Figure 10B, right) and a 4-fold reduction in 18S rRNA production (Figure 10B, bottom right). We further examined the processing of the initial rRNA transcript into the 25S and 18S forms over time. We found no defect in processing rate of 25S and 18S rRNAs in *scc2-4* mutant (Figure 10C). In summary, the *scc2-4* mutant has reduced rRNA production.

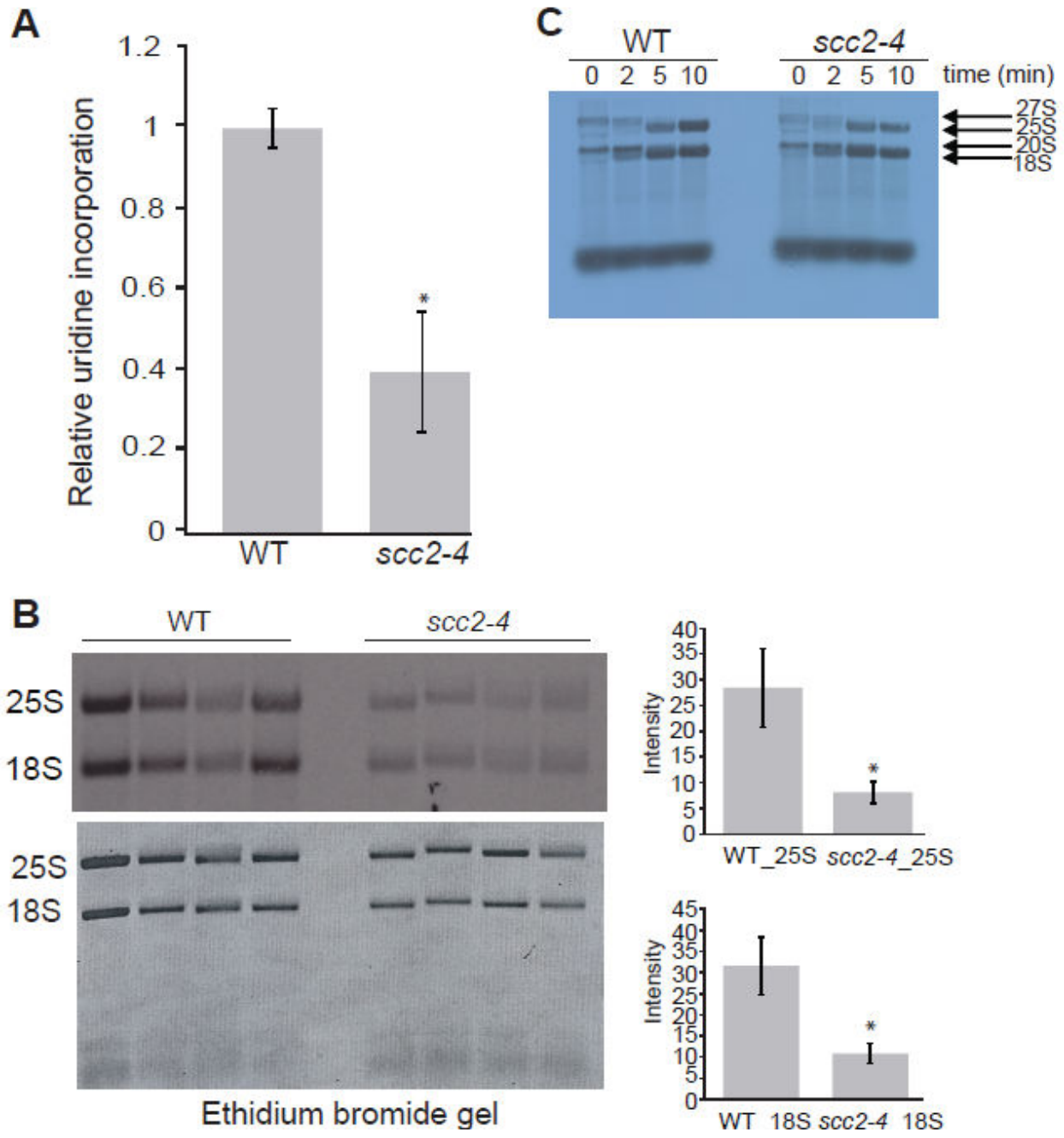


Figure 10. Ribosomal RNA production is compromised in the *scc2-4* mutant. (A) Relative RNA synthesis rate was examined by ^{3}H-uridine incorporation in WT and *scc2-4* mutant strains. Strains were grown in triplicate at 30^{o}C in SD-ura medium with minimal uracil to an approximate OD_{600} of 0.3. ^{3}H-uridine was added for 5 minutes and incorporation was quantified, averaged, and expressed relative to WT. Standard deviation are indicated for n=4. The difference between WT and mutant was calculated using a student two-tailed t-

test. * = *p<0.0001*. (B). WT and *scc2-4* mutant strains were grown in SD-met at 30^{o}C into mid-log phase. Equal numbers of cells were pulse-labeled with ^{3}H-methylmethionine for 5 min, followed by RNA extraction and electrophoresis of the RNA on a formaldehyde gel. The gel was photographed following ethidium bromide staining (bottom). RNA was transferred to a membrane and detected by autoradiography (top). 25S and 18S rRNA were cut from the membrane, quantified with a scintillation counter, and the average is expressed relative to WT (*p<0.05*). p-values were calculated using a student two-tailed t-test. Standard error is indicated for *n = 4*. (C) rRNA processing was examined by growing strains to mid-log phase, labeling with ^{3}H-methylmethionine for 2 min, chasing with 5 mM cold methionine and examining methylated rRNAs at 0, 2, 5, and 10 min time intervals. Equal amounts of RNA at each time point were compared by electrophoresis in a denaturing gel composed of 1% agarose and 16% formaldehyde. RNA was transferred to a HyBond-N^{+} nylon membrane, dried and visualized with autoradiography.

Nucleolar morphology has previously been reported to be aberrant in the *scc2-4* mutant based on visualization of the nucleolar protein Net1 fused to GFP. We extended this observation using electron microscopy (Figure 11). The dense staining nucleolar material did not adopt the normal crescent shaped structure in the mutant, consistent with the idea that reduced binding of Scc2 and defects in loading cohesin and condensin at the rDNA could result in a failure to gather the rDNA repeats into a normal nucleolar structure.

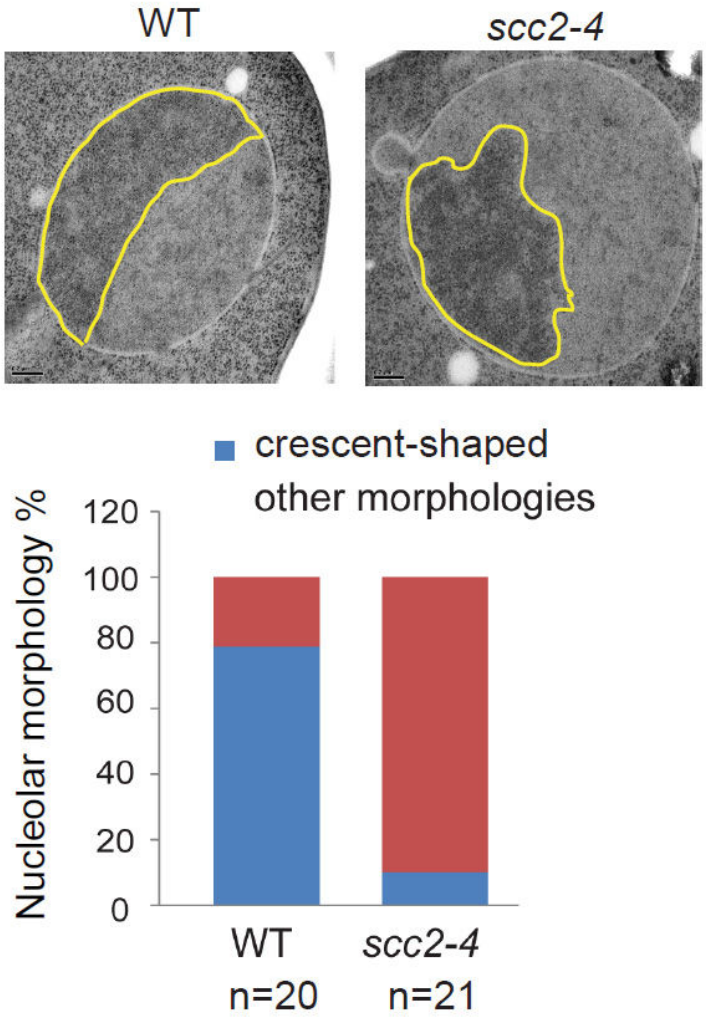


Figure 11. The *scc2-4* mutant strain shows defects in nucleolar morphology. The nucleolar morphology was examined by electron microscopy. Nucleoli were scored as aberrant if nucleoli did not have a compact crescent shape or were dispersed or undetectable. Scale bar represents 0.2 µm.

Ribosomes are partially assembled in the nucleolus and transported into the nucleus and then cytoplasm to be fully assembled and engage in translation. Since mutation in Scc2 results in differential expression of messages involved in ribosome biogenesis, we further investigated ribosome production in the *scc2-4* mutant. GFP tagged protein components of the large (Rpl25) and small (Rps2) ribosomal subunits were expressed in WT and *scc2-4* mutant strains and examined by microscopy. Instead of the normal distribution of the GFP signal in the cytoplasm,

we observed accumulation of GFP in the *scc2-4* mutant in the nucleus/nucleolus. This finding supported our hypothesis that ribosome biogenesis was affected (Figure 12A&D). By quantifying the peak fluorescence intensities of GFP in both WT and the *scc2-4* mutant using flow cytometry, we observed higher mean fluorescence for both 40S and 60S reporters in the *scc2-4* mutant (Figure 12B&E). We further analyzed the data obtained by generating a cumulative distribution function (cdf) for each sample, and calculating the longest distance between biological replicates using Kolmogorov–Smirnov test (KS) test (see materials and method and Bose et al., 2012). Mean KS-distance (depicted as box plots) between biological replicates for WT was compared with the mean KS-distance for the *scc2-4* mutant versus WT (Fig. 12C&F). Distances between the WT and *scc2-4* mutant was then used to calculate the t-test. We observed a higher KS-distance for both the 40S and 60S subunits reporter in the *scc2-4* mutant when compared to WT. This analysis confirms the aberrant ribosome biogenesis and distribution observed by microscopy. The defects in nucleolar morphology, rRNA production, and ribosome biogenesis in the *scc2-4* mutant are quite similar to those reported for the cohesin acetyltransferase mutant, suggesting cohesion is important for these aspects of nucleolar structure and function.

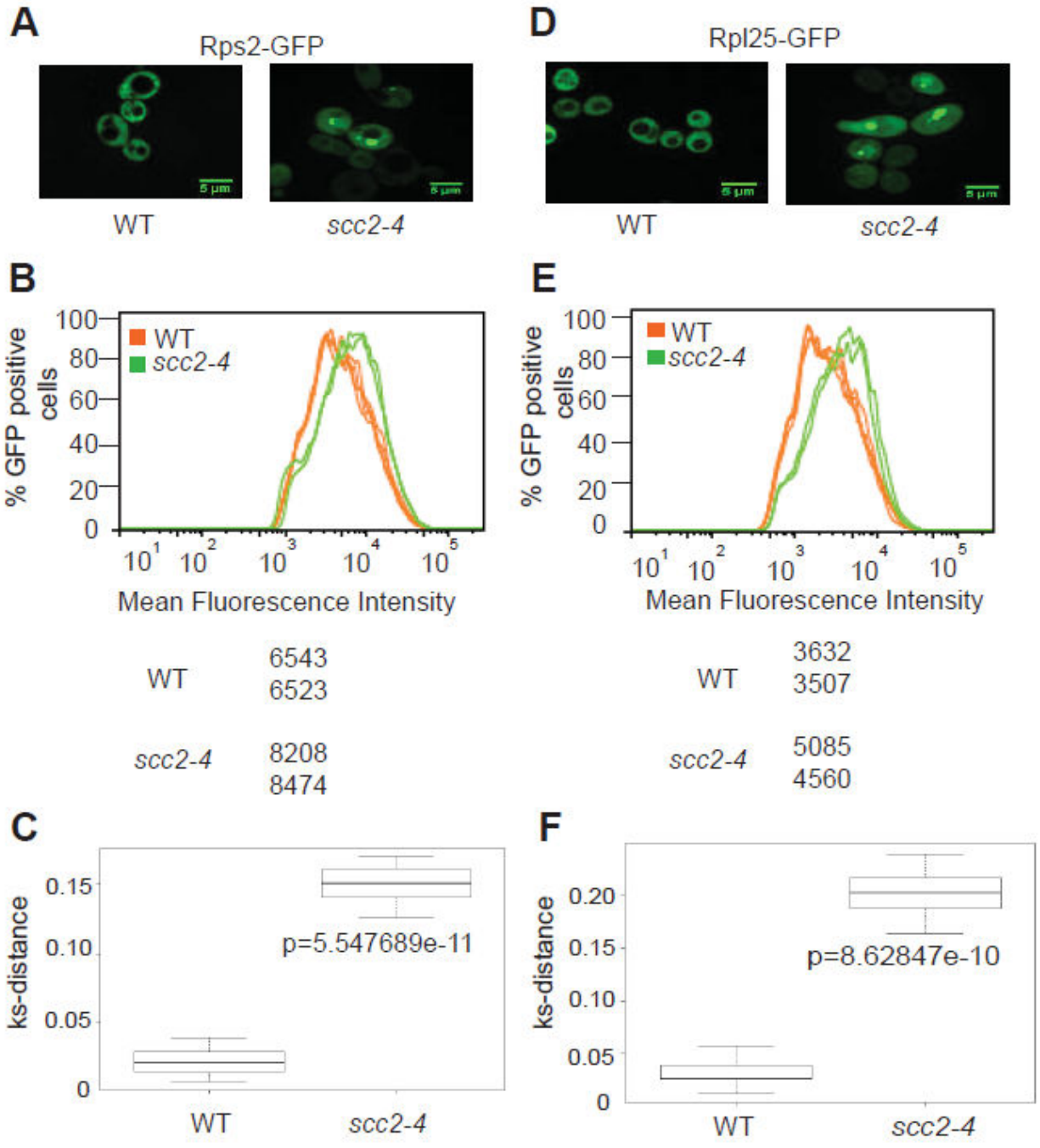


Figure 12. Ribosome biogenesis is compromised in the *scc2-4* mutant. Protein components of the small (Rps2) and large (Rpl25) ribosomal subunits were tagged with GFP and imaged. Representative images are shown for WT and mutant strains (A and D). Using flow cytometry, the peak GFP intensity was quantified for independent biological replicates (B and E). At least 10000 cells were examined per replicate. A KS-distance was calculated

from cumulative distribution frequency curves of the fluorescence which allows us to determine statistical significance using a t-test (C and F) (*p<0.0001*).

4. Down-regulation of H/ACA snoRNAs inhibits site-specific pseudouridylation in the *scc2-4* mutant

One notable group of Scc2 bound genes that was downregulated in the *scc2-4* mutant were the box H/ACA snoRNA genes (Figure 13A). This pattern was not observed in the *eco1-W216G* mutant (Bose et al., 2012). These small nucleolar rRNAs guide site-specific modification of rRNA. The snoRNAs are part of ribonucleoprotein particles (snoRNPs) along with the essential nucleolar proteins (Nhp2p, Cbf5, Nop10p and Gar1p) that together catalyze site-specific pseudouridylation of rRNAs (and other RNAs) (King et al., 2003; Ofengand, 2002). Pseudouridylation is important for RNA stability and interactions with other RNAs and proteins (Reichow et al., 2007). Mutation of the Cbf5 component of this snRNP leads to reduced pseudouridylation of rRNA and reduced translational fidelity (Jack et al., 2011; Yoon et al., 2006). These defects are thought to contribute to cases of dyskeratosis congenita caused by mutations in DKC1, the human ortholog of CBF5.

Down-regulation of box H/ACA snoRNAs could reduce pseudouridylation of rRNAs. To test this idea, cellular RNA was isolated from WT and the *scc2-4* mutant and the distribution of pseuodouridines was examined by CMC-primer extension method. Mapping of pseudo-sites on rRNA revealed down-regulation of pseudouridylation at positions Ψ1003 (guided by SNR5) and Ψ2258/60 (guided by SNR191) (Figure 13B). Pseudo-site 1123 (guided by SNR5) was however not affected (Figure 13B), implying that down-regulation of H/ACA snoRNAs may not affect the modification of all sites on rRNAs.

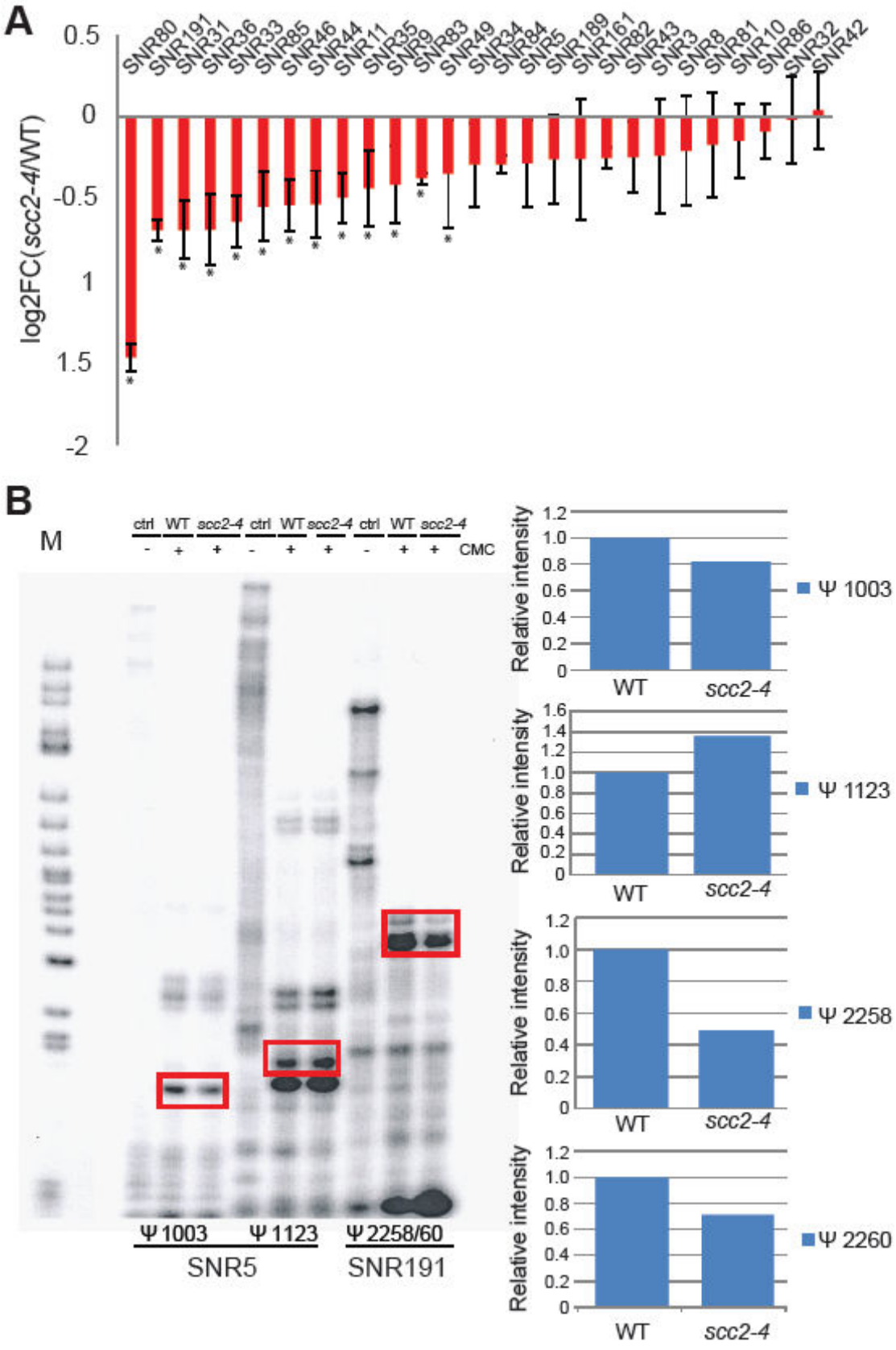

A
log2FC(scc2-4/WT)
0.5
0
-0.5
1
1.5
-2
SNR80
SNR191
SNR31
SNR36
SNR33
SNR85
SNR46
SNR44
SNR11
SNR35
SNR9
SNR83
SNR49
SNR34
SNR84
SNR5
SNR189
SNR161
SNR82
SNR43
SNR3
SNR8
SNR81
SNR10
SNR86
SNR32
SNR42
B
M
ctrl WT scc2-4 ctrl WT scc2-4 ctrl WT scc2-4
- + + - + + - + + CMC
Ψ 1003
Ψ 1123
Ψ 2258/60
SNR5
SNR191
Relative intensity
WT
scc2-4
Ψ 1003
Ψ 1123
Ψ 2258
Ψ 2260

Figure 13. Pseudouridylation of rRNA is reduced in the *scc2-4* mutant. (A) The Box H/ACA snoRNAs that guide sequence-specific pseudouridylation are down-regulated in the *scc2-4* mutant. (B) Reverse transcription with primers corresponding to residues Ψ 1003 and Ψ1123 for SNR5 and residues Ψ2258 and Ψ2260 for SNR191 was performed. Samples were treated with or without CMC, exposed to pH 10.4 for 4 hrs, and reverse transcribed. The rectangles indicate the bands quantified to the right. Pseudouridylation assays were performed at least two times.

snoRNAs are transcribed by RNA Polymerase II. The Tbf1 transcription factor has been identified at snoRNA genes (Preti et al., 2010). We examined whether the presence of either of these two protein complexes was affected at snoRNA genes in the *scc2-4* mutant. While the ChIP signal for RNA polymerase II was similar in WT and *scc2-4* mutant samples (Figure 14A), Tbf1 enrichment at snoRNAs was rather increased in the *scc2-4* mutant compared to WT..

