IDENTIFICATION AND CHARACTERIZATION OF FACTORS REQUIRED FOR REPRESSION OF THE YEAST *SER3* GENE BY *SRG1* INTERGENIC TRANSCRIPTION

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Recent studies have shown that transcription is widespread in eukaryotic genomes occurring over noncoding regions (ncDNA) as well as protein-coding genes. This pervasive transcription can have profound effects on DNA-mediated processes such as the regulation of gene expression. In one well characterized example, repression of the *Saccharomyces cerevisiae SER3* gene was shown to be dependent on transcription of *SRG1* from ncDNA initiating within intergenic DNA 5' of *SER3* and extending across the *SER3* promoter region, preventing transcription factors from binding to the *SER3* promoter.

To understand the details of this transcription interference mechanism, I performed a genetic screen in yeast to identify factors required for *SER3* repression. I identified 21 candidates including known regulators of *SER3*, factors involved in histone gene expression, and transcription elongation factors. The regulators of histone gene expression led us to discover a role for chromatin in *SER3* repression. The combined activities of the Swi/Snf chromatin remodeling factor, the HMG-like factor Spt2, and the Spt6 and Spt16 histone chaperones allow *SRG1* transcription to deposit and maintain nucleosomes over the *SER3* promoter to prevent transcription factors from binding and activating *SER3*.

Through investigations of the transcription elongation factors identified in the screen, I uncovered a role for the Paf1 transcription elongation complex in *SER3* repression. I found that *SER3* repression is primarily dependent on the Paf1 and Ctr9 subunits of this complex, with minor contributions by the Rtf1, Cdc73, and Leo1 subunits. Importantly, the defect in *SER3* repression in strains lacking Paf1 subunits is not a result of reduced *SRG1* transcription or reduced levels of known Paf1 complex-dependent histone modifications. Rather, we find that strains lacking subunits of the Paf1 complex exhibit reduced nucleosome occupancy and reduced recruitment of Spt16 and, to a lesser extent, Spt6 at the *SER3* promoter, suggesting a novel role for the complex. Taken together, my work demonstrates that *SER3* repression is mediated by nucleosome occupancy of the *SER3* promoter, which is facilitated by the disassembly and assembly of nucleosomes by Spt6 and Spt16, which requires Spt2 and the Paf1 complex during *SRG1* transcription.

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PREFACE

I would like to acknowledge the many people who have helped me over the years, without whom my accomplishments would not have been possible. First, I would like to thank Joe Martens for, not only teaching me how to be a scientist, but for being such a positive mentor for my research, teaching, and life in general. His positive attitude and eternal optimism helped keep me going every time the research hit a rough patch.

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1.0 INTRODUCTION

The precise and coordinated expression of genes lies at the heart of nearly all biological processes. Every organism, therefore, must have mechanisms in place to regulate the timing and extent of the expression of its genes. These mechanisms are complex and diverse, with some basic components conserved and other features varying from gene to gene. Transcription, the first step in gene expression, is an important target for regulation. In eukaryotes, the process of transcription and its regulation are complicated by the fact that the genetic material occurs in the context of chromatin. This necessitates a host of factors required to overcome the barrier to transcription that chromatin represents. Eukaryotic gene expression is also complicated by the widespread transcriptional activity of genomes that produce RNA transcripts, not just from protein-coding genes, but from nearly everywhere in the genome. This genome-wide transcriptional activity and the transcripts produced can have significant impacts on gene expression. These topics will be important for understanding the work presented in this dissertation and will be introduced in this chapter. Although, most of the factors and processes discussed are conserved in higher eukaryotes, I will focus discussion of these events on the model organism used in my studies, *Saccharomyces cerevisiae*, unless otherwise noted.

1.1 CHROMATIN IS AN IMPORTANT REGULATOR OF GENE EXPRESSION

1.1.1 Eukaryotic DNA is packaged into chromatin

In order to fit the large amounts of DNA of a eukaryotic genome into the relatively small space of the nucleus, a high degree of compaction is needed. This is partially mediated by the interactions of DNA with histone proteins to form nucleosomes. A nucleosome consists of ~146bp of DNA wrapped around an octamer of histone proteins containing two copies each of H2A, H2B, H3, and H4 (Figure 1) (Luger et al. 1997). Histones are small, highly conserved, negatively charged proteins consisting of a histone fold domain that forms the nucleosome core and unstructured tails that extend outside the core. Nucleosomes repeat along the length of DNA approximately every 200bp to form a "beads on a string" structure, commonly referred to as chromatin (Kornberg 1974). These nucleosome structures, with the aid of another histone protein H1, can stack and fold into progressively higher order chromatin structures to condense the DNA into the chromosome structures seen during mitosis.

1.1.2 Chromatin acts as a barrier to transcription initiation and elongation

The packaging of eukaryotic DNA into chromatin presents a major obstacle to DNA-mediated processes, such as transcription, replication, and DNA repair. Transcription initiation can be hindered by nucleosomes preventing access of transcription factors to promoter DNA (Li et al. 2007a). DNA that is encompassed in a nucleosome cannot easily be recognized by DNA binding factors (Kornberg and Lorch 1999). This can be overcome by factors that alter chromatin structure to make DNA more accessible (Cote et al. 1994; Cote et al. 1998). This barrier to

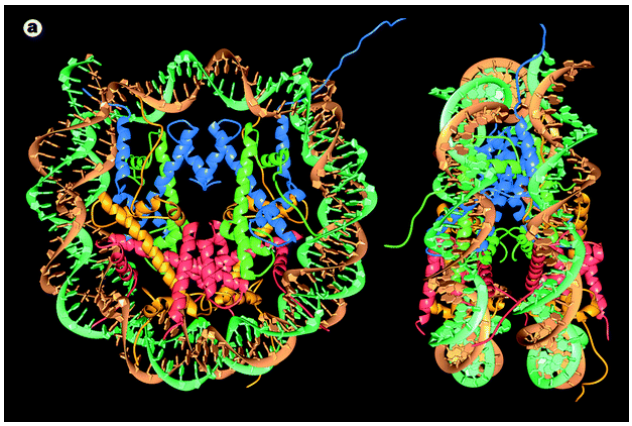

Figure 1: Structure of a nucleosome.

X-ray crystal structure of a nucleosome to 2.8 Å. The model shows the 146bp of DNA (brown and turquoise) surrounding the histone octamer made up of the core domains of histone H2A (yellow) H2B (red) H3 (green) and H4 (blue). Also seen are some of the N-terminal tail domains that extend outside of the nucleosome core, which are subject to numerous post-translational modifications, and are important for nucleosome structure and function. Figure reprinted with permission from Macmillan Publishers Ltd: Figure from Nature (Luger et al. 1997), copyright 1997.

transcription initiation has been well studied at certain yeast genes, such as *GAL1-10* and *PHO5* (Lohr 1984; Adkins et al. 2007). Genome-wide studies reveal that upstream of most yeast genes, is a region of ~200bp relatively devoid of nucleosomes, called the nucleosome free region or NFR (Lee et al. 2004; Lee et al. 2007). The NFR allows important regulatory sequences in the promoter region to remain accessible to transcription factors and may facilitate other processes such as initiation of DNA replication.

Nucleosomes are also inhibitory to transcription elongation by physically hindering the passage of RNA Polymerase II (RNA Pol II) (Li et al. 2007a). *In vitro* transcription occurs much slower on DNA reconstituted with nucleosomes compared to naked DNA (Izban and Luse 1991). Genome-wide studies also demonstrate an inverse correlation between transcription rates and nucleosome occupancy and significant pausing and backtracking of the polymerase when encountering nucleosomes (Lee et al. 2004; Churchman and Weissman 2011). In order to overcome the repressive effects of chromatin, eukaryotic cells rely on a number of mechanisms to alter chromatin architecture during transcription including: incorporation of histone variants, chromatin remodeling, nucleosome assembly and disassembly by histone chaperones, and the covalent modification of histones.

1.1.3 Canonical histones are exchanged for variants that alter chromatin structure and function

Two of the canonical histones H2A and H3 are present in the bulk of nucleosomes, but they can be replaced by less abundant alternate versions of the proteins in a DNA replication-independent manner. These histone variants perform specialized functions including the formation of chromatin boundaries (H2A.Z) and centromere formation (CenH3) in yeast and DNA damage

response (H2A.X), X chromosome inactivation (MacroH2A) and transcription activation (H3.3 and H2ABbd) in higher eukaryotes (Talbert and Henikoff 2010). H2A.Z is enriched at the nucleosomes flanking the NFR near genes and is important for establishing and maintaining the nucleosome free region (Guillemette et al. 2005; Li et al. 2005; Raisner et al. 2005; Zhang et al. 2005). Nucleosomes containing this variant have altered properties that allow them to be easily displaced during activation allowing silent genes to remain poised for rapid expression (Zhang et al. 2005).

1.1.4 Chromatin remodeling complexes displace nucleosomes in an ATP-dependent manner

The positioning of nucleosomes across a genome is determined both by the underlying DNA sequence (Segal and Widom 2009) and the activity of chromatin remodeling factors that use the energy of ATP hydrolysis to alter the position or occupancy of nucleosomes. These factors alter chromatin structure in a way that permits or restricts access to particular DNA sequences (Clapier and Cairns 2009). Chromatin remodelers belong to one of four families, 1) Swi/Snf consisting of Swi/Snf and Rsc; 2) ISWI consisting of ISW1a, ISW1b, and ISW2; 3) CHD consisting of CHD1; and 4) INO80 consisting of INO80 and SWR1. These families share some basic features, but vary in their functions and mechanisms of action. With the exception of the individual protein Chd1, chromatin remodelers are typically large complexes with many subunits that contribute unequally to the remodeling, substrate recognition, or regulatory functions of the complex (Clapier and Cairns 2009).

Functions of chromatin remodelers include altering nucleosome positioning during chromatin assembly (Polo and Almouzni 2006), replication (Vincent et al. 2008), DNA repair

and recombination (Chai et al. 2005; Papamichos-Chronakis et al. 2006; Shim et al. 2007), gene repression and activation (Cairns 2009), and transcription elongation (Morillon et al. 2003; Simic et al. 2003). They can affect these processes through a variety of mechanisms including repositioning or sliding nucleosomes, evicting histone dimers or entire octamers, or loosening the histone-DNA contacts (Clapier and Cairns 2009). Nucleosome repositioning or sliding is perhaps the most important mechanism for altering the accessibility of short DNA sequences. Repositioning is accomplished by translocating the DNA relative to the nucleosome through the formation of an intranucleosomal loop that is propagated around the histone octamer by the remodeler (Saha et al. 2002; Strohner et al. 2005; Lia et al. 2006; Zhang et al. 2006).

1.1.5 Histone chaperones alter chromatin structure in an ATP-independent manner

Another class of factors that alter chromatin is comprised of a diverse set of histone-binding proteins that function as histone chaperones. While they have no enzymatic activity, they can facilitate the disassembly and reassembly of nucleosomes required for many DNA-mediated processes (Eitoku et al. 2008). Histone chaperones are crucial for fork progression (Gambus et al. 2006; Groth et al. 2007) and deposition of new histones during DNA replication (Stillman 1986; Li et al. 2008). They also help provide access to sites of DNA damage and restore chromatin after repair (Smerdon 1991). During transcription, histone chaperones are responsible for disassembly of nucleosomes to allow passage of Pol II, as well as reassembly in its wake to re-establish proper chromatin structure (Belotserkovskaya et al. 2003; Kaplan et al. 2003; Mason and Struhl 2003; Cheung et al. 2008; Jamai et al. 2009). The growing list of histone chaperones in yeast include: Caf1, Nap1, Asf1, Vps75, Rtt106, Spt6, and Spt16. Each factor has specificity for particular histones or a specific portion of the nucleosome and facilitate different steps in the

assembly, disassembly, or shuffling of histones (Eitoku et al. 2008). Furthermore, the functions of many histone chaperones are functionally linked to post-translational histone modifications (Avvakumov et al. 2011).

1.1.6 Covalent modifications alter the structure and binding properties of histones

At the heart of many chromatin-regulated processes are histone modifying enzymes that covalently add chemical moieties to histone residues. These post-translational modifications include acetylation, mono- di- and tri-methylation, phosphorylation, ubiquitylation, sumoylation, and ribosylation and occur mostly on the unstructured N and C-terminal tails, and less frequently on the core domains (Figure 2) (Campos and Reinberg 2009). Modifications can be added and removed ensuring the state of the histones is dynamically regulated. The modification state of histones is regulated both spatially and temporally, forming a "histone code" that can signal for distinct states of DNA or functions of chromatin (Strahl and Allis 2000). Particular modification states can be associated with accessible euchromatin or inaccessible heterochromatin, active or inactive transcription, and even different stages of transcription, from initiation to elongation (Figure 3). Modifications can alter chromatin structure by altering histone-DNA contacts to make DNA sequences more or less accessible or by acting as binding sites for regulatory proteins. To facilitate many chromatin transactions there are factors that can recognize modified histones, "read" the histone code, and then produce changes in chromatin or DNA processes (Yun et al. 2011). It is important to note that, although histone modifications play important roles in gene regulation, they are not required for viability in yeast. Mutations of histone residues that remove modification sites or deletion of genes encoding histone-modifying enzymes usually does not result in inviable yeast. Therefore, modifications may not be essential

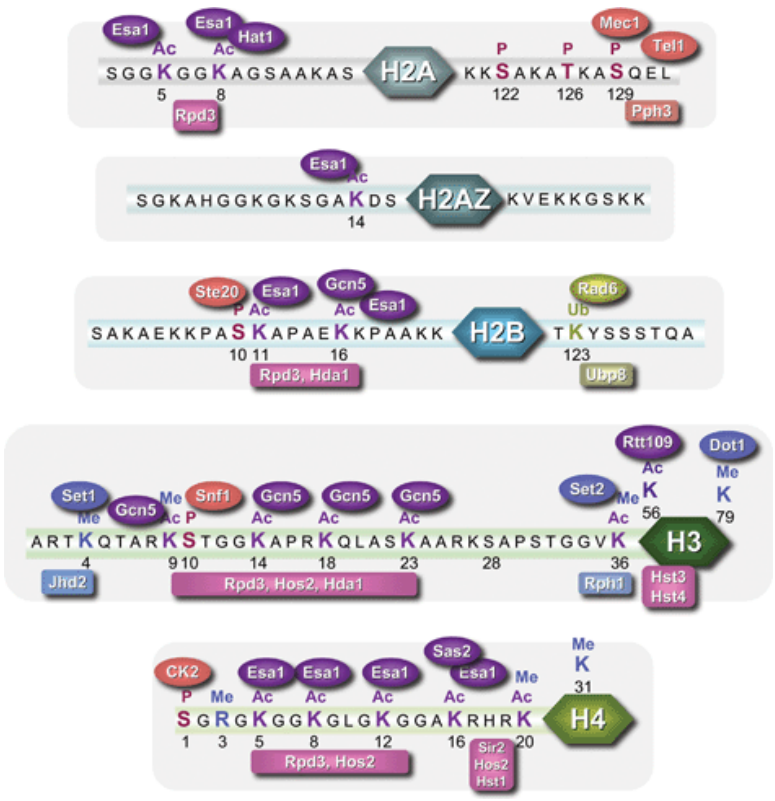

Figure 2: Map of histone modifications in yeast.

The amino acid sequences of the histone tails are shown along with the major post-translational modifications they can undergo: acetylation (Ac), methylation (Me), phosphorylation (P), and ubiquitylation (Ub). The enzymes known to add the modification are shown above the sequence and those known to remove the modification are shown below. Figure from (Krebs 2007) reproduced with permission of *The Royal Society of Chemistry*, copyright 2007.