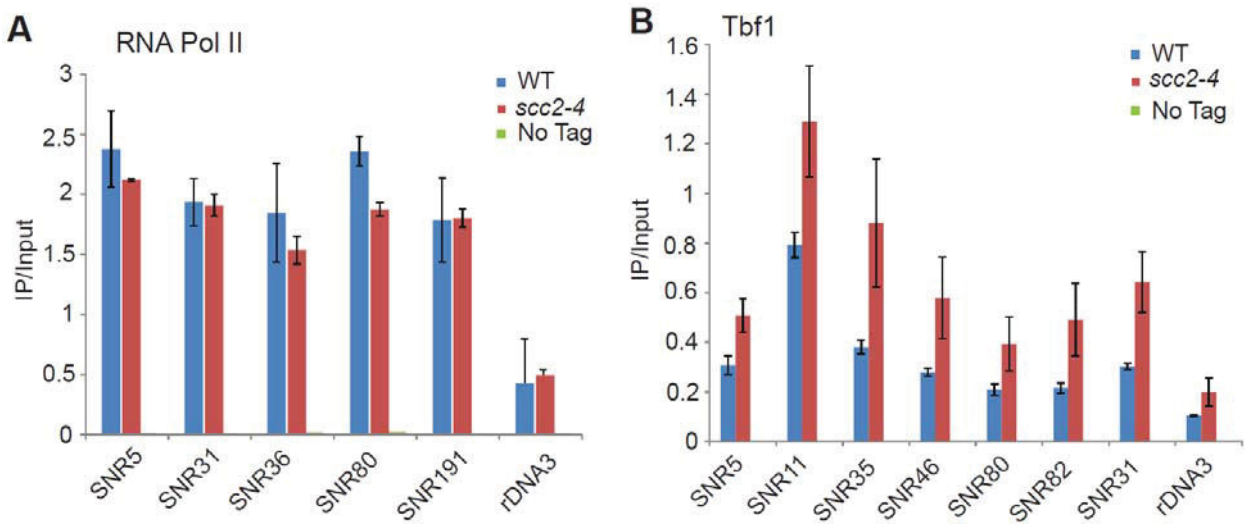
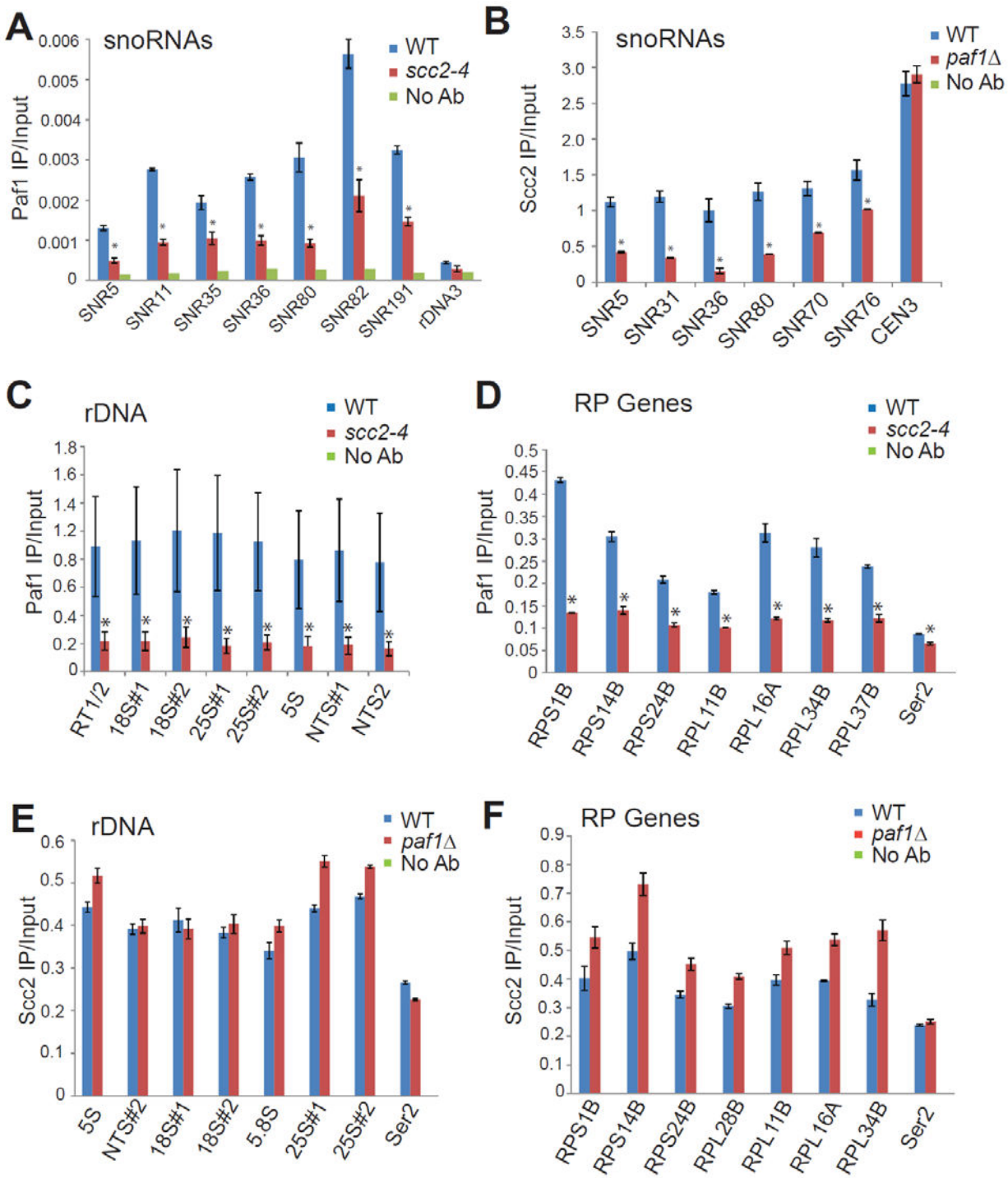


Figure 14. RNA Polymerase II and Tbf1 show normal levels of recruitment at snoDNAs in the *scc2-4* mutant strain. Overnight cultures of WT and *scc2-4* mutant strains were cultured as described in the method section and used for ChIP. A ChIP performed without the addition of antibody serves as a negative control for all forms of RNA Polymerase II ChIP. p-values were calculated using student two-tailed t-test. Standard errors is indicated for n=3. (A) ChIP/qPCR analysis shows enrichment of RNA Pol II (CTD4H8, from

Millipore) at indicated snoDNAs is similar in WT and mutant strains. (B) ChIP/qPCR analysis shows recruitment of Tbf1-Myc is similar or higher at the indicated snoDNAs in the mutant strain relative to WT (α-Myc antibody, 9B11, Cell signaling). ChIP experiments were performed at least three times for each experiment. p-values were calculated using a student t-test ($p<0.05$ for Tbf1). Standard deviation is indicated for n=3.

The Paf1 complex is important for transcription elongation. (Krogan et al., 2002; Sheldon et al., 2005; Simic et al., 2003). The Paf1 complex has previously been shown to contribute to the production of snoRNAs (Sheldon et al., 2005; Tomson et al., 2013). To assess whether Paf1 recruitment was affected, we tagged Paf1 at its endogenous locus and examined its recruitment at snoRNAs genes. In contrast to RNA Polymerase II and Tbf1, binding of Paf1 to snoDNAs (Box C/D and H/ACA snoRNAs) was significantly reduced in the *scc2-4* mutant (Figure 15A). We wondered whether Scc2 recruitment to snoRNAs was affected in the absence Paf1. Deletion of Paf1 resulted in reduced Scc2 binding at snoDNAs (Figure 15B), implying that Scc2 and Paf1 are dependent on each other for full recruitment at snoRNAs. The Paf1 complex has previously been shown to promote transcription elongation by RNA polymerase I (Zhang et al., 2009) and $Scc2^{EK}$ binds more weakly to the rDNA (Figure 5C). We therefore examined whether Paf1 recruitment to the rDNA and RP genes is dependent on Scc2 and vice versa. ChIP analysis showed that, whilst Paf1 recruitment at the rDNA and RP genes was compromised in the *scc2-4* mutant (Figure 14C&D), Paf1 deletion did not affect Scc2 recruitment (Figure 15E&F), implying that Scc2 is required for full recruitment of Paf1 at the rDNA. Having observed that the recruitment of Paf1 to snoRNAs and rDNA is compromised in the *scc2-4* mutant, we wondered if protein levels of Paf1 were affected in *scc2-4* mutant. Whilst the mRNA level of Paf1 was not affected, we observed a reduction in protein levels of Paf1 and Ctr9 in *scc2-4* (Figure 15G), implying that the stability of the Paf1 protein might be compromised in the *scc2-4* mutant. Reduction in Paf1 protein levels correlated with a general protein translation defect in *scc2-4* mutant. Surprisingly, deletion of Paf1 resulted in reduced protein levels of Scc2 (Figure 15G). This observation suggests that, Paf1 and Scc2 are dependent on each other for stability.


A
snoRNAs
Paf1 IP/Input
WT
scc2-4
No Ab
SNR5
SNR11
SNR35
SNR36
SNR80
SNR82
SNR191
rDNA3
B
snoRNAs
Scc2 IP/Input
WT
paf1Δ
No Ab
SNR5
SNR31
SNR36
SNR80
SNR70
SNR76
CEN3
C
rDNA
Paf1 IP/Input
WT
scc2-4
No Ab
RT1/2
18S#1
18S#2
25S#1
25S#2
5S
NTS#1
NTS2
D
RP Genes
Paf1 IP/Input
WT
scc2-4
No Ab
RPS1B
RPS14B
RPS24B
RPL11B
RPL16A
RPL34B
RPL37B
Ser2
E
rDNA
Scc2 IP/Input
WT
paf1Δ
No Ab
5S
NTS#2
18S#1
18S#2
5.8S
25S#1
25S#2
Ser2
F
RP Genes
Scc2 IP/Input
WT
paf1Δ
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RPS1B
RPS14B
RPS24B
RPL28B
RPL11B
RPL16A
RPL34B
Ser2

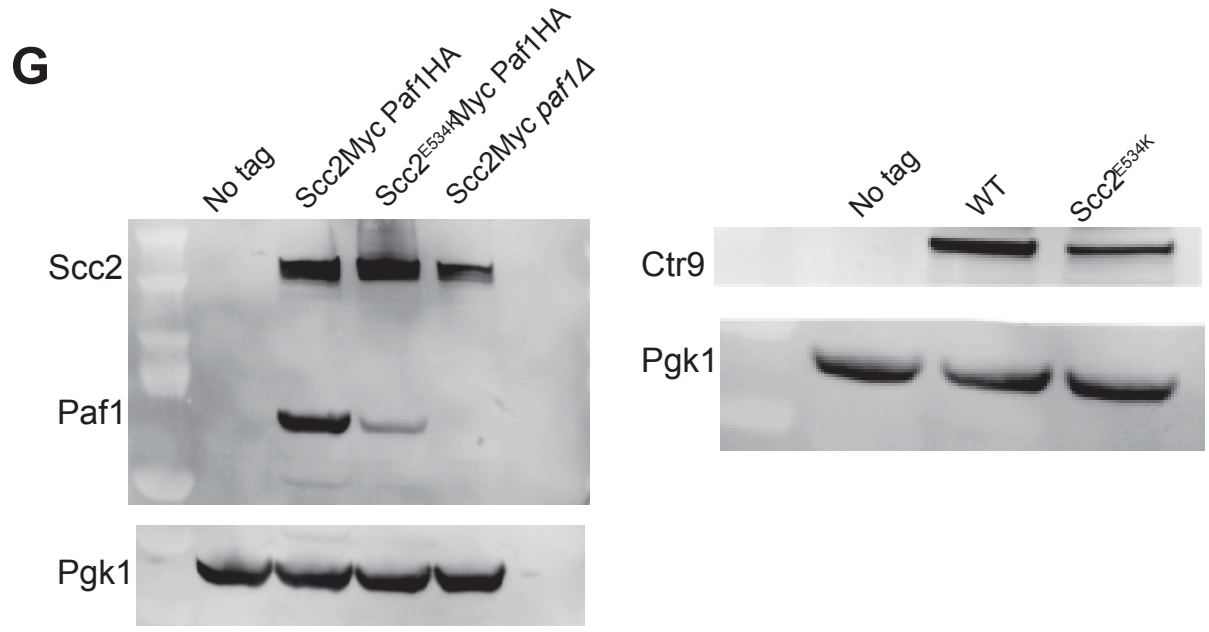


Figure 15. Scc2 and Paf1 recruitment at snoRNAs is co-dependent. WT, *scc2-4,* and *paf1Δ* mutant strains were cultured in YPD media to mid-log phase (~ OD_{600} = 0.5-0.8). Strains were cross-linked and chromatin extracted for ChIP. ChIP/qPCR analysis was carried out for Scc2-Myc and $Scc2^{E534K}$-Myc and Scc2-Myc *paf1Δ*. ChIP experiments were performed at least three times. p-values were calculated using a student t-test. Standard error bars are indicated for n=3. Values different from the WT are indicated by an asterisk (*p<0.05*). (A) ChIP/qPCR analysis shows reduced enrichment of Paf1 at indicated snoRNAs relative to WT (α-HA antibody, 12CA5, Roche). (B) ChIP/qPCR analysis shows reduced recruitment of Scc2-Myc at the indicated snoDNAs in the *paf1Δ* mutant strain relative to WT (α-Myc antibody, 9B11, Cell signaling). (C) WT, *scc2-4,* and *paf1Δ* mutant strains were cultured in YPD media to mid-log phase (~ OD_{600} = 0.5-0.8). Strains were cross-linked and chromatin extracted for ChIP. ChIP analysis was carried out for Scc2-Myc and $Scc2^{E534K}$-Myc and *paf1Δ*Scc2-Myc. (C and D) ChIP/qPCR analysis shows reduced enrichment of Paf1 at indicated rDNA and RP genes relative to WT (α-HA antibody, 12CA5, Roche). (E and F) ChIP/qPCR analysis shows almost normal recruitment of Scc2-Myc at the indicated rDNA and RP genes in the *paf1Δ* mutant strain relative to WT (α-Myc antibody, 9B11, Cell signaling). ChIP experiments were performed at least three times for each experiment. p-values were calculated using a student t-test. Standard error bars are indicated for n=3. Significant values from the WT are indicated by an asterisk (p<0.05). (G) Western blot

analysis shows that the protein level of Paf1 and Ctr9 are reduced in the *scc2-4* mutant. Scc2 levels also appear reduced in the *paf1Δ* strain. Pgk1 served as the loading control.

5. The *scc2-4* mutant has poor translational fidelity

Since mutation of *Scc2* affects ribosome biogenesis, we examined actively translating ribosomes by polysome analysis. Polysome analysis enables us determine the differential translation of individual mRNAs on a genome-wide scale. Polysome analysis of WT and *scc2-4* mutant strains showed almost identical profiles as well as similar polysome to monosome ratios (Figure 16A). To more carefully investigate the effects of *scc2-4* mutation on global protein synthesis, a ^{35}S-incorporation assay was performed. This approach enables us to follow protein synthesis by measuring the amount of incorporated radiolabeled [^{35}S]-methionine in proteins. Only a mild reduction in protein synthesis between WT and the *scc2-4* mutant was observed (Figure 16B). Consistent with a mild defect in protein synthesis, the mutant failed to grow on plates with a sub-lethal concentration of cycloheximide, an inhibitor of protein translation (Figure 16C).

We hypothesized that the down-regulation of box H/ACA snoRNAs observed in the *scc2-4* mutant might phenocopy mutation of *CBF5*. Mutations in *CBF5* show limited effects on translational efficiency, but more major effects on translational fidelity (Jack et al., 2011). We decided to examine translational fidelity in the *scc2-4* mutant. We first tested whether the mutant demonstrates an impaired ability to translate IRES-dependent mRNAs. IRES elements are used by both viral and endogenous genes to promote cap-independent translation initiation (Yoon et al., 2006). The Cricket Paralysis virus (CrPV) IRES has been shown to be active in both yeast and mammalian cells (Jack et al., 2011; Landry et al., 2009) and able to initiate translation by directly recruiting ribosomes to the initiation sites without initiation factors. Defects in ribosome biogenesis can impair cap-independent translation initiation directed by the cricket paralysis (CrPV) IGR IRES elements (Jack et al., 2011; Landry et al., 2009) and in cellular RNAs that utilize the IRES mechanism (Yoon et al., 2006).

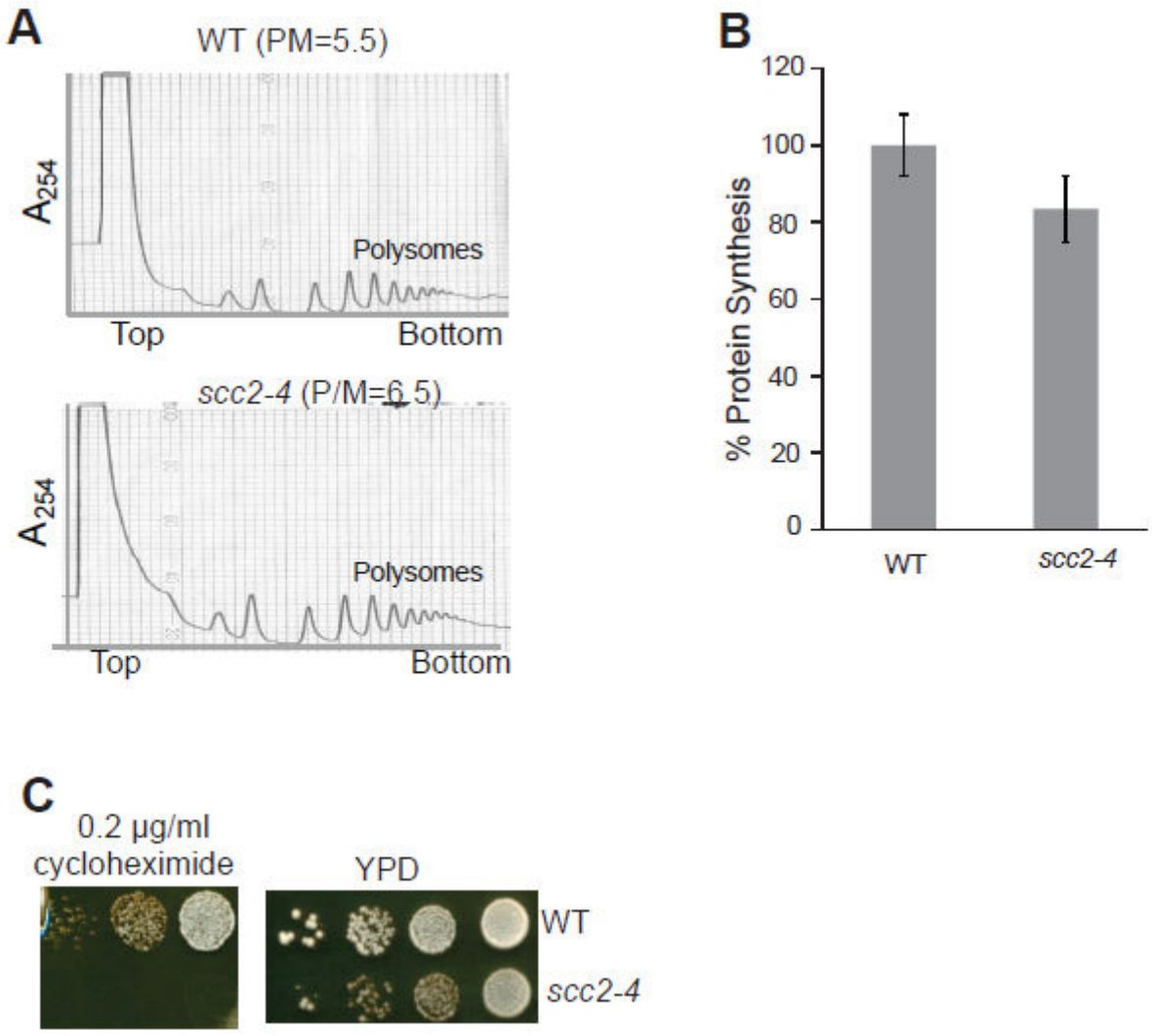


Figure 16. Protein translation is mildly affected in *scc2-4* mutant. (A) Polysome analysis of WT and *scc2-4* mutant strains. Polysome profiles from WT and scc2-4 mutant were collected from cells grown to mid-log phase in YPD+CSM medium. Polysome profiling was done twice with similar results. Profiles were calculated using Mathematica and Image J software. Polysome to monosome ratios (P/M) are indicated for WT and the *scc2-4* mutant. No significant difference in polysome profiles between WT and *scc2-4* mutant was observed. (B) No large change in overall protein translation when ^{35}S-methione labeling experiment was conducted to measure protein synthesis in WT and *scc2-4* mutant. Strains were grown to mid-log phase in SD-met supplemented with ^{35}S-methionine. Cells were lysed, protein precipitated and the amount of incorporated ^{35}S-methionine measured with a scintillation counter. Standard deviation is indicated for n=3. (C) *scc2-4* mutant is

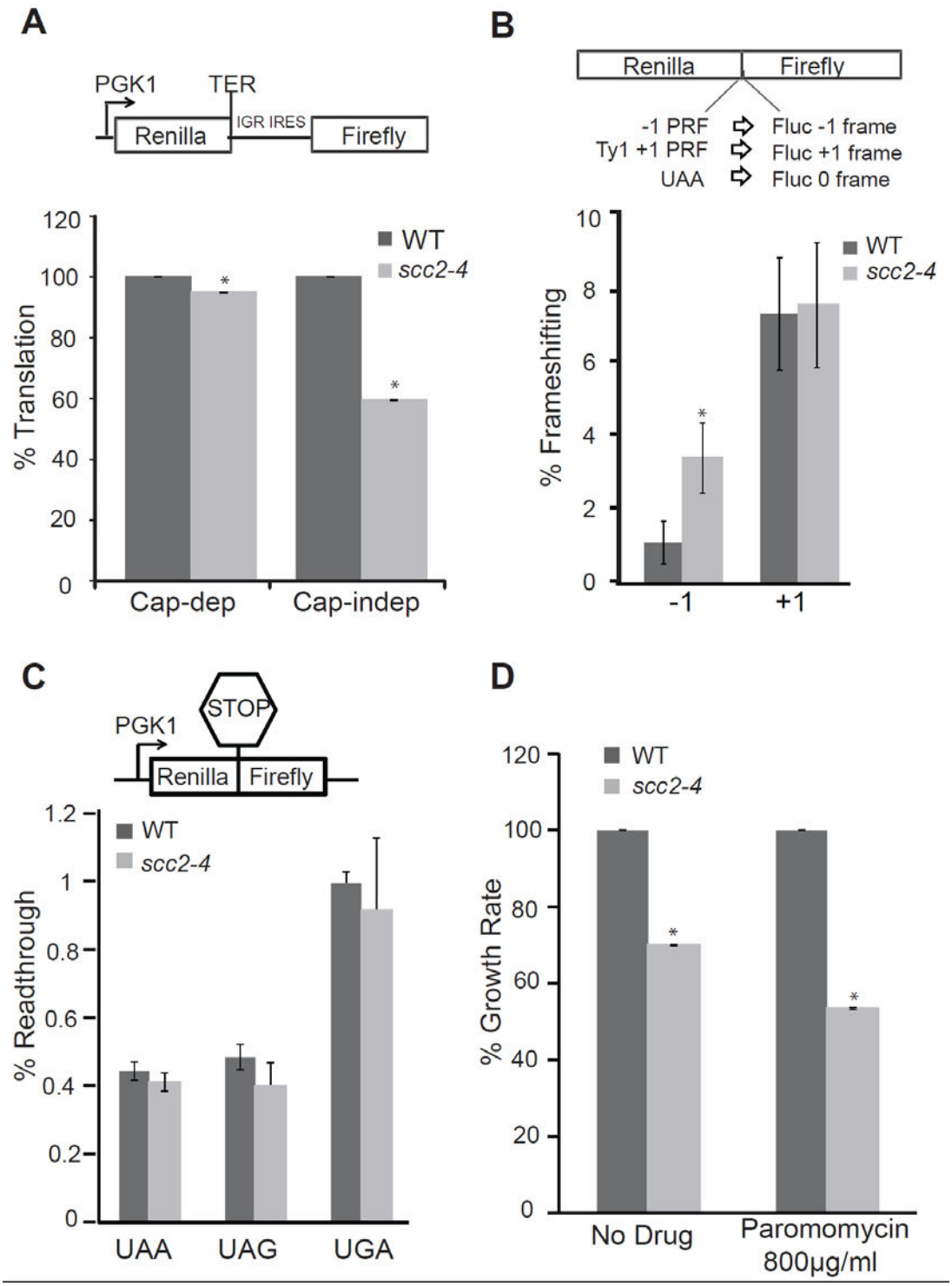
extremely sensitive to low concentration of cycloheximide. Overnight cultures of WT and *scc2-4* mutant were serially diluted and spotted on YPD plates with or without cycloheximide and incubated at 30^{o}C for 3 days. Sensitivity of the *scc2-4* mutant was examined afterwards.

We monitored translation initiation from the CrPV IGR IRES elements using a dual luciferase reporter, which is able to measure translation in vivo in yeast cells (Figure 17). While cap-dependent translation was mildly reduced in the *scc2-4* mutant, consistent with the results of ^{35}S-methionine incorporation assay (Figure 16B), IRES usage was reduced by more than 60% in the *scc2-4* mutant when compared to WT (Figure 17A), implying that Scc2 normally promotes cap-independent translation.

Faithfully maintaining the translational reading frame is one of the important functions of ribosomes. Viral mRNA signals that can induce ribosomes to shift frame by one base either in the (-1) or (+1) direction have been tremendously useful for examining translational fidelity (Dinman and Kinzy, 1997; Jack et al., 2011). We examined whether the *scc2-4* mutant has an elevation in ribosomal frameshifting. Dual luciferase reporters able to detect -1 frameshifting (LA) or +1 frameshifting (*Ty1*) were employed. Scc2 mutation resulted in approximately 3-fold increase in L-A mediated -1 PRF compared to WT (3.4% to 1.1%; *p=0.006*) (Fig. 17B). However, no change in +1 frameshifting was observed. We further examined the efficiency of stop codon recognition using dual luciferase reporters (Fig. 17C) in the *scc2-4* mutant. The *scc2-4* mutant did not show any aberrant stop codon recognition problems when compared to WT. Overall these findings are similar to the changes in translational fidelity caused by mutation of *CBF5* (Jack et al., 2011).

Changes in ribosome-tRNA interactions can affect translational fidelity and therefore influence the sensitivity of yeast to translational inhibitors. Translational inhibitors that specifically bind to the ribosomes serve as useful tools for examining changes in ribosome function. Anisomycin inhibits -1 PRF by interfering with binding of aa-tRNA to the A-site. This drug was used to probe for defects in the A-site of the ribosome. Similarly, paromomycin, which promotes -1 PRF, was used to probe interactions at the decoding center on the small subunit involving the aa-tRNA. While the *scc2-4* mutant was hypersensitive to paromomycin (Fig 17D) as might be

expected given the tendency for -1 frameshifting, inhibition of -1 PRF with anisomycin did not rescue the growth defect observed in the mutant (Figure 17E). The failure of anisomycin to rescue the growth of the *scc2-4* mutant probably reflects the fact that Scc2 contributes to growth in multiple ways. Overall our results are consistent with Scc2 normally promoting the production of rRNA and translational fidelity.


A
PGK1
TER
Renilla
IGR IRES
Firefly
% Translation
120
100
80
60
40
20
0
WT
scc2-4
*
*
Cap-dep
Cap-indep
B
Renilla
Firefly
-1 PRF
Ty1 +1 PRF
UAA
Fluc -1 frame
Fluc +1 frame
Fluc 0 frame
% Frameshifting
10
8
6
4
2
0
WT
scc2-4
*
-1
+1
C
STOP
PGK1
Renilla
Firefly
% Readthrough
1.2
1
0.8
0.6
0.4
0.2
0
WT
scc2-4
UAA
UAG
UGA
D
% Growth Rate
120
100
80
60
40
20
0
WT
scc2-4
*
*
No Drug
Paromomycin
800µg/ml

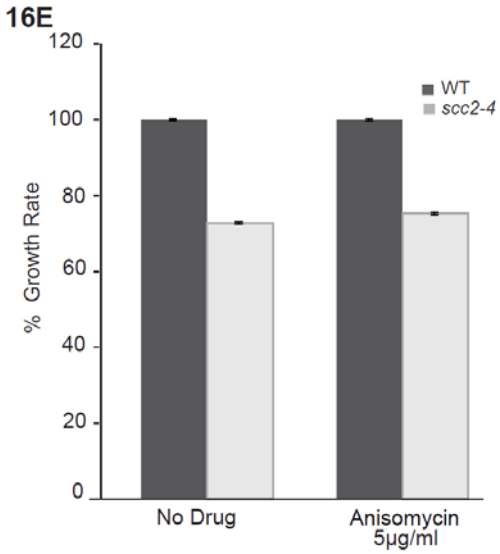


Figure 17. Translational fidelity is reduced in the *scc2-4* mutant. Dual luciferase reporters were used to measure translation. (A, top) Schematic diagram of CrPV IGR IRES containing reporter. Renilla luciferase is translated by a cap-dependent mechanism, and firefly luciferase synthesis requires cap-independent initiation mediated by the IRES. While cap-dependent translation is mildly impaired (*p=0.01*), IRES-dependent translation is more strongly inhibited in the *scc2-4* mutant (*p=0.001*). (B, top) A dual luciferase reporter was used to monitor -1 frameshifting mediated by sequence derived from the yeast L-A -1 virus, and +1 frameshifting promoted by the yeast Ty1 sequence. In-frame renilla luciferase translation serves as a normalization control, and efficiencies were determined as previously described (Landry et al., 2009) (C). Readthrough efficiency for three different stop codons (UAA, UAG, UGA) was measured for the WT and *scc2-4* strains. The translation of renilla luciferase serves as a normalization control. (D) The maximal growth rates of the WT and *scc2-4* mutant strains were compared, with the WT growth rate set to 100%. Strains were grown in YPD, YPD with paromomycin, or YPD with Anisomycin. p-values were calculated using student two-tailed t-test (*= *p<0.05*). Standard error is indicated for at least three independent measurements.

IV. Discussion

In this report, I characterized genic regions controlled by Scc2 and show that Scc2 promotes the normal expression of hundreds of genes. With the notable exception of the centromeric region, Scc2 binding at the promoter regions of ribosomal protein genes, snoDNAs, and transfer DNAs was reduced in the *scc2-4* mutant. At the snoDNAs in particular, lower levels of Scc2 recruitment correlated with lower levels of RNA, and at these genes Scc2 recruitment was partially dependent on the Paf1 transcription complex. We showed that lower levels of H/ACA snoRNAs correlated with reduced pseudouridylation in the *scc2-4* mutant and decreased translational fidelity. Therefore, Scc2 promotes a gene expression program that supports translational fidelity.

1. Scc2 coordinates events during RNA transcription and processing

Studies in human, fly, and yeast genomes show that Scc2 binds to the promoter regions of active genes (D'Ambrosio et al., 2008; Liu et al., 2009; Misulovin et al., 2008; Zuin et al., 2014). However, how Scc2 contributes to the expression of active genes is not well understood. From ChIP-seq analysis, we observed that Scc2 not only binds to the promoter regions of genes, but also in the gene body of snoRNA and tRNA genes (Fig. 2). Binding of Scc2 to both promoter and bodies of genes (in particular the snoDNAs) suggest that its role in regulating transcription might not be restricted to initiation. In support of this hypothesis, we observed that Paf1, a protein required for transcription by RNA Pol II (Sheldon et al., 2005), is significantly reduced in the *scc2-4* mutant. Studies in budding yeast have shown that Paf1 is recruited to the promoter and coding regions of genes and aids in the processing of snoRNA transcripts (Simic et al., 2003). The observation that Scc2 and Paf1 recruitment depend on each other at snoDNAs suggests the hypothesis that Paf1 and Scc2 together promote production of snoRNAs. Paf1 levels are reduced in the *scc2-4* mutant and there is a reduction in ChIP signal at all regions tested, suggesting this could contribute to differential gene expression. The observation that Ctr9 levels is also reduced in the *scc2-4* mutant supports previous findings which showed that, defects in the expression of at least one subunit of the Paf1 complex compromises the stability of other members of the complex (Mueller et al., 2004). Alternatively, the mutation in Scc2 could directly impact the stability of the Paf1 complex, for instance, at shared binding sites. The observation that deletion of Paf1 also affects the protein levels of Scc2 supports the idea that Paf1 and Scc2

are required for the stability of each other. The cooperation of Paf1 and Scc2 could have a wider implication in humans where the Paf1 complex is overexpressed in a wide range of cancers including prostate, breast, renal, and gastric cancers (Chaudhary et al., 2007; Jaehning, 2010).

Different groups of Scc2 target genes show different responses to the loss of binding. This could be due in part to other factors that operate at those gene groups. For instance, Scc2 binding to snoDNAs appears to depend on Paf1, but this is not the case at other regions of the genome such rDNA and RP genes. Instead, RP genes are expressed at higher levels with reduced Scc2 binding. The elevation may be due to competition between cohesin binding and RNA pol II binding at these promoters. As the *scc2-4* mutant decreases cohesin loading, it may allow increased access of RNA polymerase II (Patrick Grant, personal communication).

2. Mutations that affect cohesin result in differential gene expression and translation in budding yeast

The *scc2-4* mutant has translation defects. Some phenotypes are similar to what we previously reported in the acetyltransferase mutant, *eco1-W216G* (Bose et al., 2012). The *scc2-4* and *eco1-W216G* mutants share defects in nucleolar morphology, ribosome distribution, and rRNA production. In both cases, these phenotypes could result from the lack of cohesin and condensin at the rDNA. However, a closer look reveals significant differences in both gene expression and translational processes in the two mutants. For example, only the *scc2-4* mutation is associated with reduced levels of box H/ACA snoRNAs and poor translational fidelity as well as elevated levels of ribosomal protein gene messages. The acetyltransferase mutation has reduced levels of ribosomal protein gene messages, increased levels of the stress-induced transcription factor Gcn4, and a pronounced effect on translational efficiency. Reduced translational efficiency is observed in cells derived from RBS patients and in zebrafish models for CdLS, and these cohesinopathies models are partially rescued by stimulating translation (Xu et al., 2013; Xu et al., 2014b). We speculate that the differences in gene expression between Scc2 and Eco1 mutants might be based in the molecular mechanisms by which they influence gene expression. While Scc2 may maintain nucleosome-free regions and promote cohesin and condensin loading, Eco1 helps to maintain a high level of stable cohesin. Loss of function mutations in these two genes may have different outcomes for transcription. This is consistent with mutations in these two genes causing distinct human syndromes.

3. Cohesinopathies and ribosomopathies have similar characteristics

Ribosomopathies are diseases caused by mutations that affect ribosome biogenesis. One such disease is DKC. Reduced pseudouridylation of RNA is part of the etiology of DKC, a ribosomopathy characterized by bone marrow failure, skin abnormalities, and increased susceptibility to cancer (Dokal, 2000; Yoon et al., 2006). Mutations in a pseudouridine synthase that modifies rRNAs and other RNAs (*DKC1*/*CBF5*) cause some cases of DKC. Studies from X-DC patient lymphoblasts and fibroblasts show down-regulation of Box H/ACA snoRNAs (Bellodi et al., 2013), a characteristic feature in *scc2-4* mutant. Interestingly, the *scc2-4* mutant mimics DKC1 mutants, which have impaired ability to translate IRES containing genes. Our results suggest that the *scc2-4* mutation reduces rRNA modification by affecting the transcription of H/ACA snoRNAs, which are important for pseudouridylation resulting in translational fidelity problems. We previously reported that human RBS cells and zebrafish models of RBS have defects in ribosome biogenesis and protein synthesis (Bose et al., 2012; Xu et al., 2013), suggesting that RBS is at least in part a ribosomopathy. Zebrafish models for CdLS also show reduced translation (Xu et al., 2014b). Our findings suggest a shared feature of cohesinopathies and ribosomopathies is defective translation. Translation might therefore serve as a therapeutic target for CdLS.

CHAPTER 3

I. Discussion

In this study, I characterized the role of the SMC loader Scc2 in gene regulation and translational fidelity. I report that loss a of function mutation in Scc2 results in the misregulation of hundreds of genes. Among the genes affected were those important for ribosome biogenesis, RNA processing, translation, and mitochondrial function. The *scc2* mutation was associated with reduced ribosomal RNA production. Closer examination of the gene expression profile revealed that ncRNAs (in particular snoRNAs) were downregulated, and these may represent direct targets. Down-regulation of snoRNAs was associated with reduced pseudouridylation of rRNAs and translational defects in the mutant scc2. Finally, we show that Scc2 acts as a "co-activator" with the Paf1 complex in promoting the production of snoRNAs.