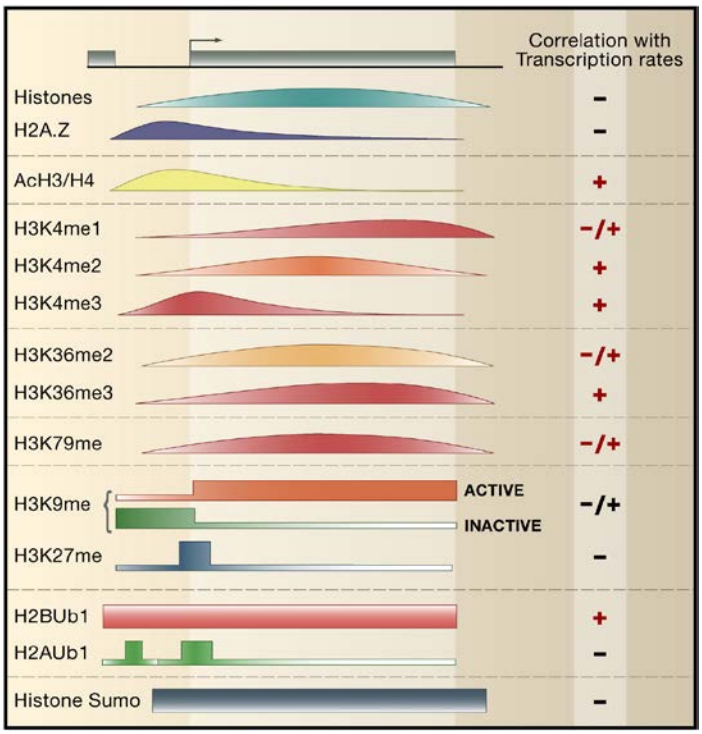

Figure 3: Distribution of histone modifications and correlation with transcription rates.

Summary of the results from numerous genome-wide studies mapping histone modifications and their correlation with transcriptional activity over protein-coding genes. Figure reprinted from *Cell*, Vol. 138, Li B, Carey M, Workman JL. The role of chromatin during transcription. Copyright (2007), with permission from Elsevier.

for gene expression or for life, but rather act as fine-tuning mechanisms to slightly alter transcription rates or chromatin transactions in a gene-specific manner.

Histone acetylation is carried out by acetyltransferases (HATs) that transfer an acetyl group from acetyl-CoA to lysine residues. Many HATs are not specific to a single residue, but rather target multiple residues, mostly in H3 and H4. Acetylation acts to partially neutralize the strong positive charge of the histones resulting in the weakening of histone-DNA contacts and easier nucleosome sliding or eviction (Campos and Reinberg 2009). Histone acetylation, therefore, has a positive influence on processes, such as transcription, while the removal of acetyl marks by histone deacetylases (HDACs) can restore tight histone-DNA binding and negatively impact transcription (Li et al. 2007a).

Histone methylation occurs on lysine and arginine residues through the activity of methyltransferases (HMTs) and can be removed by histone demethylases. Lysine methylation occurs in three distinct steps by the successive transfer of methyl groups from S-adenosyl methionine to a histone residue to form mono-, di-, and tri-methylated residues, each with their own potential for factor binding and signaling (Campos and Reinberg 2009). While methylation was previously thought to be irreversible, the discovery of two families of histone demethylases, the amine oxidase-related enzymes and the Jumonji C-terminal domain (JmjC) containing enzymes, demonstrated dynamic regulation of methylation similar to other modifications (Hou and Yu 2010). Some methylation marks, such as K4, K36, and K79, are hallmarks of active transcription while others, such as K9 and K27, are associated with inactive chromatin (Figure 3). Interestingly, K4 and K79 methylation rely on another modification, the mono-ubiquitylation of H2B K123, a mark of active transcription performed by the ubiquitin conjugase Rad6 and ubiquitin ligase Bre1 (Ng et al. 2003a; Wood et al. 2003). H2B K123ub is required for full

methylation of K4 and K79 during transcription (Ng et al. 2002; Sun and Allis 2002; Krogan et al. 2003; Ng et al. 2003a; Wood et al. 2003; Shahbazian et al. 2005). This illustrates one example of histone crosstalk where modification of one residue can influence the modification state of another residue (Suganuma and Workman 2008).

1.2 EUKARYOTIC TRANSCRIPTION IS A HIGHLY REGULATED PROCESS REQUIRING A NUMBER OF ACCESSORY FACTORS

Transcription is performed by DNA-dependent RNA polymerase enzymes (RNA Pol) that assemble RNA molecules using a DNA template. In yeast, three different polymerases transcribe different regions of the genome: Pol I synthesizes ribosomal RNA (rRNA), Pol II synthesis messenger (mRNA) and many non-coding RNAs (ncRNAs), and Pol III synthesizes transfer RNA (tRNA) and the 5S rRNA. Transcription occurs in three distinct but overlapping stages: initiation, elongation, and termination, each requiring the precise coordination and activities of numerous transcription factors.

1.2.1 RNA Polymerase II is a conserved multi-subunit enzyme

RNA Pol II is a large 514kDa complex consisting of 12 subunits that form a claw-shaped structure (Figure 4) (Cramer et al. 2008). Ten of the subunits (Rpb1-3, 5, 6, 8-12) make up the conserved enzyme core with two additional subunits (Rpb4, 7) forming a detachable heterodimer associated with the surface of Rpb1. The two largest subunits Rpb1 and Rpb2 form opposite sides of the active site cleft, connected by an alpha-helical bridge domain (Cramer et al. 2000;

Cramer et al. 2001). A portion of Rpb1 forms a flexible clamp domain that changes conformation during transcription (Cramer et al. 2001; Gnatt et al. 2001). At the catalytic core, deep in the cleft, is a coordinated magnesium ion required for enzymatic activity. DNA enters the cleft as a duplex and unwinds as it reaches the active site. Here, the template strand turns sharply, pointing the DNA base towards the active site to allow ribonucleotide selection and formation of a new phosphodiester bond onto the growing RNA chain. After ~8 nucleotides of RNA are added, the RNA-DNA hybrid interactions are split and the RNA leaves the structure via an exit channel that places it near the C-terminal domain (CTD) of Rpb1 (Gnatt et al. 2001; Kettenberger et al. 2004).

The largest Pol II subunit Rpb1 contains a flexible C-terminal tail domain (CTD) that extends outside of the enzyme structure and plays critical roles in regulating the transcription cycle. It consists of a heptapeptide with the consensus sequence YSPTSPS repeated 26 times in yeast. Progressive truncations of the CTD repeats demonstrate that at least eight repeats are required for viable yeast, while further truncations or phosphorylation site mutants are lethal (West and Corden 1995). The CTD undergoes dynamic phosphorylation of the serine residues at the 2^{nd}, 5^{th}, and 7^{th} positions (Ser2P, Ser5P, Ser7P) of the heptapeptide repeat. These phosphorylation states form the "CTD code" that can be recognized and read by binding factors to regulate steps in the transcription cycle (Figure 5) (Buratowski 2009).

In the preinitiation complex, the CTD is hypophosphorylated, which is important for Pol II recruitment and interaction with the coactivator Mediator. During the transition from initiation to elongation the CTD becomes enriched in Ser5P through the activity of the CTD kinase Kin28, a component of the general transcription factor TFIIH (Komarnitsky et al. 2000). This modification is required for recruitment and activity of the mRNA capping enzymes that add a

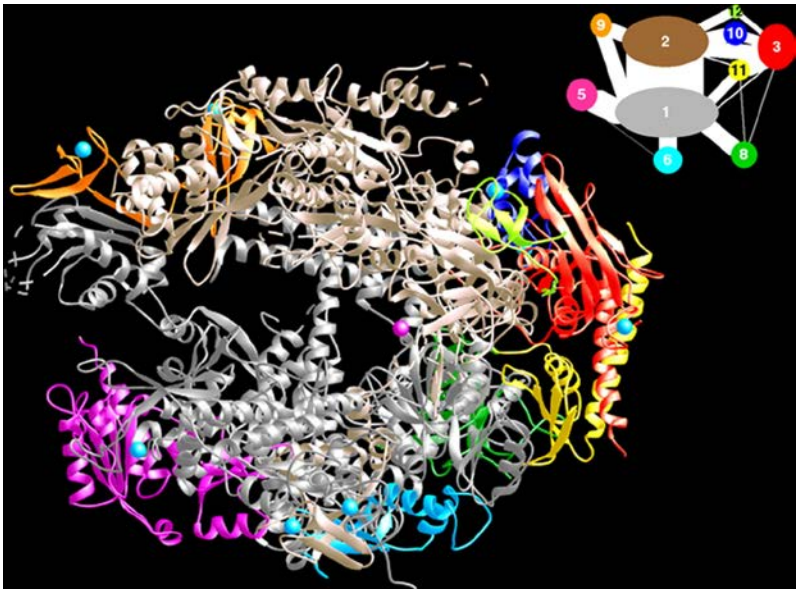

Figure 4: Structure of the RNA Polymerase II core enzyme.