1. Scc2 links transcription initiation and elongation

One of the interesting findings from this study is that Scc2 is important for transcription elongation, at least at snoRNA genes. Most studies of the role of the SMC loader Scc2 in transcription regulation have focused on transcription initiation. To date, very little is known about the role of Scc2 in transcription elongation. Our observation that Scc2 promotes transcription elongation, coupled with findings in *Drosophila* and mouse ESCs, supports the idea that the role of Scc2 in promoting transcription elongation is evolutionarily conserved from yeast to mouse. In addition, the genes bound by NIPBL in mammalian cells, such as genes involved in ribosome biogenesis, are functionally similar to the genes bound by Scc2 in our study. Scc2 could promote the release of paused RNA Polymerase II into productive elongation at these genes. It is important to note, however, that RNA Polymerase II pausing has so far not been observed in budding yeast. Collectively, our findings support the promoter-elongation model of how Scc2 may be regulating gene expression. What is currently unknown is whether the role of Scc2 in recruiting the Paf1 complex to active genes is evolutionarily conserved. It would also be interesting to know whether Scc2 is required for the recruitment of other members of elongation complexes such as FACT (facilitates chromatin transcription) and DSIF (DRB Sensitivity Including Factor) complexes and vice versa. These studies would shed further light on the role of Scc2 in transcription.

While Scc2 and Paf1 act as transcriptional coactivators at certain genomic locations, including snoDNAs, such a function was not observed at other regions, for example at the rDNA and other Pol II target genes. This dependence at snoDNAs can be explained in a number of ways; (1) Scc2 and Paf1 make independent contacts with RNA Polymerase II to promote elongation, although this hypothesis is highly unlikely since Scc2 has not been shown to interact with RNA polymerase II (2) Binding of Scc2 may bend DNA to facilitate the function of Paf1, which interacts with RNA polymerase II to promote transcription, and 3) Scc2 binding may reposition Paf1 from a location where it is unable to facilitate transcription elongation to a position where it is able to promote transcription elongation.

Another interesting observation was the reduced levels of Paf1 complex proteins in mutant scc2. Deletion of Paf1 also affected the protein levels of Scc2. Reduced protein levels of Paf1 complex and Scc2 could be caused by many factors. Scc2 and Paf1 may interact with each other, and hence the deletion of one protein may compromise the stability of the other members of the complex. This instability is observed in the Paf1 complex, where deletion of the Paf1 subunit compromises the stability of other proteins in the complex. Reduced protein levels of Paf1 may also arise because Scc2 mutation compromises general protein translation which could manifest as reduced Paf1 levels in mutant *scc2*.

2. Competition between RNA Pol II and cohesin loading at RP genes

At H/ACA snoRNAs, reduced binding of mutant scc2 correlates with down-regulation of snoRNA. In contrast, RP genes expression is upregulated (Figure 18), while Scc2 binding is reduced (Figure 6B, and Figure 19). This observation suggests that Scc2 may be regulating gene expression in a context dependent manner. At snoRNAs, Scc2 could have a positive effect on transcription by holding promoters in a nucleosome-free state, which helps maintain an open chromatin conformation and therefore, promote both transcriptional initiation and elongation (Dorsett, 2009; Lopez-Serra et al., 2014).

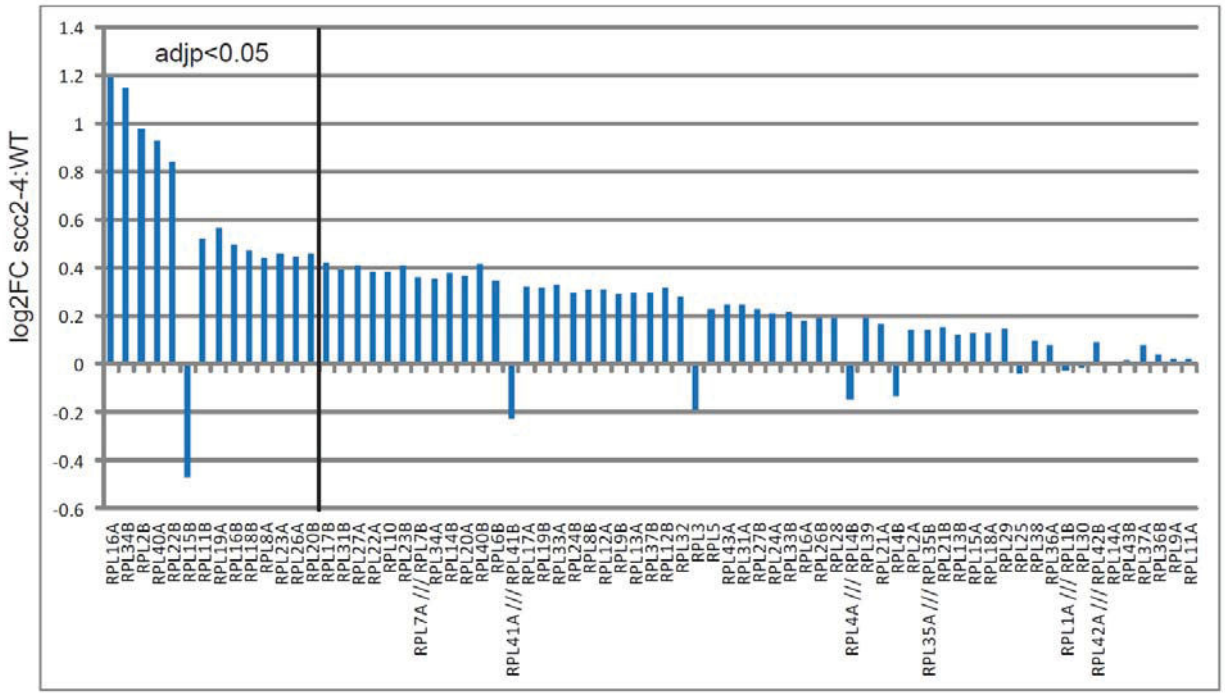


Figure 18a. Expression levels of small ribosomal subunits (RPS) are upregulated in the yeast mutant *scc2-4*. The relative expression levels (mean of triplicate samples) of RPS genes in wild type and mutant *scc2* was examined using RNA-seq.

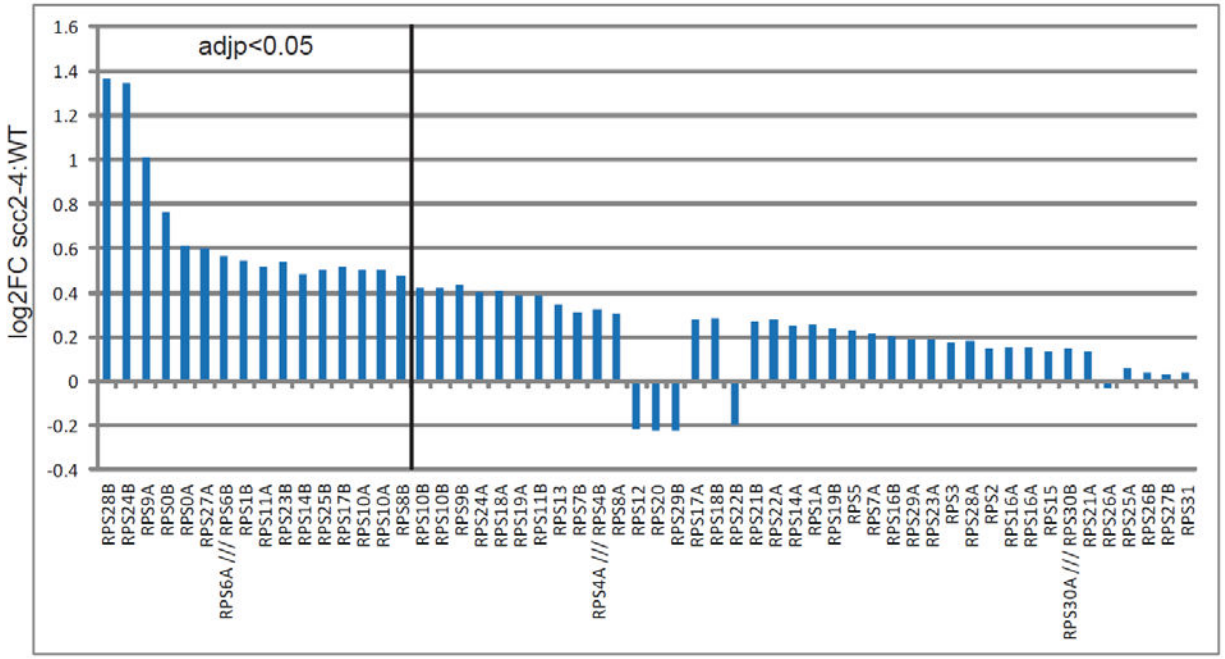


Figure 18b. Expression levels of large ribosomal subunits (RPL) are upregulated in the mutant *scc2-4*. The relative expression levels (mean of triplicate samples) of RPS genes in wild type and mutant *scc2-4* were examined using RNA-seq.

In contrast, Scc2 could influence the expression of RP genes through competition between RNA Polymerase II and cohesin. The promoters of RP genes are highly enriched with histone H3 Threonine 45 phosphorylation (H3T45ph) marks, and phosphorylation of these sites is carried out by the kinase complex Cdc7-Dbf4 (Baker et al., 2010). H3T45 phosphorylation, mediated by Cdc7-Dbf4, is important for RNA Polymerase II recruitment and full expression of RP genes (Grant, submitted). Interestingly, Scc2 binding sites also correlate with H3T45ph enriched regions of the genome. Metagene analysis of Scc2 binding from ChIP experiments shows that RP genes with the H3T45ph marks have a higher occupancy of Scc2 at the promoters (Figure 19, compare blue curve to purple). This effect is also observed with the mutant Scc2 protein (Figure 19, compare orange curve to green). This observation suggests that the H3T45ph marks correlate with more Scc2 binding. As Scc2 is recruited to nucleosome-free regions (NFRs) by the RSC complex (Sth1-3) (Lopez-Serra et al., 2014), Scc2 binding at NFRs may promote loading of the cohesin complex which could potentially influence transcription of RP genes by acting as a road-block to RNA Polymerase II. In this way, it may promote competition between cohesin binding and RNA Polymerase II binding at these promoters. As the *scc2-4* mutant decreases cohesin loading, it may allow access of RNA polymerase II (Figure 19) (Grant, submitted), resulting in the increase in RP gene expression observed. Another possibility is that the mutation triggers some pathway activation or repression that controls this gene group.

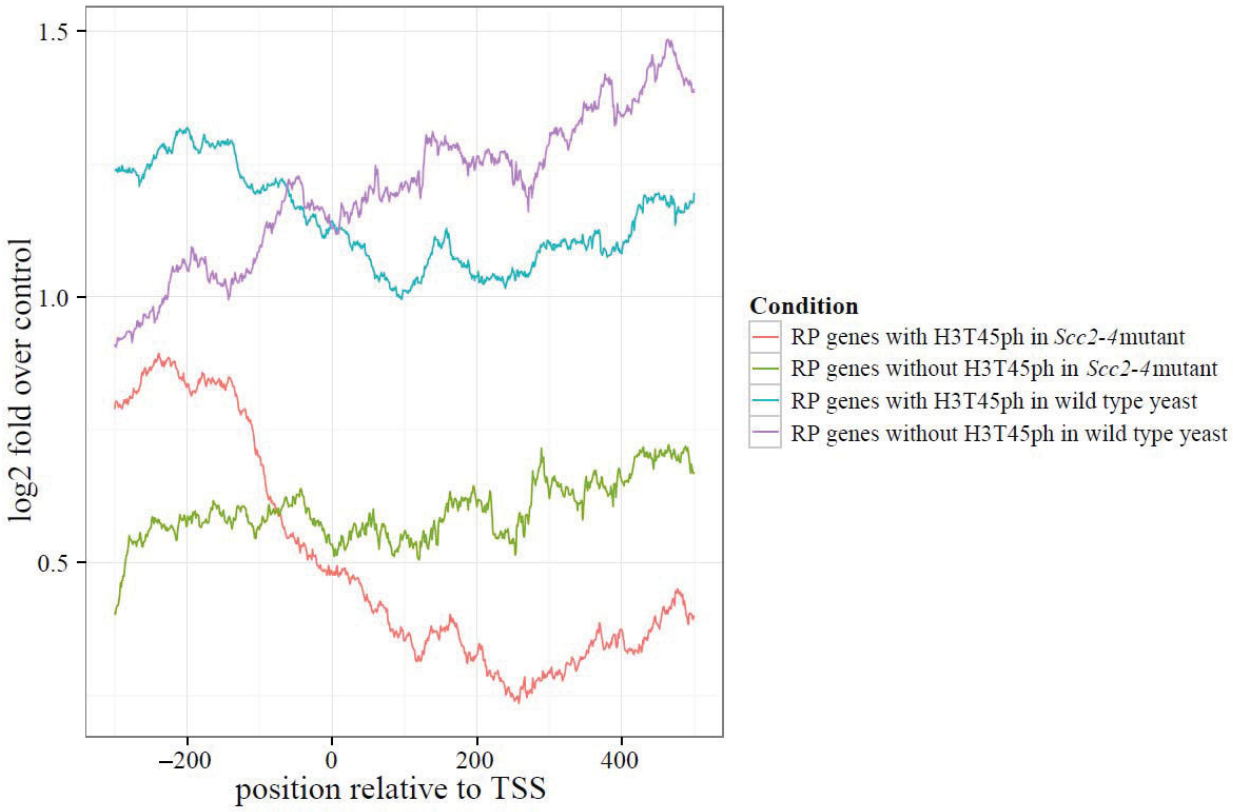


Figure 19. Mutation in Scc2 results in reduced occupancy at promoter regions of RP genes. ChIP-seq analysis was performed in mutant *scc2-4* and WT. The occupancy of Scc2 in WT and mutant *scc2-4* relative to the transcription start site (TSS) in log2 scale is shown in the metagene analysis for RP genes that bear phosphorylation of the T45 residue of histone H3. Histone H3 was used as control.

3. Implications for cancer and developmental diseases

The observation that Scc2 plays a functional role in promoting translational fidelity has wider implications for cancer and other developmental disorders. A growing number of diseases are caused by alterations in diverse components that function in the translation of mRNAs. While some of these diseases arise from mutations in components of the translation machinery such as ribosomes and tRNAs, others arise from mutations in specific mRNAs that affect translation. Loss of IRES-mediated translation is observed in mutant *scc2*, similar to what is observed in DKC1 mutants. Zebrafish models of CdLS also have defects in the phosphorylation of RPS6, and 4EBP1. The importance of Scc2 in the translation of specific mRNAs suggests that mutations in cohesin subunits could compromise the translation of mRNAs critical for development.

Another interesting observation from this study is the finding that Scc2 and Paf1 cooperate to regulate gene expression. The Paf1 complex is important for maintaining embryonic stem cell pluripotency and expression of genes involved in lineage specification (Ding et al., 2009). Paf1 is overexpressed in various cancers. Germline mutation in *CTR9*, a member of the PAF1 complex, causes Wilm's tumor in humans (Hanks et al., 2014). In zebrafish, the *paf1* complex is important for proper development of heart, ears, and neural crest cells (Akanuma et al., 2007; Langenbacher et al., 2011; Nguyen et al., 2010). Therefore, mutations in Scc2 may compromise the ability of Paf1 to regulate biological processes important for development and vice versa. Depending on the context, this defect could compromise development of body tissues or activate pathways that promote cancer development or progression.

EM analysis revealed that the nucleolus was disrupted in the yeast mutant *scc2*. The nucleolus is the site of rDNA transcription by RNA polymerase I and ribosome biogenesis. Scc2 binds to rDNA repeats, which form loops. These loops contribute to replication, and transcription. Scc2 may therefore help maintain the integrity of the genome through the organization of nucleolus. Mutations that compromise this function could affect gene expression, which results in developmental defects in patients.

4. Scc2 is important for mitochondrial function

Scc2 was also found to be important for mitochondrial function. Since the oxidative phosphorylation system is under the control of both the mitochondrial and nuclear genome, Scc2 may be important for the transcription of nuclear encoded genes needed for mitochondrial function. This hypothesis is further supported in humans, where mutation in mitochondrial ribosomal protein *MRPS22* results in a CdLS-like phenotype, with defects such as brain abnormalities and hypertrophic cardiomyopathy (Smits et al., 2011). Impaired mitochondrial gene expression is also observed in patients with myelodysplasia syndromes and acute myeloid leukemia (AML). Preliminary studies in cells from mouse *Nipbl* +/- morphants in our laboratory also show that cytochrome c oxidase activity is reduced (unpublished). Since partial loss of function mutations in cohesin subunit proteins cause AML, it is possible to speculate that mutations that compromise Scc2 function may affect mitochondria function, which could cause developmental defects and/or cancer in humans. It would be interesting to decipher how Scc2 regulates the expression of nuclear genes with mitochondria function. This would enable us to better understand the molecular etiology of these diseases.

5. Scc2 is important for splicing efficiency

Examination of the gene expression profile showed reduced splicing efficiency in the yeast mutant *scc2*. Scc2 binds to genes encoding spliceosomal RNAs, such as *SNR6*, and binding is reduced in the mutant scc2. The reduced binding of Scc2 correlates with reduced expression of *SNR6*, suggesting that Scc2 is important for their transcription. In addition, Pus1p and Pus7p, which catalyze pseudouridylation of spliceosomal snRNAs (RNA independent mechanism), are also downregulated in the mutant *scc2*. Surprisingly, mutations in the splicing factor *SFPQ* have also been in reported in acute AML (Dolnik et al., 2012), and account for 10% of AML patients. Given the importance of Box H/ACA RNPs in spliceosomal snRNA modification and pre-mRNA splicing, it is possible to hypothesize that defective spliceosomal snRNA pseudouridylation and for that matter splicing could also contribute to misregulated gene expression, which could be affecting translation in the yeast mutant *scc2*. A splicing defect could contribute to the pathogenesis of DKC and possibly cohesinopathies.

Our findings shed light on some aspect of the mechanisms by which cohesin might regulate transcription and translation. Our current findings are consistent with a role of Scc2 in transcription elongation.

II. Follow up studies

To further test our model, we propose several additional experiments. We would like to know if Scc2 directly recruits the Paf1 complex onto DNA. This experiment would be performed using Co-IP or in vitro binding assays. We would also like to know if Scc2 is required for the recruitment of other transcription elongation factors such as FACT (facilitates chromatin transcription) and DSIF complexes. These experiments would be conducted using ChIP-qPCR and Co-IP experiments. We would also like to test this idea in mouse and human cells to examine whether the role of Scc2 in elongation is evolutionarily conserved.

Recurring mutations in cohesin subunits *STAG2*, *SMC3*, *RAD21*, *NIPBL*, and *SMC1A* are observed in cancer and cohesinopathies. Studies of cohesin mutants in humans and zebrafish also show that translation is compromised (Bose et al., 2012; Xu et al., 2014b). However, it is still uncertain whether mutations in *NIPBL* affect general protein synthesis or alter the translation of specific mRNAs in humans, which could contribute to the development of these diseases. The effect of *NIPBL* mutation on global translation could be examined through Polysome profiling analysis and 35S-methionine incorporation assays in human and mouse cells. To examine if *NIPBL* mutation affects translation initiation, dicistronic reporters containing the CrPV IGR IRES inserted between *Renilla* and *Firefly* luciferase ORFs could be transformed into wild type and CdLS patient cell lines and luciferase activity measured. This way cap-dependent and cap-independent translation would be examined.

Preliminary studies in the *Nipbl*+/- mouse model show reduced expression of noncoding RNAs important for the modification of RNAs. We currently do not know if down-regulation of these noncoding RNAs is the cause of developmental defects observed in CdLS mouse models, similar to what is observed in DKC. Reduced modification could impair the expression of genes in Nipbl +/- mouse genome at posttranscriptional level, and is consistent with defects in translational defects in CdLS model organisms. To explore this hypothesis, we would like to use tissue-

specific knockout of these noncoding RNAs to examine their effect on development. This approach would enable us better understand the molecular etiology of CdLS.

In summary, this study has uncovered a hitherto unknown function of Scc2 in transcription and translational processes in budding yeast. Our findings show that, at least in budding yeast, Scc2 plays a very important role in gene activation and transcription elongation. Scc2 regulates gene expression in a context dependent manner. The transcriptional processes affected when Scc2 is mutated have a profound impact on translational fidelity, supporting the hypothesis that developmental defects observed in *NIPBL* mutants are in part caused by compromised translation. Interestingly, these translational defects are hallmarks of Scc2 mutants in other eukaryotes such as mouse. However, it is yet to be understood how translational defects ultimately result in the development defects observed in diseases. Understanding how translational defects contribute to CdLS would bring us ever closer to understanding the etiology of cohesinopathies.

CHAPTER 4

Materials and Methods

I. Yeast strains

All strains were derived from BY4741 (*MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0*). The *scc2-4* mutation was originally isolated by the Nasmyth laboratory (Michaelis et al., 1997). We constructed *scc2-4* mutant strain in the laboratory by PCR amplifying the mutation fused to a drug resistance marker from yeast genomic DNA, transforming the PCR product into the desired strain background, and isolating temperature sensitive colonies. The presence of the mutation was confirmed by sequencing.

II. Measurement of RNA synthesis in vivo

Ribosomal RNA production was examined as previously described with some modifications (Snyder et al., 2009). Briefly, duplicate cultures of WT and *scc2-4* mutant strains were grown to mid-log phase (OD_{600}=0.3) in SD-ura medium supplemented with 6.7ng/µl uracil. ^{3}H-uridine (5 µCi) was added to 500 µL of each culture and incubated at 30°C for 5 min with aeration. Samples were treated with 2.5 mL 10% trichloroacetic acid (TCA) and 2.5 mg/ml of uridine. After filtration through nitrocellulose, each membrane was washed with 5% TCA, dried, and counted in a Beckman LS 6500 multipurpose scintillation counter.

III. Metabolic labeling-protein

WT and *scc2-4* strains were grown to mid-log phase in YPD+CSM medium. Cells were pelleted, washed in PBS, and resuspended in a similar volume of pre-warmed SD-met medium. Aliquot for zero time point was taken. Samples were then supplemented with 27.5 µCi ^{35}S-methionine and 1mg/ml unlabeled-methionine. Samples were withdrawn at 15 min intervals for 2 hr. Amount of incorporated ^{35}S-methionine in proteins was measured by adapting Kang and Hershey approach (Kang and Hershey, 1994). Briefly, cells were lysed in 1.8 N NaOH buffer containing 0.2 M β-mercaptoethanol. Proteins were precipitated with hot 10% TCA acid and precipitate washed twice in acetone. The precipitates were dissolved in 1% SDS and boiled for 10 min. Aliquots of samples were counted for ^{35}S-methionine incorporation using a scintillation counter.

IV. rRNA processing

WT and *scc2-4* mutants strains were grown in SD-methionine medium to mid-log phase (OD_{600}=0.3). Cells were pulse-labeled for 2 min with 250 μCi/ml ^{3}H-methylmethionine and chased with 5mM cold methionine. Samples were removed at 0, 2, 5 and 15 min intervals and flash-frozen in liquid nitrogen. RNA was extracted from cells, run on 1% formaldehyde agarose gel, transferred to a Hybond-N^{+} nylon membrane (GE Healthcare), and detected by autoradiography.

V. RNA sequencing

WT and *scc2-4* mutant strains were grown in triplicate to mid-log phase (~OD_{600}= 0.8) in YPD+CSM medium. Cells were pelleted and RNA was extracted. RNA integrity was checked following isolation using an Agilent Technologies 2100 Bioanalyzer and a 1.2% formaldehyde agarose gel. Samples were depleted of ribosomal RNA using the Epicentre Ribo-zero Gold kit (human/mouse/rat, cat# RZG1224) according to manufacturer's protocol. Libraries for sequencing were prepared using the Illumina Truseq RNA library preparation kit (cat# RS-930-2001) according to the manufacturer's protocol. Libraries were pooled and run on an agarose gel, size selected from 200-400bp (with adapters), and run on the Hi-seq. Reads were mapped to saccCer2 using tophat to generate bam alignment files. Gene coordinates from ensembl biomart were then iterated over to count the number of reads mapping to each feature. These counts were then used to generate gene expression coefficients and statistics using the DESeq package in R. The GO term enrichment analysis was carried out using the hypergeometric test as implemented in the GeneAnswers package from Bioconductor. For the metagene analysis, reads were extended to 150 base pairs before calculating coverage. Coverage was adjusted to reads per million. For a group of genes, RPM coverage was extracted for 600 bp on either side of the TSS and the values were averaged per base pair for the group.

VI. Splicing analysis

The generated fastq files from RNA sequencing were aligned using tophat (Langmead et al., 2009) against the *S. cerevisiae* genome assembly EF4 and gene annotation from the Ensembl release 69 (Oct. 2012) with the default options. Between 17.4M and 26.0M reads were aligned with 82.2% to 85.1% uniquely mapped reads per library. Splicing efficiency (expressed as

Percent Spliced Out or PSO) was measured by computing the mean nucleotide coverage over every spliced junctions divided by the mean nucleotide coverage for reads falling within the exonic splicing unit (i.e. counting all the reads overlapping within the two exons flaking the splice junctions and dividing by the length of the two exons). Significant changes in PSO were computed by performing ANOVA for each splicing units. The computed probabilities of the difference between the means where adjusted for multiple testing using the Benjamini and Hochberg method.

VII. Chromatin immunoprecipitation (ChIP)

Genome-wide chromatin immunoprecipitation sequencing (ChIP-seq) was performed as previously described (Glynn et al., 2004). Briefly, yeast strains were cultured in duplicate in YPD to mid-log phase (OD_{600}=0.8). Strains were cross-linked for either 15 min or 2 hrs (for Scc2 ChIP) with 1% formaldehyde at room temperature with occasional swirling. Cultures were pelleted, washed with cold PBS, and resuspended in PBS and placed at 4^{o}C overnight. Cells were spheroplasted with 2.5mg/ml zymolyase. Spheroplasts were washed and sonicated in SDS lysis buffer (1 mM PMSF, 0.8µg/ml pepstatin A, 0.6 µg/ml leupeptin) to between 300-1000 bp in length. Chromatin extracts were diluted in immunoprecipitation (IP) buffer, debris was pelleted, and the supernatant was decanted into conical tubes. This chromatin solution was aliquoted for IPs. Chromatin extracts were immunoprecipitated overnight with antibody at 4^{o}C followed by several wash step, and DNA was recovered in 1%SDS/0.1 M $NaHCO_3$ elution buffer. Crosslinking was reversed by incubation at 65^{o}C overnight, followed by protease treatment, phenol chloroform extraction, and ethanol precipitation of the recovered DNA. IPs were performed in duplicate for each sample. Sequence libraries were constructed and validated. Reads were mapped to saccCer2 using tophat to generate bam alignment files. Antibodies used for ChIP experiments are as follows: Myc antibody from Cell signaling (Cat #2276), HA antibody (12CA5) from Roche, and RNA Polymerase II CTD4H8 antibody from Millipore (4 ul per 1mg protein IP).

VIII. Polysome analysis

Polysome analysis was done as previously described (Landry et al., 2009). Briefly, yeast strains were grown in synthetic minimal medium to mid-log phase (OD_{600}=0.8). Cycloheximide was

added to the cultures to a final concentration of 100µg/ml and cultured for additional 15 min. Cells were harvested by centrifugation (7,250 rpm in a SLA-1500 rotor) for 5 min at 4°C and washed once with ice cold lysis buffer (80 µg/mL cycloheximide, 200 µg/mL heparin, 0.2% DEPC, and 10 mM Tris-HCl pH 7.5, 0.1M NaCl, 30mM $MgCl_2$). Cells were resuspended in lysis buffer after centrifugation and lysed in baked acid-washed glass beads. Lysates were cleared by centrifugation and loaded onto 7%-47% sucrose gradients (50mM NH4Cl, 50mM Tris-Acetate pH 7.0 and 12mM $MgCl_2$, 1mM DTT) at 4°C. Gradients were processed by centrifugation and in a Beckmann SW40 rotor at 13,000 rpm for 150 min at 4°C. Fractions were collected, and the A_{254} was recorded using ISCO UA-5 absorbance monitor (Teledyne).

IX. Microscopy and cytometry

Accumulation of ribosomal protein GFP fusion proteins in strains was measured with spinning disc confocal microscope (Zeiss). The GFP intensity in WT and *scc2-4* mutant strains was quantified as previously described (Bose et al., 2012). Briefly, peak GFP fluorescence intensity in each cell was calculated by measuring the pulse height in the cell using a B1 detector (525/50 emission). For each sample, approximately 10,000 cells were measured. To quantify the distance between the empirical distribution function of two samples, the Kolmogorov-Smirnov (KS) statistic was used. This statistic enables us to calculate the distance between two biological replicates (same genotype) and between samples with different genotypes since the distribution of fluorescence intensity among GFP positive cells is non-Gaussian. Using KS-distance enables us to determine whether the distance between WT and mutant samples is significantly greater than between replicates.

X. Luciferase assays

The IRES and frameshifting assays were performed as previously described (Deniz et al., 2009; Landry et al., 2009). Briefly, yeast strains were transformed with the indicated reporter plasmids. To examine IRES activity, overnight cultures of strains harboring CrPV IGR IRES and mutant cc-gg constructs were subcultured in SD-leu media to mid-log phase (approx. OD_{600}=0.5 to 1.0). Cells were lysed with 100 µl 1X passive lysis buffer (PLB) for 2 min. Dual luciferase assay kit (Promega) was used to measure luminescence according to manufacturer's protocol. IRES

activity was measured using Firefly/Renilla ratio normalized to the Firefly/Renilla ratio of the wild type strain.

The frameshifting and readthrough assays were conducted with dual luciferase constructs reporters (a generous gift from Dori Landry) (Landry et al., 2009). Translational fidelity assays employed the dual-luciferase reporter system using pJD375 (the 0-frame control), pJD377 (containing the Ty1 +1 PRF signal), pJD376 (containing the yeast L-A virus -1 PRF signal). Luciferase activities were determined as previously described (Jacobs and Dinman, 2004). To measure frameshifting, firefly/renilla luciferase ratio of the frameshifting reporter was divided by the firefly/renilla luciferase ratio of the control which has both luciferases in the same reading frame and lacks frameshifting signal. For readthrough assay, overnight cultures of strains transformed with dual luciferase construct were subcultured and grown to mid-log phase, pelleted, lysed with 1X passive lysis buffer, and dual luciferase assay performed in triplicates according to manufacturer's protocol (Promega). Percentage readthrough was then calculated as previously described (Landry et al., 2009).

XI. Antibiotic sensitivity

Growth and temperature sensitivity assays were performed using 10-fold serial dilutions of cells spotted onto the indicated medium and incubated at 30ºC for 2-3 days. Maximal growth rates and sensitivity to translational inhibitors were determined using the TECAN machine in YPD media containing 5μg/mL anisomycin, or 800μg/mL paromomycin.

XII. Western blotting

Western blot experiments were conducted using TCA precipitation as previously described (Kim and Koepp, 2012; Kim et al., 2012). Briefly, overnight cultures were subcultured in YPD medium until mid-log phase (OD_{600}=0.6). Cell were pelleted and washed 2 times in 20% tricholoroacetic acid (TCA). Cells were resuspended in residual TCA and glass beads were added. Cells were vortexed for 4 min at 3000g and spun for 5 min at 14000 rpm. To each sample, 100 μl sample loading buffer was added and vortexed. Tris-HCl pH 8.8 or more was added and vortexed until the color changed to purple blue. Samples were boiled at 95^{o}C for 5 min and loaded onto an SDS-PAGE gel.

XIII. Pseudouridylation assay

Pseudouridylation assay was performed as previously described with a few modifications (Bakin and Ofengand, 1993).

1. Reaction with CMC

Overnight cultures of yeast strains were subcultured and grown to mid-log phase (OD_{600}=0.6). Cells were pelleted and RNA isolated from strains. RNA was dried and 8µg RNA sample were treated with 30µl of 0.17M *N-cyclohexyl-N'-β-*(4-methylmorpholinium)-ethylcarbodiimide p-*tosylate* (CMC) in 50 mM Bicine, pH 8.3, 4 mM EDTA, and 7 M urea at 37^{o}C for 20 min. Reaction was stopped with 100 µl of 0.3 M Sodium acetate (NaOAc) and 0.1 mM EDTA, pH 5.6 (buffer A) and 700ul 100% ethanol. Pellets were recovered, washed with 70% ethanol, dissolved in 100 µl buffer A, and reprecipitated with 300 µl 100% ethanol. Pellets were dried, dissolved in 40 µl 50 mM Na_2CO_3, pH 10.4, and incubated at 37^{o}C for 2 hrs. RNA was then precipitated with 100 µl buffer A and 700 µl of ethanol. Pellets were washed twice with 70% ethanol, dried, and dissolved in 40 µl water.

XIV. Reverse transcription and gel analysis

RNA was reversed transcribed using 5'-end labeled primers. Hybridization was performed in a mixture containing 8 µg RNA, 4 * 10^5 cpm/pmol 5'-labeled primer, and hybridization buffer (50mM Tris-HCl, pH 8.5, 20mM KCl) in a total volume of 5 µl. The mixture was incubated at 70^{o}C for 3 min, transferred to a 37^{o}C for 5 min, and then chilled on ice for 2 min or longer. Extension reaction was performed by adding 5 µl of a mixture containing 100 mM Tris-HCl, pH 8.5, 20 mM $MgCl_2$, 20 mM DTT, 5 nM each of dATP, dGTP, dCTP, and dTTP, and 1.6 units of reverse transcriptase to the hybridization mixture and incubated at 37^{o}C for 30 min. The extension reaction was stopped by adding 2 µl of a solution containing 0.5 mg/ml RNase A and 0.1 M EDTA, pH 6.5 and incubated at 37^{o}C for 45 min. The sample mixture was then precipitated with 3 volumes of ethanol, dried, and dissolved in 15 µl 75% Formamide, 1.4 mM Tris-HCl, pH 7.4, 1.4 mM EDTA, and 0.08% each of xylene cyanol FF and bromophenol blue. Samples were run on 8% polyacrylamide-7 M urea gel and developed with phosphoImager screens. Oligonucleotides for monitoring pseudouridylation were designed as previously described (Ganot et al., 1997).

XV. Chromatin fractionation

Cells were grown in YPD at 30°C to approximate OD_{600}=0.8 and harvested by centrifugation. Cells were washed in 100 ml cold water and SB buffer (20 mM Tris, 1M sorbitol). Cell pellets were resuspended in 2 ml SB, frozen in liquid nitrogen, and stored at -80oC. Cells were thawed the following day on ice and spun down. Cells were resuspended in 2 ml PSB buffer (200 mM Tris, 20mM EDTA, 1M NaCl, 100 mM β-Me) and incubated at room temperature for 10 min. Cells were collected by centrifugation, washed in wash buffer (20 mM Tris, 1M NaCl) and SB.Cells were again resuspended in in 2 ml SB with 40 ul glusulase, incubated 30°C 1 hr to spheroplast, and centrifuge at 7000rpm for 7 min and pellet was washed 2X in SB. Pellets were resuspended in 4 ml EBX-0.1 (20 mM Tris, 0.1 M NaCl, 0.25% triton X-100, 15 mM β-Me, protease inhibitors), 20% Triton X-100 added to a final concentration of 0.5% (50 ul). Mixture was gently swirled for 10 min on ice and lysates were layered on 10 ml cushion of NIB (20 mM Tris, 0.1 M NaCl, 1.2 M sucrose, 15 mM B-Me) and centrifuged at 12,000 g for 20 min. Nuclear pellet was resuspended in 1.8 ml EBX-0.1 (with protease inhibitors). Nuclei was lysed by 20% Triton to a final concentration of 1% (0.1 ml), swirled on ice 15 min and centrifuged at 15,000 g for 20 min (save 30 ul out of 2 ml as soluble nuclear proteins). Pellets were each resuspended in 0.2 ml EBX-0.1 (0.5 mM B-Me). Nuclear lysate (15 ul/0.75%/1.5), soluble nuclear proteins (30 ul, 1.5%/3), and pellet (30ul/ 15%/30) were loaded for westerning and probed with Anti-HA (Paf1), anti-Myc (Scc2).

XVI. Cytochrome c oxidase assay

Mitochondria were isolated according manufacturers protocol (Sigma, Catalog No# MITOISO3). Briefly, WT and mutant *scc2* were grown in YPD medium to mid-log phase (approx. OD_{600}=0.8). Cells were pelleted, washed in water, and resuspended in 2 ml 1X Buffer A. Cells were incubated for 15 minutes at 30 °C with gentle shaking, centrifuged at 1,500g for 5 minutes and supernatant discarded. Cell pellets were resuspended in 1 ml 1X Buffer B. 10 ml of the resuspended cell sample were added to 990 ml of ultrapure water and OD read at 600 nm. ODs of the cell suspensions were used as a reference values. Lyticase solution was added to sample suspensions. Cells were incubated at 30 °C with gentle shaking and OD measured every 5 min during incubation period. 10 ml of the samples was added to 990 ml of ultrapure water and OD

read at 600 nm. When the OD decreased to 30–40% of the initial value (pre-lysed sample), reaction was stopped by centrifuging at 1,200g for 5 minutes at 4°C. After centrifugation, supernatants were discarded. Samples were then kept on ice. Spheroplasts were mixed to a uniform suspension in 1 ml Lysis Buffer and incubated on ice for 5 minutes. During the 5 minute incubation, spheroplasts were mixed every minute by a single inversion of the tube. At the end of the incubation period, 2 volumes of 1X Storage Buffer were added and samples were centrifuged at 600 g for 10 minutes at 4 °C. Supernatant liquid was carefully transferred to a fresh tube and centrifuged at 6,500g for 10 minutes at 4 °C. Supernatant was carefully removed and discarded. Pellet was resuspended in 1X storage buffer. Cytochrom c oxidase activity was measured using manufacturers protocol (Sigma, Catalog No# CYTOCOX1).

VI. References

Akanuma, T., Koshida, S., Kawamura, A., Kishimoto, Y., Takada, S., 2007. Paf1 complex homologues are required for Notch-regulated transcription during somite segmentation. EMBO Rep 8, 858-863.

Alter, B.P., Giri, N., Savage, S.A., Rosenberg, P.S., 2009. Cancer in dyskeratosis congenita. Blood 113, 6549-6557.

Arumugam, P., Gruber, S., Tanaka, K., Haering, C.H., Mechtler, K., Nasmyth, K., 2003. ATP hydrolysis is required for cohesin's association with chromosomes. Current biology : CB 13, 1941-1953.

Baker, S.P., Phillips, J., Anderson, S., Qiu, Q., Shabanowitz, J., Smith, M.M., Yates, J.R., 3rd, Hunt, D.F., Grant, P.A., 2010. Histone H3 Thr 45 phosphorylation is a replication-associated post-translational modification in S. cerevisiae. Nature cell biology 12, 294-298.

Bakin, A., Ofengand, J., 1993. Four newly located pseudouridylate residues in Escherichia coli 23S ribosomal RNA are all at the peptidyltransferase center: analysis by the application of a new sequencing technique. Biochemistry 32, 9754-9762.