Crystal structure of RNA Pol II along with a key showing the locations of the 10 core subunits and their interactions. The magnesium ion at the catalytic core is shown as a pink dot. Figure from (Kornberg 2007), reprinted according to guidelines of the National Academy of Sciences (http://www.pnas.org/site/misc/rightperm.shtml). Copyright (2007) National Academy of Sciences, USA.

7-methylguanylate cap to the 5' end of the growing RNA chain. Ser5P also stimulates the methylation of H3 K4 by the Set1 methyltransferase at the 5' ends of genes (Ng et al. 2003b) and the stimulation of the Ser2 kinases. Interestingly, Ser7 is also phosphorylated near the 5' end of genes by TFIIH (Chapman et al. 2007; Egloff et al. 2007; Akhtar et al. 2009). Although its role is not clear, Ser7P may be involved in the processing of certain short snRNAs (Egloff et al. 2007). As transcription progresses from 5' to 3' Ser5P decreases, as it is removed by the phosphatase Rtr1 (Mosley et al. 2009), and Ser2P increases through the combined kinase activities of Ctk1 and Bur1 (Yao et al. 2000; Cho et al. 2001; Murray et al. 2001; Qiu et al. 2009). In the middle of genes there is a double phosphorylation state Ser2P/5P that can stimulate H3 K36 methylation by Set2 (Kizer et al. 2005). Near the end of genes Ser2P predominates, signaling for polyadenylation of the RNA and transcription termination (Buratowski 2009). After termination, the CTD becomes dephosphorylated by Fcp1 and Ssu72 to allow the cycle to be repeated (Cho et al. 2001; Krishnamurthy et al. 2004).

1.2.2 Transcription initiation occurs through the binding of transcription factors to regulatory DNA sequences and the assembly of the pre-initiation complex

Genes are made up of coding sequences that specify the nucleotide or amino acid sequence of the final RNA or protein product, and regulatory sequences that control when and how the gene gets expressed. In yeast, the regulatory sequences are typically found in the promoter region within ~500bp upstream of the transcription start site (TSS) (Figure 6). The TSS, also called the initiator element (Inr) consists of an A/G rich region neighboring the start site, usually an adenine. Approximately 20% of yeast genes contain a regulatory element called the TATA box with a consensus sequence TATA(A/T)A(A/T)N located 40-120bp upstream

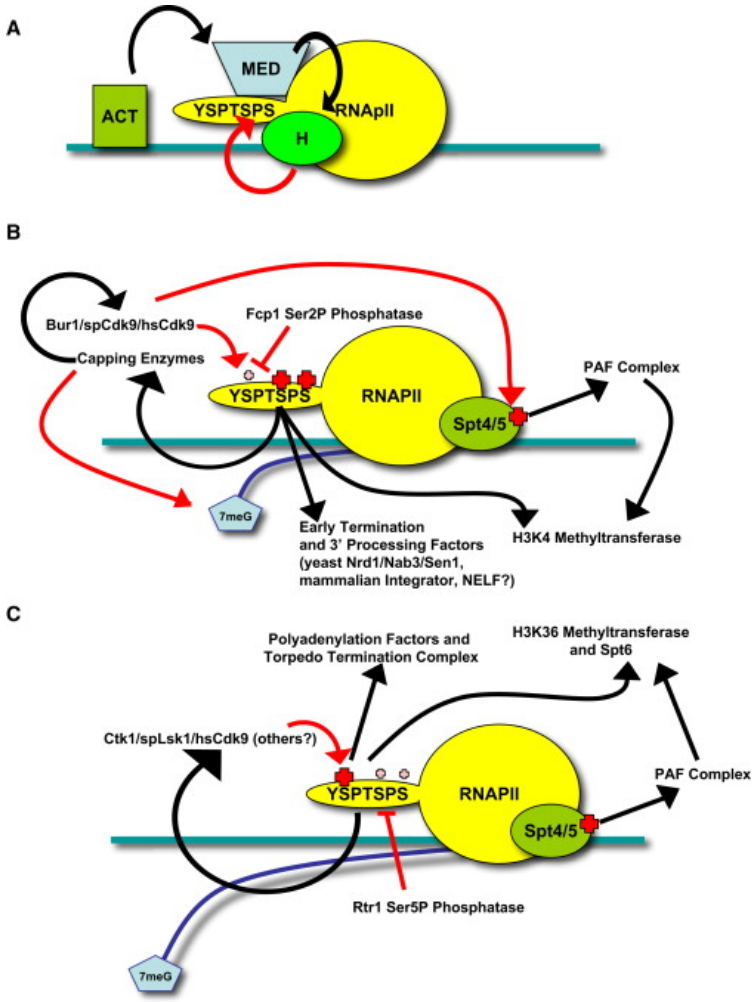

Figure 5: The phosphorylation events on the Pol II CTD during transcription.

Model of the regulation of the transcription cycle by the Pol II CTD during preinitiation (A), early elongation (B), and late elongation or termination (C). Red lines indicate the activity of the kinases or phosphatases that modify the CTD, indicating other targets of these factors as well. Black lines indicate factors or processes that are affected by the particular phosphorylation state of the CTD. Figure reprinted from Molecular Cell, Vol. 36, Buratowski S. Progression through the RNA polymerase II CTD cycle. Copyright (2009), with permission from Elsevier.

of the TSS (Struhl 1989; Basehoar et al. 2004). These elements signal the location and direction of preinitiation complex (PIC) formation. Other elements, such as the downstream core promoter element (DPE) and TFIIB recognition element (BRE) facilitate binding of general transcription factors, especially at TATA-less genes. Further upstream of these core promoter elements are upstream activating and repressing sequences (UAS and URS) that can be bound by sequence-specific activator and repressor proteins, respectively (Krishnamurthy and Hampsey 2009).

While RNA Pol II is sufficient for transcription, it cannot recognize promoters and initiate efficiently without help from accessory factors collectively known as general transcription factors (GTFs) (Figure 6) (Sikorski and Buratowski 2009). TATA Binding Protein (TBP), a subunit of TFIID binds to the TATA box and initiations PIC formation with the help of TBP-associated factors (TAFs) that make up the rest of TFIID and bind to other DNA sequences. TFIIB associates with the TBP-DNA complex, which is then able to recruit Pol II and TFIIF. TFIIE follows Pol II to the PIC and stimulates the binding and activity of the last initiation factor TFIIH. TFIIH has helicase activity that melts the promoter DNA, as well as CTD kinase activity that helps transition the preinitiation complex into elongation (Sikorski and Buratowski 2009).