Barber, T.D., McManus, K., Yuen, K.W., Reis, M., Parmigiani, G., Shen, D., Barrett, I., Nouhi, Y., Spencer, F., Markowitz, S., Velculescu, V.E., Kinzler, K.W., Vogelstein, B., Lengauer, C., Hieter, P., 2008. Chromatid cohesion defects may underlie chromosome instability in human colorectal cancers. Proceedings of the National Academy of Sciences of the United States of America 105, 3443-3448.

Beckouet, F., Hu, B., Roig, M.B., Sutani, T., Komata, M., Uluocak, P., Katis, V.L., Shirahige, K., Nasmyth, K., 2010. An Smc3 acetylation cycle is essential for establishment of sister chromatid cohesion. Molecular cell 39, 689-699.

Bellodi, C., Krasnykh, O., Haynes, N., Theodoropoulou, M., Peng, G., Montanaro, L., Ruggero, D., 2010. Loss of function of the tumor suppressor DKC1 perturbs p27 translation control and contributes to pituitary tumorigenesis. Cancer research 70, 6026-6035.

Bellodi, C., McMahon, M., Contreras, A., Juliano, D., Kopmar, N., Nakamura, T., Maltby, D., Burlingame, A., Savage, S.A., Shimamura, A., Ruggero, D., 2013. H/ACA small RNA dysfunctions in disease reveal key roles for noncoding RNA modifications in hematopoietic stem cell differentiation. Cell reports 3, 1493-1502.

Bellows, A.M., Kenna, M.A., Cassimeris, L., Skibbens, R.V., 2003. Human EFO1p exhibits acetyltransferase activity and is a unique combination of linker histone and Ctf7p/Eco1p chromatid cohesion establishment domains. Nucleic acids research 31, 6334-6343.

Bernard, P., Maure, J.F., Partridge, J.F., Genier, S., Javerzat, J.P., Allshire, R.C., 2001. Requirement of heterochromatin for cohesion at centromeres. Science 294, 2539-2542.

Beygo, J., Citro, V., Sparago, A., De Crescenzo, A., Cerrato, F., Heitmann, M., Rademacher, K., Guala, A., Enklaar, T., Anichini, C., Cirillo Silengo, M., Graf, N., Prawitt, D., Cubellis, M.V., Horsthemke, B., Buiting, K., Riccio, A., 2013. The molecular function and clinical phenotype of partial deletions of the IGF2/H19 imprinting control region depends on the spatial arrangement of the remaining CTCF-binding sites. Human molecular genetics 22, 544-557.

Birkenbihl, R.P., Subramani, S., 1992. Cloning and characterization of rad21 an essential gene of Schizosaccharomyces pombe involved in DNA double-strand-break repair. Nucleic acids research 20, 6605-6611.

Blat, Y., Kleckner, N., 1999. Cohesins bind to preferential sites along yeast chromosome III, with differential regulation along arms versus the centric region. Cell 98, 249-259.

Boeger, H., Griesenbeck, J., Strattan, J.S., Kornberg, R.D., 2003. Nucleosomes unfold completely at a transcriptionally active promoter. Molecular cell 11, 1587-1598.

Borck, G., Zarhrate, M., Bonnefont, J.P., Munnich, A., Cormier-Daire, V., Colleaux, L., 2007. Incidence and clinical features of X-linked Cornelia de Lange syndrome due to SMC1L1 mutations. Human mutation 28, 205-206.

Borges, V., Lehane, C., Lopez-Serra, L., Flynn, H., Skehel, M., Rolef Ben-Shahar, T., Uhlmann, F., 2010. Hos1 deacetylates Smc3 to close the cohesin acetylation cycle. Molecular cell 39, 677-688.
Bose, T., Lee, K.K., Lu, S., Xu, B., Harris, B., Slaughter, B., Unruh, J., Garrett, A., McDowell, W., Box, A., Li, H., Peak, A., Ramachandran, S., Seidel, C., Gerton, J.L., 2012. Cohesin proteins promote ribosomal RNA production and protein translation in yeast and human cells. PLoS genetics 8, e1002749.
Carlile, T.M., Rojas-Duran, M.F., Zinshteyn, B., Shin, H., Bartoli, K.M., Gilbert, W.V., 2014. Pseudouridine profiling reveals regulated mRNA pseudouridylation in yeast and human cells. Nature.
Cena, A., Skoneczny, M., Chelstowska, A., Kowalec, P., Natorff, R., Kurlandzka, A., 2013. Cohesin Irr1/Scc3 is likely to influence transcription in Saccharomyces cerevisiae via interaction with Mediator complex. Acta Biochim. Pol. 60, 233-238.
Chan, K.L., Roig, M.B., Hu, B., Beckouet, F., Metson, J., Nasmyth, K., 2012. Cohesin's DNA exit gate is distinct from its entrance gate and is regulated by acetylation. Cell 150, 961-974.
Chaudhary, K., Deb, S., Moniaux, N., Ponnusamy, M.P., Batra, S.K., 2007. Human RNA polymerase II-associated factor complex: dysregulation in cancer. Oncogene 26, 7499-7507.
Ciosk, R., Shirayama, M., Shevchenko, A., Tanaka, T., Toth, A., Shevchenko, A., Nasmyth, K., 2000. Cohesin's binding to chromosomes depends on a separate complex consisting of Scc2 and Scc4 proteins. Molecular cell 5, 243-254.
Conaway, R.C., Conaway, J.W., 2013. The Mediator complex and transcription elongation. Biochimica et biophysica acta 1829, 69-75.
D'Ambrosio, C., Schmidt, C.K., Katou, Y., Kelly, G., Itoh, T., Shirahige, K., Uhlmann, F., 2008. Identification of cis-acting sites for condensin loading onto budding yeast chromosomes. Genes & development 22, 2215-2227.
D'Amours, D., Jackson, S.P., 2002. The Mre11 complex: at the crossroads of dna repair and checkpoint signalling. Nat. Rev. Mol. Cell Biol. 3, 317-327.
Deardorff, M.A., Bando, M., Nakato, R., Watrin, E., Itoh, T., Minamino, M., Saitoh, K., Komata, M., Katou, Y., Clark, D., Cole, K.E., De Baere, E., Decroos, C., Di Donato, N., Ernst, S., Francey, L.J., Gyftodimou, Y., Hirashima, K., Hullings, M., Ishikawa, Y., Jaulin, C., Kaur, M., Kiyono, T., Lombardi, P.M., Magnaghi-Jaulin, L., Mortier, G.R., Nozaki, N., Petersen, M.B., Seimiya, H., Siu, V.M., Suzuki, Y., Takagaki, K., Wilde, J.J., Willems, P.J., Prigent, C., Gillessen-Kaesbach, G., Christianson, D.W., Kaiser, F.J., Jackson, L.G., Hirota, T., Krantz, I.D., Shirahige, K., 2012a. HDAC8 mutations in Cornelia de Lange syndrome affect the cohesin acetylation cycle. Nature 489, 313-317.
Deardorff, M.A., Kaur, M., Yaeger, D., Rampuria, A., Korolev, S., Pie, J., Gil-Rodriguez, C., Arnedo, M., Loeys, B., Kline, A.D., Wilson, M., Lillquist, K., Siu, V., Ramos, F.J., Musio, A., Jackson, L.S., Dorsett, D., Krantz, I.D., 2007. Mutations in cohesin complex members SMC3 and SMC1A cause a mild variant of cornelia de Lange syndrome with predominant mental retardation. American journal of human genetics 80, 485-494.
Deardorff, M.A., Wilde, J.J., Albrecht, M., Dickinson, E., Tennstedt, S., Braunholz, D., Monnich, M., Yan, Y., Xu, W., Gil-Rodriguez, M.C., Clark, D., Hakonarson, H., Halbach, S., Michelis, L.D., Rampuria, A., Rossier, E., Spranger, S., Van Maldergem, L., Lynch, S.A., Gillessen-Kaesbach, G., Ludecke, H.J., Ramsay, R.G., McKay, M.J., Krantz, I.D., Xu, H., Horsfield, J.A., Kaiser, F.J., 2012b. RAD21 mutations cause a human cohesinopathy. American journal of human genetics 90, 1014-1027.
Decatur, W.A., Fournier, M.J., 2003. RNA-guided nucleotide modification of ribosomal and other RNAs. The Journal of biological chemistry 278, 695-698.
Deniz, N., Lenarcic, E.M., Landry, D.M., Thompson, S.R., 2009. Translation initiation factors are not required for Dicistroviridae IRES function in vivo. Rna 15, 932-946.
Ding, L., Paszkowski-Rogacz, M., Nitzsche, A., Slabicki, M.M., Heninger, A.K., de Vries, I., Kittler, R., Junqueira, M., Shevchenko, A., Schulz, H., Hubner, N., Doss, M.X., Sachinidis, A., Hescheler, J., Iacone, R., Anastassiadis, K., Stewart, A.F., Pisabarro, M.T., Caldarelli, A., Poser, I., Theis, M., Buchholz, F., 2009. A

genome-scale RNAi screen for Oct4 modulators defines a role of the Paf1 complex for embryonic stem cell identity. Cell Stem Cell 4, 403-415.
Dinman, J.D., Kinzy, T.G., 1997. Translational misreading: mutations in translation elongation factor 1alpha differentially affect programmed ribosomal frameshifting and drug sensitivity. Rna 3, 870-881.
Dokal, I., 2000. Dyskeratosis congenita in all its forms. British journal of haematology 110, 768-779.
Dolnik, A., Engelmann, J.C., Scharfenberger-Schmeer, M., Mauch, J., Kelkenberg-Schade, S., Haldemann, B., Fries, T., Kronke, J., Kuhn, M.W., Paschka, P., Kayser, S., Wolf, S., Gaidzik, V.I., Schlenk, R.F., Rucker, F.G., Dohner, H., Lottaz, C., Dohner, K., Bullinger, L., 2012. Commonly altered genomic regions in acute myeloid leukemia are enriched for somatic mutations involved in chromatin remodeling and splicing. Blood 120, e83-92.
Donze, D., Adams, C.R., Rine, J., Kamakaka, R.T., 1999. The boundaries of the silenced HMR domain in Saccharomyces cerevisiae. Genes & development 13, 698-708.
Dorsett, D., 2009. Cohesin, gene expression and development: lessons from Drosophila. Chromosome research : an international journal on the molecular, supramolecular and evolutionary aspects of chromosome biology 17, 185-200.
Dorsett, D., Krantz, I.D., 2009. On the molecular etiology of Cornelia de Lange syndrome. Annals of the New York Academy of Sciences 1151, 22-37.
Dowen, J.M., Bilodeau, S., Orlando, D.A., Hubner, M.R., Abraham, B.J., Spector, D.L., Young, R.A., 2013. Multiple structural maintenance of chromosome complexes at transcriptional regulatory elements. Stem cell reports 1, 371-378.
Ebmeier, C.C., Taatjes, D.J., 2010. Activator-Mediator binding regulates Mediator-cofactor interactions. Proceedings of the National Academy of Sciences of the United States of America 107, 11283-11288.
Fay, A., Misulovin, Z., Li, J., Schaaf, C.A., Gause, M., Gilmour, D.S., Dorsett, D., 2011. Cohesin selectively binds and regulates genes with paused RNA polymerase. Current biology : CB 21, 1624-1634.
Filippova, G.N., Fagerlie, S., Klenova, E.M., Myers, C., Dehner, Y., Goodwin, G., Neiman, P.E., Collins, S.J., Lobanenkov, V.V., 1996. An exceptionally conserved transcriptional repressor, CTCF, employs different combinations of zinc fingers to bind diverged promoter sequences of avian and mammalian c-myc oncogenes. Molecular and cellular biology 16, 2802-2813.
Fryns, J.P., 1986. On the nosology of the Cornelia de Lange and Coffin-Siris syndromes. Clinical genetics 29, 263-264.
Galande, S., Purbey, P.K., Notani, D., Kumar, P.P., 2007. The third dimension of gene regulation: organization of dynamic chromatin loopscape by SATB1. Current opinion in genetics & development 17, 408-414.
Gandhi, R., Gillespie, P.J., Hirano, T., 2006. Human Wapl is a cohesin-binding protein that promotes sister-chromatid resolution in mitotic prophase. Current biology : CB 16, 2406-2417.
Ganot, P., Bortolin, M.L., Kiss, T., 1997. Site-specific pseudouridine formation in preribosomal RNA is guided by small nucleolar RNAs. Cell 89, 799-809.
Gard, S., Light, W., Xiong, B., Bose, T., McNairn, A.J., Harris, B., Fleharty, B., Seidel, C., Brickner, J.H., Gerton, J.L., 2009. Cohesinopathy mutations disrupt the subnuclear organization of chromatin. The Journal of cell biology 187, 455-462.
Gerton, J.L., 2012. Translational mechanisms at work in the cohesinopathies. Nucleus 3, 520-525.
Gimigliano, A., Mannini, L., Bianchi, L., Puglia, M., Deardorff, M.A., Menga, S., Krantz, I.D., Musio, A., Bini, L., 2012. Proteomic profile identifies dysregulated pathways in Cornelia de Lange syndrome cells with distinct mutations in SMC1A and SMC3 genes. Journal of proteome research 11, 6111-6123.
Gligoris, T.G., Scheinost, J.C., Burmann, F., Petela, N., Chan, K.L., Uluocak, P., Beckouet, F., Gruber, S., Nasmyth, K., Lowe, J., 2014. Closing the cohesin ring: structure and function of its Smc3-kleisin interface. Science 346, 963-967.

Glynn, E.F., Megee, P.C., Yu, H.G., Mistrot, C., Unal, E., Koshland, D.E., DeRisi, J.L., Gerton, J.L., 2004. Genome-wide mapping of the cohesin complex in the yeast Saccharomyces cerevisiae. PLoS biology 2, E259.
Gordillo, M., Vega, H., Jabs, E.W., 1993. Roberts Syndrome, in: Pagon, R.A., Adam, M.P., Ardinger, H.H., Bird, T.D., Dolan, C.R., Fong, C.T., Smith, R.J.H., Stephens, K. (Eds.), GeneReviews(R), Seattle (WA).
Gordillo, M., Vega, H., Trainer, A.H., Hou, F., Sakai, N., Luque, R., Kayserili, H., Basaran, S., Skovby, F., Hennekam, R.C., Uzielli, M.L., Schnur, R.E., Manouvrier, S., Chang, S., Blair, E., Hurst, J.A., Forzano, F., Meins, M., Simola, K.O., Raas-Rothschild, A., Schultz, R.A., McDaniel, L.D., Ozono, K., Inui, K., Zou, H., Jabs, E.W., 2008. The molecular mechanism underlying Roberts syndrome involves loss of ESCO2 acetyltransferase activity. Human molecular genetics 17, 2172-2180.
Gruber, S., Arumugam, P., Katou, Y., Kuglitsch, D., Helmhart, W., Shirahige, K., Nasmyth, K., 2006. Evidence that loading of cohesin onto chromosomes involves opening of its SMC hinge. Cell 127, 523-537.
Gruber, S., Haering, C.H., Nasmyth, K., 2003. Chromosomal cohesin forms a ring. Cell 112, 765-777.
Guacci, V., Koshland, D., 2012. Cohesin-independent segregation of sister chromatids in budding yeast. Molecular biology of the cell 23, 729-739.
Guacci, V., Koshland, D., Strunnikov, A., 1997. A direct link between sister chromatid cohesion and chromosome condensation revealed through the analysis of MCD1 in S. cerevisiae. Cell 91, 47-57.
Gullerova, M., Proudfoot, N.J., 2008. Cohesin complex promotes transcriptional termination between convergent genes in S. pombe. Cell 132, 983-995.
Hadjur, S., Williams, L.M., Ryan, N.K., Cobb, B.S., Sexton, T., Fraser, P., Fisher, A.G., Merkenschlager, M., 2009. Cohesins form chromosomal cis-interactions at the developmentally regulated IFNG locus. Nature 460, 410-413.
Haering, C.H., Lowe, J., Hochwagen, A., Nasmyth, K., 2002. Molecular architecture of SMC proteins and the yeast cohesin complex. Molecular cell 9, 773-788.
Hakimi, M.A., Bochar, D.A., Schmiesing, J.A., Dong, Y., Barak, O.G., Speicher, D.W., Yokomori, K., Shiekhattar, R., 2002. A chromatin remodelling complex that loads cohesin onto human chromosomes. Nature 418, 994-998.
Han, M., Grunstein, M., 1988. Nucleosome loss activates yeast downstream promoters in vivo. Cell 55, 1137-1145.
Hanks, S., Perdeaux, E.R., Seal, S., Ruark, E., Mahamdallie, S.S., Murray, A., Ramsay, E., Del Vecchio Duarte, S., Zachariou, A., de Souza, B., Warren-Perry, M., Elliott, A., Davidson, A., Price, H., Stiller, C., Pritchard-Jones, K., Rahman, N., 2014. Germline mutations in the PAF1 complex gene CTR9 predispose to Wilms tumour. Nat Commun 5, 4398.
Hansen, J.C., 2002. Conformational dynamics of the chromatin fiber in solution: determinants, mechanisms, and functions. Annu Rev Biophys Biomol Struct 31, 361-392.
Harris, B., Bose, T., Lee, K.K., Wang, F., Lu, S., Ross, R.T., Zhang, Y., French, S.L., Beyer, A.L., Slaughter, B.D., Unruh, J.R., Gerton, J.L., 2014. Cohesion promotes nucleolar structure and function. Molecular biology of the cell 25, 337-346.
Hartley, P.D., Madhani, H.D., 2009. Mechanisms that specify promoter nucleosome location and identity. Cell 137, 445-458.
Hauf, S., Roitinger, E., Koch, B., Dittrich, C.M., Mechtler, K., Peters, J.M., 2005. Dissociation of cohesin from chromosome arms and loss of arm cohesion during early mitosis depends on phosphorylation of SA2. PLoS biology 3, e69.
Heidinger-Pauli, J.M., Mert, O., Davenport, C., Guacci, V., Koshland, D., 2010. Systematic reduction of cohesin differentially affects chromosome segregation, condensation, and DNA repair. Current biology : CB 20, 957-963.