Activator proteins bind specific UAS elements within promoters to stimulate transcription in response to environmental cues or signaling pathways. They facilitate transcription initiation beyond the basal level signaled by a core promoter alone. Activators recruit co-activator complexes such as SAGA, Swi/Snf, and Mediator. SAGA is a histone acetyltransferase complex capable of loosening histone-DNA contacts through acetylation (Rodriguez-Navarro 2009), allowing chromatin remodelers like Swi/Snf to more easily displace nucleosomes to allow initiation (Schwabish and Struhl 2007). PIC formation is also facilitated

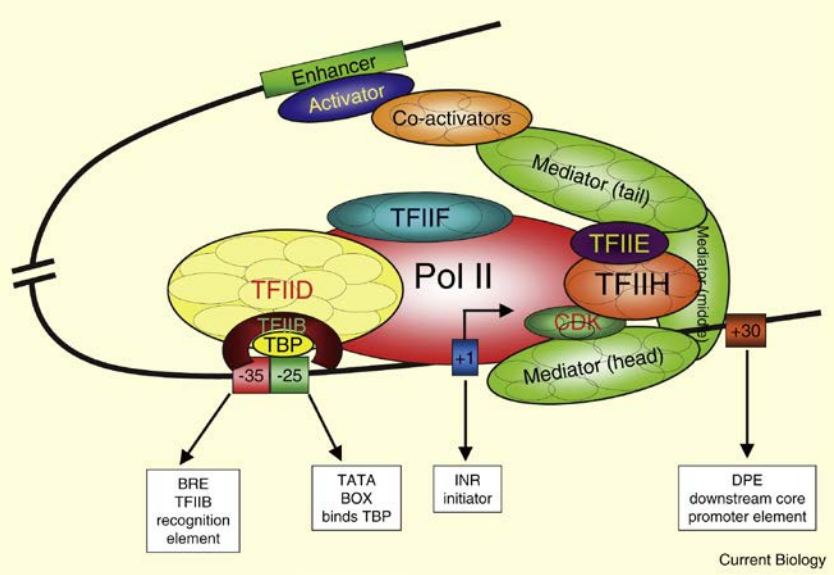

Figure 6: The transcription preinitiation complex.

Model of the regulatory sequences of a typical yeast promoter along with the general transcription machinery assembled into a preinitiation complex. Figure reprinted from Current Biology, Vol. 19, Krishnamurthy S, Hampsey M. Eukaryotic transcription initiation. Copyright (2009), with permission from Elsevier.

by the large multifunctional Mediator complex, which connects activators to Pol II and the PIC (Kornberg 2005). RNA Pol II, the GTFs, and Mediator, together nearly 60 protein subunits and nearly 3 megadaltons in size, comprise the complete preinitiation complex (Figure 6) (Kornberg 2007).

1.2.3 Transcription elongation factors travel with RNA Pol II and facilitate elongation

Upon formation of the preinitiation complex, productive transcription does not proceed automatically. Pol II must overcome a stage of abortive initiation in which short nucleotide segments are repeatedly made and released (Liu et al. 2011). Promoter clearance, the act of overcoming abortive initiation, is facilitated by strong binding of Pol II to DNA, plentiful nucleotide pools, and TFIIH-stimulated phosphorylation of the Pol II CTD on Ser5 residues (Dvir 2002; Max et al. 2007). Once the RNA chain reaches ~25bp, productive transcription elongation begins and nucleotides are rapidly added to the growing RNA molecule (Kornberg 2007).

Transcription elongation is facilitated by a number of elongation factors, including Spt4/5, Bur1/2, and the Paf1 complex that are evolutionarily conserved. Spt4 and Spt5 form a complex that associates with Pol II over actively transcribed genes, has numerous physical and genetic interactions with other transcription factors, and facilitates elongation through chromatin (Hartzog et al. 1998; Squazzo et al. 2002; Rondon et al. 2003; Simic et al. 2003). Bur1 and Bur2 form a cyclin-dependent kinase/cyclin complex that plays a variety of roles in transcription through the phosphorylation of substrates including the CTD of Pol II (Murray et al. 2001; Qiu et al. 2009), the ubiquitin conjugating enzyme Rad6 (Wood et al. 2005), and the C-terminal repeat region of Spt5 (Liu et al. 2009; Zhou et al. 2009). Phosphorylation by Bur1/2 activates the Spt5

protein, which promotes the recruitment of the Paf1 complex to chromatin (Liu et al. 2009; Zhou et al. 2009). The Paf1 complex is a conserved, multi-subunit complex consisting of Paf1, Ctr9, Rtf1, Cdc73, and Leo1, that plays a number of important roles in the transcription cycle (Jaehning 2010). The Paf1 complex facilitates elongation largely through the regulation of histone modifications associated with active transcription: H2B ubiquitylation and H3 K4, K36, and K79 methylation (Krogan et al. 2003; Ng et al. 2003a; Wood et al. 2003; Chu et al. 2006).

Much of the requirement for elongation factors is to help Pol II overcome the nucleosome barrier. One general elongation factor TFIIS helps Pol II overcome pausing at nucleosomes by stimulating the cleavage of the RNA molecule in the active site and backtracking of the polymerase to allow elongation to resume (Kettenberger et al. 2004; Churchman and Weissman 2011). Other elongation factors such as Spt6 and FACT can either partially or fully disassemble nucleosomes ahead of Pol II to allow its passage (Belotserkovskaya et al. 2003; Kaplan et al. 2003; Mason and Struhl 2003; Cheung et al. 2008; Jamai et al. 2009). This is facilitated by HATs that acetylate nucleosomes over the transcribed region to loosen histone-DNA contacts.

These mechanisms for allowing Pol II passage through chromatin also require the restoration of the nucleosome structure in its wake. Without proper reassembly of nucleosomes, transcription initiation can occur from cryptic promoter elements within coding sequences (Kaplan et al. 2003). Cryptic initiation is prevented through two major mechanisms, the reassembly and deacetylation of nucleosomes. As Spt6 and FACT disassemble nucleosomes ahead of Pol II they also reassemble them behind the transcribing polymerase (Mason and Struhl 2003; Kaplan et al. 2009). The coding sequence-associated nucleosomes are also methylated on H3 K36 by Set2, which travels with Pol II (Pokholok et al. 2005; Rao et al. 2005). Nucleosomes trimethylated on H3 K36 are bound by the Rco1 subunit of the Rpd3s HDAC complex, which

deacetylates the nucleosomes to restore chromatin and prevent internal initiation (Carrozza et al. 2005; Joshi and Struhl 2005; Keogh et al. 2005).

1.2.4 Transcription termination and 3' end processing result in a mature transcript

The termination of transcription, 3' end processing of the transcript, and release of Pol II must be precisely coordinated in order to ensure the proper length, localization, stability, and function of the RNA product (Kuehner et al. 2011). Proper termination is also required to prevent read-through of the transcription machinery into adjacent genomic regions, which can interfere with the expression of neighboring genes (Palmer et al. 2011). Processing and termination are largely regulated by a diverse set of factors that bind to the Ser2P CTD near the ends of genes. Termination of most protein-coding genes is coupled with cleavage of the transcript and addition of a poly-adenosine, Poly(A), tail to the 3' end. After the Poly(A) site on the DNA template is transcribed, Pol II pauses, allowing the ~20 subunit termination machinery, including the CPF and Rat1-Rai1-Rtt103 complexes, to cleave and polyadenylate the transcript and release Pol II (Figure 7A) (Park et al. 2004). An alternative pathway of termination exists for many non-coding transcripts lacking Poly(A) tails, called the Sen1-dependent pathway. Sen1 is an essential factor, recruited by the RNA binding factors Nrd1 and Nab3, that uses its helicase activity to disrupt the DNA-RNA complex and release Pol II (Figure 7B) (Steinmetz et al. 2006). After release, the Pol II is free to bind another promoter to repeat the transcription cycle. Gene looping events have been observed in yeast where terminator regions are held in physical proximity to promoter regions to facilitate Pol II recycling (O'Sullivan et al. 2004; Ansari and Hampsey 2005).