Horsfield, J.A., Anagnostou, S.H., Hu, J.K., Cho, K.H., Geisler, R., Lieschke, G., Crosier, K.E., Crosier, P.S., 2007. Cohesin-dependent regulation of Runx genes. Development 134, 2639-2649.
Hou, F., Zou, H., 2005. Two human orthologues of Eco1/Ctf7 acetyltransferases are both required for proper sister-chromatid cohesion. Molecular biology of the cell 16, 3908-3918.
Hu, B., Itoh, T., Mishra, A., Katoh, Y., Chan, K.L., Upcher, W., Godlee, C., Roig, M.B., Shirahige, K., Nasmyth, K., 2011. ATP hydrolysis is required for relocating cohesin from sites occupied by its Scc2/4 loading complex. Current biology : CB 21, 12-24.
Huang, C.E., Milutinovich, M., Koshland, D., 2005. Rings, bracelet or snaps: fashionable alternatives for Smc complexes. Philos Trans R Soc Lond B Biol Sci 360, 537-542.
Huang, J., Hsu, J.M., Laurent, B.C., 2004. The RSC nucleosome-remodeling complex is required for Cohesin's association with chromosome arms. Molecular cell 13, 739-750.
Ivanov, D., Schleiffer, A., Eisenhaber, F., Mechtler, K., Haering, C.H., Nasmyth, K., 2002. Eco1 is a novel acetyltransferase that can acetylate proteins involved in cohesion. Current biology : CB 12, 323-328.
Izban, M.G., Luse, D.S., 1992. Factor-stimulated RNA polymerase II transcribes at physiological elongation rates on naked DNA but very poorly on chromatin templates. The Journal of biological chemistry 267, 13647-13655.
Jack, K., Bellodi, C., Landry, D.M., Niederer, R.O., Meskauskas, A., Musalgaonkar, S., Kopmar, N., Krasnykh, O., Dean, A.M., Thompson, S.R., Ruggero, D., Dinman, J.D., 2011. rRNA pseudouridylation defects affect ribosomal ligand binding and translational fidelity from yeast to human cells. Molecular cell 44, 660-666.
Jacobs, J.L., Dinman, J.D., 2004. Systematic analysis of bicistronic reporter assay data. Nucleic acids research 32, e160.
Jaehning, J.A., 2010. The Paf1 complex: platform or player in RNA polymerase II transcription? Biochimica et biophysica acta 1799, 379-388.
Jahnke, P., Xu, W., Wulling, M., Albrecht, M., Gabriel, H., Gillessen-Kaesbach, G., Kaiser, F.J., 2008. The Cohesin loading factor NIPBL recruits histone deacetylases to mediate local chromatin modifications. Nucleic acids research 36, 6450-6458.
Jenuwein, T., Allis, C.D., 2001. Translating the histone code. Science 293, 1074-1080.
Kagey, M.H., Newman, J.J., Bilodeau, S., Zhan, Y., Orlando, D.A., van Berkum, N.L., Ebmeier, C.C., Goossens, J., Rahl, P.B., Levine, S.S., Taatjes, D.J., Dekker, J., Young, R.A., 2010. Mediator and cohesin connect gene expression and chromatin architecture. Nature 467, 430-435.
Kang, H.A., Hershey, J.W., 1994. Effect of initiation factor eIF-5A depletion on protein synthesis and proliferation of Saccharomyces cerevisiae. The Journal of biological chemistry 269, 3934-3940.
Kaur, M., DeScipio, C., McCallum, J., Yaeger, D., Devoto, M., Jackson, L.G., Spinner, N.B., Krantz, I.D., 2005. Precocious sister chromatid separation (PSCS) in Cornelia de Lange syndrome. American journal of medical genetics. Part A 138, 27-31.
Kim, D.H., Koepp, D.M., 2012. Hect E3 ubiquitin ligase Tom1 controls Dia2 degradation during the cell cycle. Molecular biology of the cell 23, 4203-4211.
Kim, D.H., Zhang, W., Koepp, D.M., 2012. The Hect domain E3 ligase Tom1 and the F-box protein Dia2 control Cdc6 degradation in G1 phase. The Journal of biological chemistry 287, 44212-44220.
Kim, J., Guermah, M., McGinty, R.K., Lee, J.S., Tang, Z., Milne, T.A., Shilatifard, A., Muir, T.W., Roeder, R.G., 2009. RAD6-Mediated transcription-coupled H2B ubiquitylation directly stimulates H3K4 methylation in human cells. Cell 137, 459-471.
Kim, J., Guermah, M., Roeder, R.G., 2010. The human PAF1 complex acts in chromatin transcription elongation both independently and cooperatively with SII/TFIIS. Cell 140, 491-503.
King, T.H., Liu, B., McCully, R.R., Fournier, M.J., 2003. Ribosome structure and activity are altered in cells lacking snoRNPs that form pseudouridines in the peptidyl transferase center. Molecular cell 11, 425-435.

Kitamura, T., Inoue, D., Okochi-Watanabe, N., Kato, N., Komeno, Y., Lu, Y., Enomoto, Y., Doki, N., Uchida, T., Kagiyama, Y., Togami, K., Kawabata, K.C., Nagase, R., Horikawa, S., Hayashi, Y., Saika, M., Fukuyama, T., Izawa, K., Oki, T., Nakahara, F., Kitaura, J., 2014. The molecular basis of myeloid malignancies. Proc Jpn Acad Ser B Phys Biol Sci 90, 389-404.
Klein, F., Mahr, P., Galova, M., Buonomo, S.B., Michaelis, C., Nairz, K., Nasmyth, K., 1999. A central role for cohesins in sister chromatid cohesion, formation of axial elements, and recombination during yeast meiosis. Cell 98, 91-103.
Knezetic, J.A., Luse, D.S., 1986. The presence of nucleosomes on a DNA template prevents initiation by RNA polymerase II in vitro. Cell 45, 95-104.
Kondrashov, N., Pusic, A., Stumpf, C.R., Shimizu, K., Hsieh, A.C., Xue, S., Ishijima, J., Shiroishi, T., Barna, M., 2011. Ribosome-mediated specificity in Hox mRNA translation and vertebrate tissue patterning. Cell 145, 383-397.
Koshland, D., Strunnikov, A., 1996. Mitotic chromosome condensation. Annual review of cell and developmental biology 12, 305-333.
Krantz, I.D., McCallum, J., DeScipio, C., Kaur, M., Gillis, L.A., Yaeger, D., Jukofsky, L., Wasserman, N., Bottani, A., Morris, C.A., Nowaczyk, M.J., Toriello, H., Bamshad, M.J., Carey, J.C., Rappaport, E., Kawauchi, S., Lander, A.D., Calof, A.L., Li, H.H., Devoto, M., Jackson, L.G., 2004. Cornelia de Lange syndrome is caused by mutations in NIPBL, the human homolog of Drosophila melanogaster Nipped-B. Nature genetics 36, 631-635.
Krogan, N.J., Kim, M., Ahn, S.H., Zhong, G., Kobor, M.S., Cagney, G., Emili, A., Shilatifard, A., Buratowski, S., Greenblatt, J.F., 2002. RNA polymerase II elongation factors of Saccharomyces cerevisiae: a targeted proteomics approach. Molecular and cellular biology 22, 6979-6992.
Kueng, S., Hegemann, B., Peters, B.H., Lipp, J.J., Schleiffer, A., Mechtler, K., Peters, J.M., 2006. Wapl controls the dynamic association of cohesin with chromatin. Cell 127, 955-967.
Laloraya, S., Guacci, V., Koshland, D., 2000. Chromosomal addresses of the cohesin component Mcd1p. The Journal of cell biology 151, 1047-1056.
Landry, D.M., Hertz, M.I., Thompson, S.R., 2009. RPS25 is essential for translation initiation by the Dicistroviridae and hepatitis C viral IRESs. Genes & development 23, 2753-2764.
Langenbacher, A.D., Nguyen, C.T., Cavanaugh, A.M., Huang, J., Lu, F., Chen, J.N., 2011. The PAF1 complex differentially regulates cardiomyocyte specification. Developmental biology 353, 19-28.
Langmead, B., Trapnell, C., Pop, M., Salzberg, S.L., 2009. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome biology 10, R25.
Lau, A., Blitzblau, H., Bell, S.P., 2002. Cell-cycle control of the establishment of mating-type silencing in S. cerevisiae. Genes & development 16, 2935-2945.
Lee, W., Tillo, D., Bray, N., Morse, R.H., Davis, R.W., Hughes, T.R., Nislow, C., 2007. A high-resolution atlas of nucleosome occupancy in yeast. Nature genetics 39, 1235-1244.
Lengronne, A., Katou, Y., Mori, S., Yokobayashi, S., Kelly, G.P., Itoh, T., Watanabe, Y., Shirahige, K., Uhlmann, F., 2004. Cohesin relocation from sites of chromosomal loading to places of convergent transcription. Nature 430, 573-578.
Lengronne, A., McIntyre, J., Katou, Y., Kanoh, Y., Hopfner, K.P., Shirahige, K., Uhlmann, F., 2006. Establishment of sister chromatid cohesion at the S. cerevisiae replication fork. Molecular cell 23, 787-799.
Liang, X.H., Liu, Q., Fournier, M.J., 2009. Loss of rRNA modifications in the decoding center of the ribosome impairs translation and strongly delays pre-rRNA processing. Rna 15, 1716-1728.
Lindahl, T., 1993. Instability and decay of the primary structure of DNA. Nature 362, 709-715.
Lindgren, E., Hagg, S., Giordano, F., Bjorkegren, J., Strom, L., 2014. Inactivation of the Budding Yeast Cohesin Loader Scc2 alters Gene Expression both Globally and in Response to a Single DNA Double Strand Break. Cell cycle, 0.

Liu, J., Krantz, I.D., 2009. Cornelia de Lange syndrome, cohesin, and beyond. Clinical genetics 76, 303-314.
Liu, J., Zhang, Z., Bando, M., Itoh, T., Deardorff, M.A., Clark, D., Kaur, M., Tandy, S., Kondoh, T., Rappaport, E., Spinner, N.B., Vega, H., Jackson, L.G., Shirahige, K., Krantz, I.D., 2009. Transcriptional dysregulation in NIPBL and cohesin mutant human cells. PLoS biology 7, e1000119.
Lopez-Serra, L., Kelly, G., Patel, H., Stewart, A., Uhlmann, F., 2014. The Scc2-Scc4 complex acts in sister chromatid cohesion and transcriptional regulation by maintaining nucleosome-free regions. Nature genetics 46, 1147-1151.
Lopez-Serra, L., Lengronne, A., Borges, V., Kelly, G., Uhlmann, F., 2013. Budding yeast Wapl controls sister chromatid cohesion maintenance and chromosome condensation. Current biology : CB 23, 64-69.
Lorch, Y., Maier-Davis, B., Kornberg, R.D., 2006. Chromatin remodeling by nucleosome disassembly in vitro. Proceedings of the National Academy of Sciences of the United States of America 103, 3090-3093.
Ma, Z., Lin, M., Li, K., Fu, Y., Liu, X., Yang, D., Zhao, Y., Zheng, J., Sun, B., 2013. Knocking down SMC1A inhibits growth and leads to G2/M arrest in human glioma cells. Int J Clin Exp Pathol 6, 862-869.
Mager, W.H., Winderickx, J., 2005. Yeast as a model for medical and medicinal research. Trends Pharmacol. Sci. 26, 265-273.
Mannini, L., Cucco, F., Quarantotti, V., Krantz, I.D., Musio, A., 2013. Mutation spectrum and genotype-phenotype correlation in Cornelia de Lange syndrome. Human mutation 34, 1589-1596.
Marygold, S.J., Roote, J., Reuter, G., Lambertsson, A., Ashburner, M., Millburn, G.H., Harrison, P.M., Yu, Z., Kenmochi, N., Kaufman, T.C., Leevers, S.J., Cook, K.R., 2007. The ribosomal protein genes and Minute loci of Drosophila melanogaster. Genome biology 8, R216.
Mayan, M., Aragon, L., 2010. Cis-interactions between non-coding ribosomal spacers dependent on RNAP-II separate RNAP-I and RNAP-III transcription domains. Cell cycle 9, 4328-4337.
Mehta, A., Haber, J.E., 2014. Sources of DNA double-strand breaks and models of recombinational DNA repair. Cold Spring Harb Perspect Biol 6, a016428.
Melby, T.E., Ciampaglio, C.N., Briscoe, G., Erickson, H.P., 1998. The symmetrical structure of structural maintenance of chromosomes (SMC) and MukB proteins: long, antiparallel coiled coils, folded at a flexible hinge. The Journal of cell biology 142, 1595-1604.
Merkenschlager, M., Odom, D.T., 2013. CTCF and cohesin: linking gene regulatory elements with their targets. Cell 152, 1285-1297.
Michaelis, C., Ciosk, R., Nasmyth, K., 1997. Cohesins: chromosomal proteins that prevent premature separation of sister chromatids. Cell 91, 35-45.
Misulovin, Z., Schwartz, Y.B., Li, X.Y., Kahn, T.G., Gause, M., MacArthur, S., Fay, J.C., Eisen, M.B., Pirrotta, V., Biggin, M.D., Dorsett, D., 2008. Association of cohesin and Nipped-B with transcriptionally active regions of the Drosophila melanogaster genome. Chromosoma 117, 89-102.
Morey, L., Helin, K., 2010. Polycomb group protein-mediated repression of transcription. Trends in biochemical sciences 35, 323-332.
Mueller, C.L., Jaehning, J.A., 2002. Ctr9, Rtf1, and Leo1 are components of the Paf1/RNA polymerase II complex. Molecular and cellular biology 22, 1971-1980.
Mueller, C.L., Porter, S.E., Hoffman, M.G., Jaehning, J.A., 2004. The Paf1 complex has functions independent of actively transcribing RNA polymerase II. Molecular cell 14, 447-456.
Musio, A., Selicorni, A., Focarelli, M.L., Gervasini, C., Milani, D., Russo, S., Vezzoni, P., Larizza, L., 2006. X-linked Cornelia de Lange syndrome owing to SMC1L1 mutations. Nature genetics 38, 528-530.
Muto, A., Ikeda, S., Lopez-Burks, M.E., Kikuchi, Y., Calof, A.L., Lander, A.D., Schilling, T.F., 2014. Nipbl and mediator cooperatively regulate gene expression to control limb development. PLoS genetics 10, e1004671.
Nasmyth, K., 2011. Cohesin: a catenase with separate entry and exit gates? Nature cell biology 13, 1170-1177.

Nasmyth, K., Haering, C.H., 2005. The structure and function of SMC and kleisin complexes. Annu Rev Biochem 74, 595-648.
Ng, H.H., Dole, S., Struhl, K., 2003. The Rtf1 component of the Paf1 transcriptional elongation complex is required for ubiquitination of histone H2B. The Journal of biological chemistry 278, 33625-33628.
Nguyen, C.T., Langenbacher, A., Hsieh, M., Chen, J.N., 2010. The PAF1 complex component Leo1 is essential for cardiac and neural crest development in zebrafish. Developmental biology 341, 167-175.
Ocampo-Hafalla, M.T., Uhlmann, F., 2011. Cohesin loading and sliding. Journal of cell science 124, 685-691.
Ofengand, J., 2002. Ribosomal RNA pseudouridines and pseudouridine synthases. FEBS letters 514, 17-25.
Ong, C.T., Corces, V.G., 2014. CTCF: an architectural protein bridging genome topology and function. Nature reviews. Genetics 15, 234-246.
Panizza, S., Tanaka, T., Hochwagen, A., Eisenhaber, F., Nasmyth, K., 2000. Pds5 cooperates with cohesin in maintaining sister chromatid cohesion. Current biology : CB 10, 1557-1564.
Parelho, V., Hadjur, S., Spivakov, M., Leleu, M., Sauer, S., Gregson, H.C., Jarmuz, A., Canzonetta, C., Webster, Z., Nesterova, T., Cobb, B.S., Yokomori, K., Dillon, N., Aragon, L., Fisher, A.G., Merkenschlager, M., 2008. Cohesins functionally associate with CTCF on mammalian chromosome arms. Cell 132, 422-433.
Pauli, A., Althoff, F., Oliveira, R.A., Heidmann, S., Schuldiner, O., Lehner, C.F., Dickson, B.J., Nasmyth, K., 2008. Cell-type-specific TEV protease cleavage reveals cohesin functions in Drosophila neurons. Developmental cell 14, 239-251.
Preti, M., Ribeyre, C., Pascali, C., Bosio, M.C., Cortelazzi, B., Rougemont, J., Guarnera, E., Naef, F., Shore, D., Dieci, G., 2010. The telomere-binding protein Tbf1 demarcates snoRNA gene promoters in Saccharomyces cerevisiae. Molecular cell 38, 614-620.
Reichow, S.L., Hamma, T., Ferre-D'Amare, A.R., Varani, G., 2007. The structure and function of small nucleolar ribonucleoproteins. Nucleic acids research 35, 1452-1464.
Rhodes, D.R., Yu, J., Shanker, K., Deshpande, N., Varambally, R., Ghosh, D., Barrette, T., Pandey, A., Chinnaiyan, A.M., 2004. Large-scale meta-analysis of cancer microarray data identifies common transcriptional profiles of neoplastic transformation and progression. Proceedings of the National Academy of Sciences of the United States of America 101, 9309-9314.
Riles, L., Shaw, R.J., Johnston, M., Reines, D., 2004. Large-scale screening of yeast mutants for sensitivity to the IMP dehydrogenase inhibitor 6-azauracil. Yeast 21, 241-248.
Rollins, R.A., Korom, M., Aulner, N., Martens, A., Dorsett, D., 2004. Drosophila nipped-B protein supports sister chromatid cohesion and opposes the stromalin/Scc3 cohesion factor to facilitate long-range activation of the cut gene. Molecular and cellular biology 24, 3100-3111.
Rollins, R.A., Morcillo, P., Dorsett, D., 1999. Nipped-B, a Drosophila homologue of chromosomal adherins, participates in activation by remote enhancers in the cut and Ultrabithorax genes. Genetics 152, 577-593.
Schaaf, C.A., Misulovin, Z., Sahota, G., Siddiqui, A.M., Schwartz, Y.B., Kahn, T.G., Pirrotta, V., Gause, M., Dorsett, D., 2009. Regulation of the Drosophila Enhancer of split and invected-engrailed gene complexes by sister chromatid cohesion proteins. PloS one 4, e6202.
Schleiffer, A., Kaitna, S., Maurer-Stroh, S., Glotzer, M., Nasmyth, K., Eisenhaber, F., 2003. Kleisins: a superfamily of bacterial and eukaryotic SMC protein partners. Molecular cell 11, 571-575.
Schuldiner, O., Berdnik, D., Levy, J.M., Wu, J.S., Luginbuhl, D., Gontang, A.C., Luo, L., 2008. piggyBac-based mosaic screen identifies a postmitotic function for cohesin in regulating developmental axon pruning. Developmental cell 14, 227-238.

Schwartz, S., Bernstein, D.A., Mumbach, M.R., Jovanovic, M., Herbst, R.H., Leon-Ricardo, B.X., Engreitz, J.M., Guttman, M., Satija, R., Lander, E.S., Fink, G., Regev, A., 2014. Transcriptome-wide Mapping Reveals Widespread Dynamic-Regulated Pseudouridylation of ncRNA and mRNA. Cell 159, 148-162.
Sheldon, K.E., Mauger, D.M., Arndt, K.M., 2005. A Requirement for the Saccharomyces cerevisiae Paf1 complex in snoRNA 3' end formation. Molecular cell 20, 225-236.
Shi, X., Finkelstein, A., Wolf, A.J., Wade, P.A., Burton, Z.F., Jaehning, J.A., 1996. Paf1p, an RNA polymerase II-associated factor in Saccharomyces cerevisiae, may have both positive and negative roles in transcription. Molecular and cellular biology 16, 669-676.
Simic, R., Lindstrom, D.L., Tran, H.G., Roinick, K.L., Costa, P.J., Johnson, A.D., Hartzog, G.A., Arndt, K.M., 2003. Chromatin remodeling protein Chd1 interacts with transcription elongation factors and localizes to transcribed genes. The EMBO journal 22, 1846-1856.
Sjogren, C., Nasmyth, K., 2001. Sister chromatid cohesion is required for postreplicative double-strand break repair in Saccharomyces cerevisiae. Current biology : CB 11, 991-995.
Skibbens, R.V., Colquhoun, J.M., Green, M.J., Molnar, C.A., Sin, D.N., Sullivan, B.J., Tanzosh, E.E., 2013. Cohesinopathies of a feather flock together. PLoS genetics 9, e1004036.
Skibbens, R.V., Corson, L.B., Koshland, D., Hieter, P., 1999. Ctf7p is essential for sister chromatid cohesion and links mitotic chromosome structure to the DNA replication machinery. Genes & development 13, 307-319.
Smits, P., Saada, A., Wortmann, S.B., Heister, A.J., Brink, M., Pfundt, R., Miller, C., Haas, D., Hantschmann, R., Rodenburg, R.J., Smeitink, J.A., van den Heuvel, L.P., 2011. Mutation in mitochondrial ribosomal protein MRPS22 leads to Cornelia de Lange-like phenotype, brain abnormalities and hypertrophic cardiomyopathy. European journal of human genetics : EJHG 19, 394-399.
Snyder, M., Huang, X.Y., Zhang, J.J., 2009. The minichromosome maintenance proteins 2-7 (MCM2-7) are necessary for RNA polymerase II (Pol II)-mediated transcription. The Journal of biological chemistry 284, 13466-13472.
Solomon, D.A., Kim, T., Diaz-Martinez, L.A., Fair, J., Elkahloun, A.G., Harris, B.T., Toretsky, J.A., Rosenberg, S.A., Shukla, N., Ladanyi, M., Samuels, Y., James, C.D., Yu, H., Kim, J.S., Waldman, T., 2011. Mutational inactivation of STAG2 causes aneuploidy in human cancer. Science 333, 1039-1043.
Squazzo, S.L., Costa, P.J., Lindstrom, D.L., Kumer, K.E., Simic, R., Jennings, J.L., Link, A.J., Arndt, K.M., Hartzog, G.A., 2002. The Paf1 complex physically and functionally associates with transcription elongation factors in vivo. The EMBO journal 21, 1764-1774.
Strunnikov, A.V., Larionov, V.L., Koshland, D., 1993. SMC1: an essential yeast gene encoding a putative head-rod-tail protein is required for nuclear division and defines a new ubiquitous protein family. The Journal of cell biology 123, 1635-1648.
Sumara, I., Vorlaufer, E., Stukenberg, P.T., Kelm, O., Redemann, N., Nigg, E.A., Peters, J.M., 2002. The dissociation of cohesin from chromosomes in prophase is regulated by Polo-like kinase. Molecular cell 9, 515-525.
Thota, S., Viny, A.D., Makishima, H., Spitzer, B., Radivoyevitch, T., Przychodzen, B., Sekeres, M.A., Levine, R.L., Maciejewski, J.P., 2014. Genetic alterations of the cohesin complex genes in myeloid malignancies. Blood 124, 1790-1798.
Tomson, B.N., Crisucci, E.M., Heisler, L.E., Gebbia, M., Nislow, C., Arndt, K.M., 2013. Effects of the Paf1 complex and histone modifications on snoRNA 3'-end formation reveal broad and locus-specific regulation. Molecular and cellular biology 33, 170-182.
Tonkin, E.T., Wang, T.J., Lisgo, S., Bamshad, M.J., Strachan, T., 2004. NIPBL, encoding a homolog of fungal Scc2-type sister chromatid cohesion proteins and fly Nipped-B, is mutated in Cornelia de Lange syndrome. Nature genetics 36, 636-641.