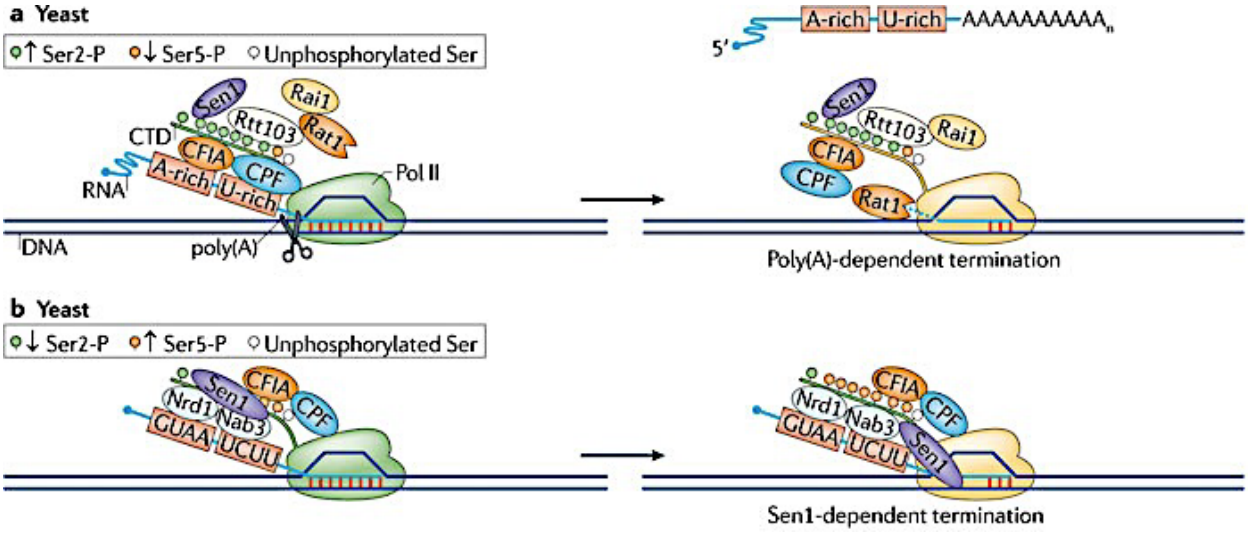

Figure 7: Factors involved in Poly(A)-dependent and Sen1-dependent termination.

Model of the Poly(A)-dependent (A) and Sen1-dependent (B) termination pathways in yeast. Reprinted with modifications by permission from Macmillan Publishers Ltd: Nature Reviews Molecular Cell Biology (Kuehner et al. 2011), copyright 2011.

1.3 TRANSCRIPTION IS PERVASIVE IN EUKARYOTIC GENOMES PRODUCING A VARIETY OF NON-CODING TRANSCRIPTS THAT CAN IMPACT EXPRESSION OF PROTEIN-CODING GENES

1.3.1 The majority of the transcriptional output of cells is non-protein coding

One of the biggest surprises in the field of gene expression over the past decade is the finding that transcription is not limited to protein-coding genes, but rather occurs throughout entire genomes and often involves both DNA strands (Kapranov et al. 2007; Pheasant and Mattick 2007; Berretta and Morillon 2009; Jacquier 2009). This conclusion is the result of numerous genome-wide expression studies using a variety of techniques, such as Pol II chromatin immunoprecipitation (ChIP), tiled microarray analysis, RNA sequencing, and more. These studies have revealed that over 85% of the yeast genome is transcribed, with non-coding regions making up the vast majority of the transcriptional activity of the cell (David et al. 2006; Miura et al. 2006; Nagalakshmi et al. 2008). Similar results have been seen in higher eukaryotes as well (Kim et al. 2005; Guttman et al. 2009; Guttman et al. 2010).

The extent of transcription of ncDNA has recently been questioned, as some transcripts appear to be false positives (van Bakel et al. 2010), or artifacts of the experimental design (Perocchi et al. 2007). It is also likely that many bona fide ncRNAs may represent transcriptional noise, the byproducts of bidirectional promoters (Morris et al. 2008; Neil et al. 2009; Xu et al. 2009) or imprecise transcription initiation (Struhl 2007) and may not be regulatory. While future studies will be needed to resolve the extent and significance of non-coding transcription, it is clear that eukaryotes produce many RNA molecules that likely do not encode proteins. It is also becoming clear that transcription of non-coding regions plays

important biological functions, primarily in regulating gene expression (Goodrich and Kugel 2009; Harrison et al. 2009; Mercer et al. 2009).

1.3.2 Some well-characterized ncRNAs including rRNAs, tRNAs, snRNAs, and snoRNAs play important biological functions

Many families of non-protein coding RNAs have been well characterized and play diverse and important roles in the cell. Ribosomal RNAs (rRNAs) are responsible for much of the structure of the ribosome as well as its catalytic protein synthesis activity (Ban et al. 2000; Nissen et al. 2000). The 60-300nt small nucleolar RNAs (snoRNAs) associate with proteins in the nucleolus forming ribonucleoprotein (RNP) particles essential for rRNA processing and ribosome biogenesis (Kiss 2002). Small nuclear RNAs (snRNAs) also from a large RNP complex, called the spliceosome, involved in the splicing of introns from pre-mRNA molecules (Will and Luhrmann 2011). Transfer RNAs (tRNAs) play a crucial role in translation by pairing the correct amino acid with the mRNA codon for incorporation into the growing polypeptide chain. Together these RNAs function as some of the core components of the cell's gene expression machinery (Figure 8) (Collins 2011).

1.3.3 Most classes of ncRNAs are largely uncharacterized

With the exception of the well-studied classes of ncRNAs discussed in the last section, the biological functions of many ncRNAs are only beginning to be understood. ncRNAs have diverse properties of both size and stability. Many of the ncRNAs, called stable unannotated transcripts (SUTs), are relatively long-lived and can be detected in wild type cells

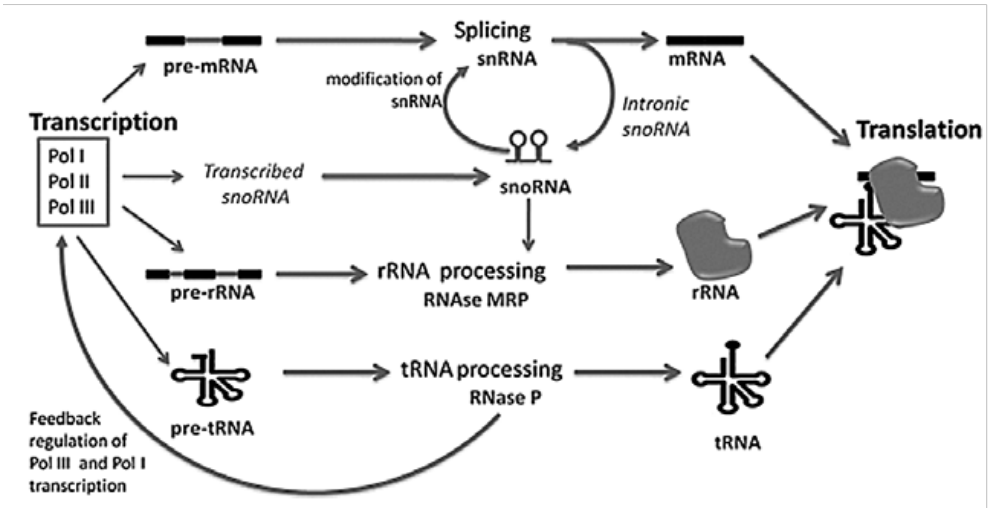

Figure 8: Regulation of core gene expression activities by ncRNAs.

Overview of the major classes of well-characterized ncRNAs and their role in gene expression. Figure adapted and reprinted with permission from Springer Science + Business media: *RNA Infrastructure and Networks*, The RNA Infrastructure: and introduction to RNA networks, 2011, by Lesley J. Collins, Page 3, Figure 1.

(Neil et al. 2009; Xu et al. 2009). Other ncRNAs called cryptic unstable transcripts (CUTs) are rapidly degraded and short-lived in the cell (Davis and Ares 2006). CUTs are only detected when mutations disable the RNA surveillance machinery responsible for degrading aberrant RNAs. The exosome is the major RNA degradation machinery responsible for the 3' to 5' exonucleolytic degradation of CUTs in the nucleus. The exosome works with another complex, the Trf4-Air2-Mtr4p polyadenylation (TRAMP) complex that prepares ncRNAs for cleavage by the exosome (Callahan and Butler 2010). Mutations in the gene encoding the Rrp6 subunit of the exosome, alone or in combination, with *trf4* mutants allow for detection of many CUTs (LaCava et al. 2005; Vanacova et al. 2005; Wyers et al. 2005; Davis and Ares 2006). Another class of CUTs, called XUTs, are detected by the disabling of a cytoplasmic 5' to 3' endonuclease Xrn1 (van Dijk et al. 2011).