Toth, A., Ciosk, R., Uhlmann, F., Galova, M., Schleiffer, A., Nasmyth, K., 1999. Yeast cohesin complex requires a conserved protein, Eco1p(Ctf7), to establish cohesion between sister chromatids during DNA replication. Genes & development 13, 320-333.
Uhlmann, F., Lottspeich, F., Nasmyth, K., 1999. Sister-chromatid separation at anaphase onset is promoted by cleavage of the cohesin subunit Scc1. Nature 400, 37-42.
Unal, E., Arbel-Eden, A., Sattler, U., Shroff, R., Lichten, M., Haber, J.E., Koshland, D., 2004. DNA damage response pathway uses histone modification to assemble a double-strand break-specific cohesin domain. Molecular cell 16, 991-1002.
Van Den Berg, D.J., Francke, U., 1993. Roberts syndrome: a review of 100 cases and a new rating system for severity. American journal of medical genetics 47, 1104-1123.
van der Lelij, P., Chrzanowska, K.H., Godthelp, B.C., Rooimans, M.A., Oostra, A.B., Stumm, M., Zdzienicka, M.Z., Joenje, H., de Winter, J.P., 2010. Warsaw breakage syndrome, a cohesinopathy associated with mutations in the XPD helicase family member DDX11/ChlR1. American journal of human genetics 86, 262-266.
van Steensel, B., 2011. Chromatin: constructing the big picture. The EMBO journal 30, 1885-1895.
Vega, H., Waisfisz, Q., Gordillo, M., Sakai, N., Yanagihara, I., Yamada, M., van Gosliga, D., Kayserili, H., Xu, C., Ozono, K., Jabs, E.W., Inui, K., Joenje, H., 2005. Roberts syndrome is caused by mutations in ESCO2, a human homolog of yeast ECO1 that is essential for the establishment of sister chromatid cohesion. Nature genetics 37, 468-470.
Vignali, M., Hassan, A.H., Neely, K.E., Workman, J.L., 2000. ATP-dependent chromatin-remodeling complexes. Molecular and cellular biology 20, 1899-1910.
Wade, P.A., Werel, W., Fentzke, R.C., Thompson, N.E., Leykam, J.F., Burgess, R.R., Jaehning, J.A., Burton, Z.F., 1996. A novel collection of accessory factors associated with yeast RNA polymerase II. Protein expression and purification 8, 85-90.
Waizenegger, I.C., Hauf, S., Meinke, A., Peters, J.M., 2000. Two distinct pathways remove mammalian cohesin from chromosome arms in prophase and from centromeres in anaphase. Cell 103, 399-410.
Wang, P., Bowl, M.R., Bender, S., Peng, J., Farber, L., Chen, J., Ali, A., Zhang, Z., Alberts, A.S., Thakker, R.V., Shilatifard, A., Williams, B.O., Teh, B.T., 2008. Parafibromin, a component of the human PAF complex, regulates growth factors and is required for embryonic development and survival in adult mice. Molecular and cellular biology 28, 2930-2940.
Watanabe, Y., Nurse, P., 1999. Cohesin Rec8 is required for reductional chromosome segregation at meiosis. Nature 400, 461-464.
Weitzer, S., Lehane, C., Uhlmann, F., 2003. A model for ATP hydrolysis-dependent binding of cohesin to DNA. Current biology : CB 13, 1930-1940.
Welch, J.S., Ley, T.J., Link, D.C., Miller, C.A., Larson, D.E., Koboldt, D.C., Wartman, L.D., Lamprecht, T.L., Liu, F., Xia, J., Kandoth, C., Fulton, R.S., McLellan, M.D., Dooling, D.J., Wallis, J.W., Chen, K., Harris, C.C., Schmidt, H.K., Kalicki-Veizer, J.M., Lu, C., Zhang, Q., Lin, L., O'Laughlin, M.D., McMichael, J.F., Delehaunty, K.D., Fulton, L.A., Magrini, V.J., McGrath, S.D., Demeter, R.T., Vickery, T.L., Hundal, J., Cook, L.L., Swift, G.W., Reed, J.P., Alldredge, P.A., Wylie, T.N., Walker, J.R., Watson, M.A., Heath, S.E., Shannon, W.D., Varghese, N., Nagarajan, R., Payton, J.E., Baty, J.D., Kulkarni, S., Klco, J.M., Tomasson, M.H., Westervelt, P., Walter, M.J., Graubert, T.A., DiPersio, J.F., Ding, L., Mardis, E.R., Wilson, R.K., 2012. The origin and evolution of mutations in acute myeloid leukemia. Cell 150, 264-278.
Whelan, G., Kreidl, E., Peters, J.M., Eichele, G., 2012a. The non-redundant function of cohesin acetyltransferase Esco2: some answers and new questions. Nucleus 3, 330-334.
Whelan, G., Kreidl, E., Wutz, G., Egner, A., Peters, J.M., Eichele, G., 2012b. Cohesin acetyltransferase Esco2 is a cell viability factor and is required for cohesion in pericentric heterochromatin. The EMBO journal 31, 71-82.

Wood, A., Schneider, J., Dover, J., Johnston, M., Shilatifard, A., 2003. The Paf1 complex is essential for histone monoubiquitination by the Rad6-Bre1 complex, which signals for histone methylation by COMPASS and Dot1p. The Journal of biological chemistry 278, 34739-34742.
Xiong, B., Lu, S., Gerton, J.L., 2010. Hos1 is a lysine deacetylase for the Smc3 subunit of cohesin. Current biology : CB 20, 1660-1665.
Xu, B., Lee, K.K., Zhang, L., Gerton, J.L., 2013. Stimulation of mTORC1 with L-leucine rescues defects associated with Roberts syndrome. PLoS genetics 9, e1003857.
Xu, B., Lu, S., Gerton, J.L., 2014a. Roberts syndrome: A deficit in acetylated cohesin leads to nucleolar dysfunction. Rare diseases 2, e27743.
Xu, B., Sowa, N., Cardenas, M.E., Gerton, J.L., 2014b. l-leucine partially rescues translational and developmental defects associated with zebrafish models of Cornelia de Lange syndrome. Human molecular genetics.
Yoon, A., Peng, G., Brandenburger, Y., Zollo, O., Xu, W., Rego, E., Ruggero, D., 2006. Impaired control of IRES-mediated translation in X-linked dyskeratosis congenita. Science 312, 902-906.
Yuan, G.C., Liu, Y.J., Dion, M.F., Slack, M.D., Wu, L.F., Altschuler, S.J., Rando, O.J., 2005. Genome-scale identification of nucleosome positions in S. cerevisiae. Science 309, 626-630.
Zhang, N., Kuznetsov, S.G., Sharan, S.K., Li, K., Rao, P.H., Pati, D., 2008. A handcuff model for the cohesin complex. The Journal of cell biology 183, 1019-1031.
Zhang, Y., Sikes, M.L., Beyer, A.L., Schneider, D.A., 2009. The Paf1 complex is required for efficient transcription elongation by RNA polymerase I. Proceedings of the National Academy of Sciences of the United States of America 106, 2153-2158.
Zhou, Q., Li, T., Price, D.H., 2012. RNA polymerase II elongation control. Annu Rev Biochem 81, 119-143.
Zuin, J., Franke, V., van Ijcken, W.F., van der Sloot, A., Krantz, I.D., van der Reijden, M.I., Nakato, R., Lenhard, B., Wendt, K.S., 2014. A cohesin-independent role for NIPBL at promoters provides insights in CdLS. PLoS genetics 10, e1004153.

Appendix 1. Strains

Strain	Genotype	Reference
BY4741	MATa his3Δ leu2Δ0 met15Δ0 ura3Δ0	
MZ23	MATa his3Δ leu2Δ0 met15Δ0 ura3Δ0 scc2-4::HYG	This study
MZ68	MATa his3Δ leu2Δ0 met15Δ0 ura3Δ0 carrying pJD375 CEN URA plasmid	This study
MZ69	MATa his3Δ leu2Δ0 met15Δ0 ura3Δ0 carrying pJD376 CEN URA plasmid	This study
MZ70	MATa his3Δ leu2Δ0 met15Δ0 ura3Δ0 carrying pJD377 CEN URA plasmid	This study
MZ70	MATa his3Δ leu2Δ0 met15Δ0 ura3Δ0 carrying pJD431 CEN URA plasmid	This study
MZ71	MATa his3Δ leu2Δ0 met15Δ0 ura3Δ0 carrying pJD432 CEN URA plasmid	This study
MZ72	MATa his3Δ leu2Δ0 met15Δ0 ura3Δ0 carrying pJD433 CEN URA plasmid	This study
MZ30	MATa his3Δ leu2Δ0 met15Δ0 ura3Δ0 paf1Δ::NATMx6 SCC2::KanMx6	This study
MZ74	MATa his3Δ leu2Δ0 met15Δ0 ura3Δ0 scc2-4::HYGMx6 carrying pJD433 CEN URA plasmid	This study
MZ75	MATa his3Δ leu2Δ0 met15Δ0 ura3Δ0 scc2-4::HYGMx6 carrying pJD433 CEN URA plasmid	This study
MZ76	MATa his3Δ leu2Δ0 met15Δ0 ura3Δ0 scc2-4::HYGMx6 carrying pJD433 CEN URA plasmid	This study

MZ77	MATa his3Δ leu2Δ0 met15Δ0 ura3Δ0 scc2-4::HYGMx6 carrying pJD433 CEN URA plasmid	This study
MZ78	MATa his3Δ leu2Δ0 met15Δ0 ura3Δ0 scc2-4::HYGMx6 carrying pJD433 CEN URA plasmid	This study
MZ79	MATa his3Δ leu2Δ0 met15Δ0 ura3Δ0 scc2-4::HYGMx6 carrying pJD433 CEN URA plasmid	This study
MZ11	MATa his3Δ leu2Δ0 met15Δ0 ura3Δ0 scc2-4::HYGMx6 carrying Rps2-GFP CEN LEU plasmid	This study
MZ13	MATa his3Δ leu2Δ0 met15Δ0 ura3Δ0 scc2-4::HYGMx6 carrying Rpl25-GFP CEN LEU plasmid	This study
MZ12	MATa his3Δ leu2Δ0 met15Δ0 ura3Δ0 scc2-4::HYGMx6 carrying Rps2-GFP CEN LEU plasmid	This study
MZ14	MATa his3Δ leu2Δ0 met15Δ0 ura3Δ0 scc2-4::HYGMx6 carrying Rpl25-GFP CEN LEU plasmid	This study
MZ216	MATa his3Δ leu2Δ0 met15Δ0 ura3Δ0 SCC2-13MYC::KanMx6 PAF1-3HA::HISMx6	This study
MZ217	MATa his3Δ leu2Δ0 met15Δ0 ura3Δ0 scc2-4-13MYC::KanMx6 PAF1-3HA::HisMx6	This study
MZ219	MATa his3Δ leu2Δ0 met15Δ0 ura3Δ0 SCC2-13MYC::KanMx6 CTR9-3HA::HisMx6	This study
MZ220	MATa his3Δ leu2Δ0 met15Δ0 ura3Δ0 scc2-4-13MYC::KanMx6 CTR9-3HA::HisMx6	This study

Appendix 2. Published Papers and Conference Abstracts

Publications

1**. Zakari M,** Ross RT, Peak A, Blanchette M, Seidel C, and Gerton JL. "*The SMC loader Scc2 promotes ncRNA biogenesis and Translational fidelity*". (*manuscript under revision*) ***PLoS Genet.***

2. Sendinc M, Hoang SA, **Zakari** M, Gerton JL, Bekiranov S, Grant PA. "*The Replication Kinase Cdc7 marks histones to regulate biosynthesis genes"* (*manuscript under revision*) ***Nat Struct Mol Biol.***

3. **Zakari M,** Chiyung K, and Gerton JL. "*The Etiology and Pathogenesis of Cohesinopathies*". *(accepted for publication)* ***Dev. Bio***

4. **Zakari M,** and Gerton JL. *"The SMC loader Scc2 regulates gene expression" (accepted for publication)* ***Cell Cycle***

5. Antonie D. Kline, Anne L. Calof, Arthur Lander, Jennifer L. Gerton, Ian D. Krantz, Dale Dorsett, Matthew A. Deardorff, Natalie Blagowidow, Kyoko Yokomori, Katsuhiko Shirahige, Rosaysela Santos, Julie Woodman, Paul C. Megee, Julia T. O'Connor, Alena Egense, Sarah Noon, Maurice Belote, Marjorie T. Goodban, Blake D. Hansen, Jenni Glad Timmons, Antonio Musio, Stacey L. Ishman, Yvon Bryan, Yaning Wu, Laura R. Bettini, Devanshi Mehta, **Musinu Zakari**, Jason A. Mills, Siddharth Srivastava, Richard E. Haaland*," Clinical, Developmental and Molecular Update on Cornelia de Lange Syndrome and the Cohesin Complex: Abstracts from the 2014 Scientific and Educational Symposium"* ***American Journal of Medical Genetics***

Conference Presentation

Zakari M, Gerton JL. The Cohesin loader Scc2 is required for non-coding RNA biogenesis and translational fidelity in yeast. 6th Cornelia de Lange Syndrome Scientific and Educational Symposia. Costa Mesa, California. June 2014 (Speaker)

Conference Abstracts

Zakari M, Ooi SK, Blanchette M, Seidel C, and Gerton JL. 2013 H Foundation Basic Science Symposium. Northwestern University Physical Sciences-Oncology center, Evanston IL. June 2013 (Poster)

Zakari M, Ooi SK, Blanchette M, Seidel C, and Gerton JL. The Cohesin loader Scc2 promotes translational fidelity. Stowers Institute for Medical Research Young Investigator's Research Day. Kansas city, March 2013 (Poster)

Zakari Musinu - Biologie Cellulaire et moléculaire – 2015

Résumé:

Le complexe Scc2-Scc4 est essentiel pour l'association du complexe cohésine sur l'ADN. Les proteines Cohésine génèrent la cohésion entre les chromatides sœurs, ce qui est essentiel pour la ségrégation des chromosomes. Scc2 (également connu sous le nom NIPBL) est muté chez les patients atteints du syndrome de Cornelia de Lange, une maladie multi-organique caractérisée par des anomalies du développement du visage, de la developpement mental cardiaque et du tractus gastro-intestinal. Comment les mutations localisées au niveau du gène codant pour la proteine Scc2 conduisent à des anomalies du développement chez les patients n'a pas encore été élucidé. Une des hypothèses est que la liaison de Scc2 / cohésine à différentes régions du génome a une incidence sur la transcription. Chez la levure de bière, il a été montre que Scc2 se lie aux genes transcrits par l'ARN Pol III (les ARNt et spliceosomals) , ainsi qu'aux gènes transcrits par l'ARN Pol II codant pour des petits ARN nucléolaires et nucléaires (snARN et snoARNs) et des gènes de protéines ribosomiques. Nous rapportons ici que Scc2 est important pour l'expression de ces gènes. Scc2 et le régulateur transcriptionnel Paf1 collaborent pour promouvoir la production de Box H / ACA snoARNs qui guident la pseudouridylation des ARN y compris l'ARN ribosomal. Une mutation de Scc2 a été associée à des défauts dans la production d'ARN ribosomal, la biogenèse des ribosomes, et del'épissage. Alors que le mutant Scc2 n'a pas de défaut général de la synthèse protéique, il montre un déphasage accrue et une réduction de l'utilisation du site interne d'entrée ribosomale (IRES)/ coiffe-indépendante. Ces résultats suggèrent que Scc2 favorise normalement un programme d'expression génétique qui prend en charge la fidélité de la traduction. Nous émettons l'hypothèse que le dysfonctionnement de traduction peut contribuer au syndrome de Cornelia de Lange, qui est causé par des mutations dans Scc2.

Mots clés: Scc2, translationnelle fidélité, pseudouridylation, frameshifting, biogenèse des ribosomes, utilisation IRES, Paf1

The SMC loader Scc2 promotes ncRNA biogenesis and translational fidelity in Saccharomyces cerevisiae

Abstract :

The Scc2-Scc4 complex is essential for loading the cohesin complex onto DNA. Cohesin generates cohesion between sister chromatids, which is critical for chromosome segregation. Scc2 (also known as NIPBL) is mutated in patients with Cornelia de Lange syndrome, a multi-organ disease characterized by developmental defects in head, limb, cognition, heart, and the gastrointestinal tract. How mutations in Scc2 lead to developmental defects in patients is yet to be elucidated. One hypothesis is that the binding of Scc2/cohesin to different regions of the genome will affect transcription. In budding yeast, Scc2 has been shown to bind to RNA Pol III transcribed genes (tRNAs, and spliceosomal), as well as RNA Pol II-transcribed genes encoding

small nuclear and nucleolar RNAs (snRNAs and snoRNAs) and ribosomal protein genes. Here, we report that Scc2 is important for gene expression. Scc2 and the transcriptional regulator Paf1 collaborate to promote the production of Box H/ACA snoRNAs which guide pseudouridylation of RNAs including ribosomal RNA. Mutation of Scc2 was associated with defects in the production of ribosomal RNA, ribosome biogenesis, and splicing. While the scc2 mutant does not have a general defect in protein synthesis, it shows increased frameshifting and reduced internal ribosomal entry site (IRES) usage/cap-independent translation. These findings suggest Scc2 normally promotes a gene expression program that supports translational fidelity. We hypothesize that translational dysfunction may contribute to the human disorder Cornelia de Lange syndrome, which is caused by mutations in Scc2.

Keywords: Scc2, translational fidelity, pseudouridylaton, frameshifting, ribosome biogenesis, IRES usage, Paf1