Another important class of non-coding transcription occurs antisense to protein-coding genes. An estimated 55% of yeast SUTs are antisense to genes (Xu et al. 2009) and 25% of human protein-coding genes contain antisense transcripts (He et al. 2008). The roles of this antisense transcription are not well characterized, but it can often interfere with transcription on the sense strand. Genome-wide analysis of antisense transcription in yeast suggest it increases expression variability of protein-coding genes, and can act to spread regulatory signals from one promoter to neighboring loci (Xu et al. 2011). Since antisense transcripts are complementary to sense transcripts, they may also act through an RNA interference mechanism to degrade the sense mRNA or prevent its translation (Willingham and Gingeras 2006).

1.3.4 Many ncRNAs regulate the expression of protein coding genes *in trans*

Significant advances have been made in understanding the widely diverse mechanisms by which transcription of ncDNAs regulate gene expression. In some cases, it is the ncRNA product that regulates gene expression. One well-studied class in higher eukaryotes is made up of small ncRNAs that act in RNA interference (RNAi) pathways, such as microRNAs (miRNA), small interfering RNAs (siRNA), and Piwi-interacting RNAs (piRNA) (Ghildiyal and Zamore 2009). These 20-30nt RNAs can either be encoded in the genome (miRNAs and piRNAs) or added exogenously to cells (siRNAs) to mediate specific down-regulation of gene expression. These ncRNAs bind to complementary, or near complementary, sequences in mRNAs forming a substrate for the RNAi machinery that processes the ncRNA/substrate complex leading either to degradation of the target mRNA or inhibition of its translation (Ghildiyal and Zamore 2009). These RNAi pathways do not appear to be conserved in *S. cerevisiae* suggesting alternative mechanisms may perform these functions of regulating gene expression (Harrison et al. 2009).

Outside of RNA interference, many long ncRNAs have been shown to regulate gene expression *in trans* through a variety of mechanisms. They can recruit complexes that modify chromatin, interact with activator and coactivator proteins and modulate their function, titrate away transcription factors or miRNAs, and interact with RNA Pol II and other basal transcription factors to control their activity (Figure 9) (Goodrich and Kugel 2009; Harrison et al. 2009; Mercer et al. 2009; Wang and Chang 2011). Some examples of *trans*-acting long ncRNAs that regulate important processes include the Xist/Tsix RNAs involved in mammalian X-inactivation (Lee 2009), the roX1 and roX2 RNAs involved in dosage compensation in *Drosophila* (Gelbart and Kuroda 2009), the human HOTAIR involved in the regulation of developmental genes (Rinn et al. 2007), the mouse Air and Kcnq1ot1 RNAs involved in establishing genomic imprinting

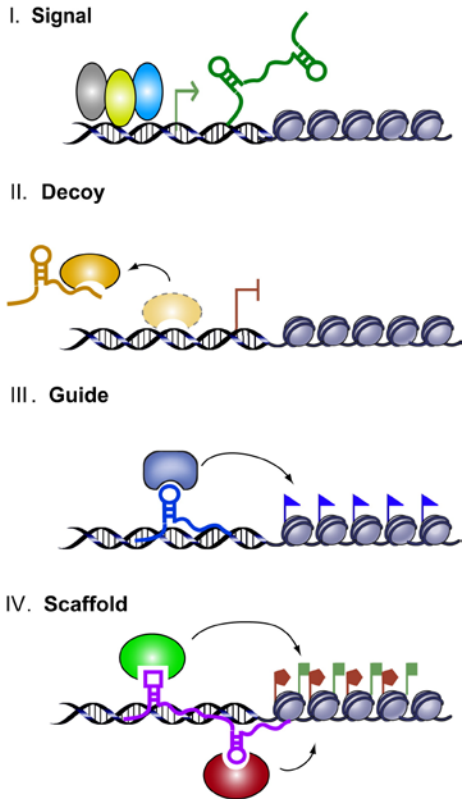

Figure 9: Possible mechanisms of *trans*-acting ncRNAs.

Long ncRNAs can adopt important secondary structural conformations that allow them to act as, (I) signaling molecules, (II) decoys that can titrate away transcription factors, (III) guides to recruit chromatin remodeling or histone modifying complexes, and (IV) scaffolds to assemble protein complexes. Figure reprinted from Molecular Cell, Vol. 43, Wang KC, Chang HY. Molecular mechanisms of long noncoding RNAs. Copyright (2011), with permission from Elsevier.

(Royo and Cavaille 2008), and the mouse VL30 RNA and human PSF-binding ncRNAs that regulate cell proliferation and tumorigenesis (Ardehali et al. 2009; Wang et al. 2009).

1.3.5 Transcription of non-coding regions of the genome can alter gene expression *in cis*

Aside from ncRNAs acting *in trans*, the act of transcribing ncDNA has also been shown to both positively and negatively regulate local gene expression (Hainer and Martens 2011b). In most of these cases, a transcription interference mechanism has been proposed. The earliest examples include the mouse and human globin genes (Ashe et al. 1997; Gribnau et al. 2000), and have since been seen for other genes in higher eukaryotes, such as the *Drosophila* Hox genes (Schmitt et al. 2005; Mazo et al. 2007), but these mechanisms are not well understood. Perhaps the most characterized examples of *cis*-regulation occur in budding yeast, beginning with studies of the regulation of *SER3* by *SRG1* (Discussed in detail in Chapter 1.4). Studies have since discovered mechanisms of non-coding transcription contributing to the regulation of numerous yeast genes.

These mechanisms of gene regulation can occur by non-coding transcription on the same strand as the protein-coding gene. Genes encoding two zinc-dependent alcohol dehydrogenases, *ADH1* and *ADH3*, are both repressed by intergenic transcription in response to low zinc levels, in a mechanism similar to *SER3* repression (Bird et al. 2006). The transcription termination factor *NRD1* is autoregulated; when present in sufficient levels it causes premature termination of the *NRD1* gene while low Nrd1 protein levels lead to proper expression of the gene (Arigo et al. 2006). The *IMD2* gene encodes an enzyme required for guanine nucleotide synthesis and is regulated by short unstable transcripts produced from start sites upstream of the normal *IMD2* transcription start site (TSS). When guanine levels are high, these non-coding transcripts prevent *IMD2* expression, while low guanine levels cause a shift to the functional *IMD2* TSS (Jenks et al.

2008; Kuehner and Brow 2008). The *FLO11* gene is repressed by non-coding transcription, *ICR1*, over its promoter. Interestingly, *ICR1* itself is repressed by antisense transcription, *PWR1*, which relieves *ICR1* repression of *FLO11* (Bumgarner et al. 2009). The *ASP3* gene is regulated by intragenic transcription, initiating within the gene, that somehow feeds back to the *ASP3* promoter to facilitate its expression (Huang et al. 2010).

Antisense transcription also participates in many *cis*-regulatory mechanisms. The *IME4* gene, required for entry of diploids into meiosis, is repressed in haploid cells by antisense transcription initiated downstream of the *IME4* gene (Hongay et al. 2006). Conversely, antisense transcription initiated downstream of the *PHO5* gene is thought to enhance *PHO5* transcription by facilitating chromatin remodeling over its promoter (Uhler et al. 2007). Antisense transcription has been shown to silence the expression of *PHO84* by a mechanism that requires Hda1/2/3-dependent deacetylation of histones located at the *PHO84* promoter (Camblong et al. 2007; Camblong et al. 2009). Finally, two recent studies provide evidence that transcription of DNA antisense to the *GAL10* gene alters post-translational modifications of histones that facilitate repression of the divergently transcribed *GAL10* and *GAL1* genes (Houseley et al. 2008; Pinskaya et al. 2009). Both the variety of mechanisms used and the number of important cellular processes affected by the transcription of ncDNA emphasizes its importance in gene regulation.

1.4 THE YEAST GENE *SER3* IS REGULATED BY NON-CODING INTERGENIC TRANSCRIPTION ACROSS ITS PROMOTER

In the budding yeast *Saccharomyces cerevisiae*, the *SER3* and *SER33* genes encode nearly identical (92%) enzymes that catalyze the first step of serine and glycine biosynthesis (Figure 10)

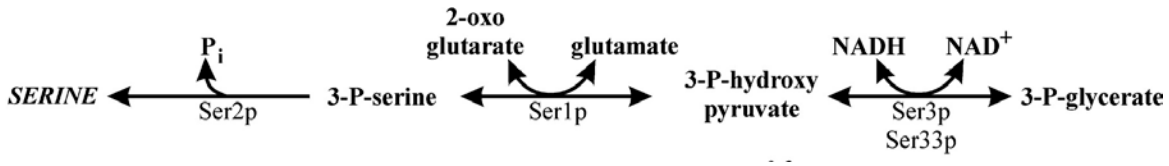

Figure 10: The major serine biosynthesis pathway in yeast.

This schematic depicts the production of serine from 3-phosphoglycerate. In the first step, 3-phosphoglycerate is converted to 3-phosphohydroxypyruvate by the redundant phosphoglycerate dehydrogenases, Ser3 and Ser33. This product is converted to 3-phosphoserine by the phosphoserine transaminase Ser1. The final step, catalyzed by the Ser2 phosphoserine phosphatase, converts phosphoserine to serine. Figure modified with permission from (Albers et al. 2003), copyright 2003, by the American Society for Biochemistry and Molecular Biology.

(Albers et al. 2003). The two enzymes have redundant functions, as cells containing a deletion of either gene alone can still grow in the absence of serine, while the double mutant cannot. Moreover, Ser33p is expressed regardless of the presence or absence of serine, similar to the other serine biosynthesis enzymes Ser1 and Ser2, and therefore appears to be a housekeeping enzyme (Albers et al. 2003). Ser3, on the other hand, is only expressed when serine is absent from the media, but the mechanism behind this regulation was previously not understood.

The regulation of *SER3* expression became of interest during investigations of the Swi/Snf chromatin remodeling complex. Genome-wide identification of genes regulated by the complex revealed many genes that require Swi/Snf for activation, as expected, but also genes that seem to be repressed by Swi/Snf, including *SER3* (Holstege et al. 1998; Sudarsanam et al. 2000). Studies of the mechanism of Swi/Snf in *SER3* repression suggested the complex acted directly in repression and depended largely on the Snf2 subunit, as opposed to activation that requires most of Swi/Snf subunits (Martens and Winston 2002). These investigations led to the discovery that the *SER3* gene was regulated by a previously uncharacterized mechanism (Martens et al. 2004).

1.4.1 Transcription occurs in the intergenic region upstream of *SER3* and overlaps the *SER3* promoter and coding region

While *SER3* was repressed in rich growth conditions (high serine) a number of factors associated with active transcription; TBP, RNA polymerase II, and mRNA capping factors were present on DNA upstream of the *SER3* promoter by ChIP. Further investigations discovered a Pol II transcript being produced in the intergenic region between *SER3* and its neighboring gene *AIM9* (formerly YER080w) (Martens et al. 2004). This intergenic transcription occurs on the same

strand as *SER3* and is initiated from a promoter upstream of the *SER3* promoter that contains elements, such as a TATA box and UAS (upstream activating sequence) that are conserved across related yeast species (Figure 11A). By Northern analysis, a probe specific to the *AIM9-SER3* intergenic region detects a diffuse band of ~550bp as well as a much larger band corresponding to a read-through to the end of *SER3* (Figure 11B). Mapping of these transcripts showed they initiate at -475 relative to the *SER3* translational start (ATG = +1), and the diffuse band is actually two separate transcripts terminating 75bp 5' and 25bp 3' of *SER3* ATG, respectively (Martens et al. 2004; Thompson and Parker 2007). While the intergenic transcripts appear to be capped and polyadenylated like mRNAs, they are considered CUTs. Although they are stable enough to detect by Northern analysis, they can be further stabilized by mutations in certain RNA degradation pathways. The two smaller transcripts can be degraded in the nucleus by the exosome (Davis and Ares 2006), but are primarily degraded in the cytoplasm by decapping and 5' to 3' exonucleolytic digestion. Degradation of the read-through product is largely performed by the cytoplasmic nonsense mediated decay (NMD) pathway (Thompson and Parker 2007). Since the intergenic transcription overlapping the *SER3* promoter was shown to have a regulatory function (Chapter 1.4.2) it was named *SRG1* for *SER3 regulatory gene 1* (Martens et al. 2004).

1.4.2 *SRG1* intergenic transcription is required to repress *SER3* through a transcription interference mechanism

Under serine rich conditions when *SER3* transcription levels are low, *SRG1* intergenic transcript levels are high. It became clear that the intergenic transcription was playing an important regulatory role because when it was abolished, by mutating the upstream TATA element, *SER3*

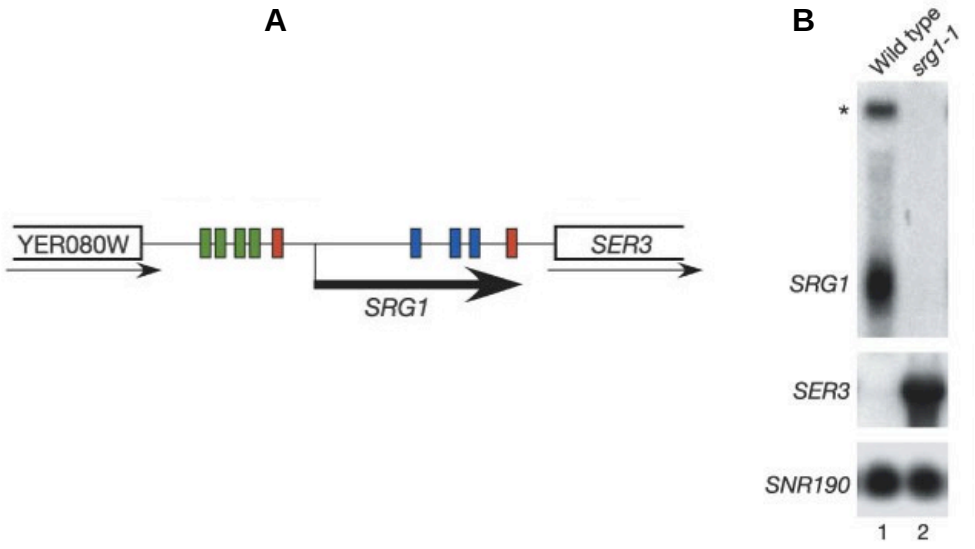

Figure 11: Repression of *SER3* by *SRG1* intergenic transcription.

A) Schematic diagram of the *SRG1-SER3* locus. The arrows indicate sites and direction of transcription. Blocks represent conserved regulatory elements: Red indicates TATA sequences, Blue indicates potential *SER3* activating sequences, and Green indicates potential *SRG1* activating sequences. Note that *YER080w* has been renamed *AIM9* and will be referred to as such throughout this document. B) Northern analysis of *SRG1*, *SER3*, and the loading control *SNR190* in a wild type strain and a strain in which the *SRG1* TATA sequence has been mutated. Figure reprinted by permission from Macmillan Publishers Ltd: Nature (Martens et al. 2004), copyright 2004.

was highly upregulated (Figure 11B) (Martens et al. 2004). This negative correlation between the two regions of transcription suggested a role for *SRG1* in the repression of *SER3* through a number of possible mechanisms. One possibility is a mechanism requiring the ncRNA produced from *SRG1* transcription that could act either locally or at a distance to regulate *SER3*. Alternatively, the *SRG1* promoter could repress *SER3* by competing transcription factors away from the *SER3* promoter. A third possibility is that *SRG1* transcription prevents transcription factors from binding to the *SER3* promoter by a transcription interference mechanism (Martens et al. 2004).

To distinguish among these possible models, first a *cis/trans* test was performed to determine if *SER3* repression requires *SRG1* to be adjacent to *SER3* or if it can repress from another genomic location (Figure 12). Diploid strains were created with two copies of the *SRG1* locus (either wild-type or the nonfunctional *srg1-1*) regulating both *SER3* and *URA3* at two different genomic locations. Northern analysis of the diploid strains confirmed that both *SER3* and *URA3* were repressed when *SRG1* was expressed immediately upstream, but neither gene could be repressed by a functional *SRG1* produced at the other locus (Figure 12A) (Martens et al. 2004). These results suggest *SRG1* can only repress *SER3 in cis*. To further distinguish between the *cis*-regulatory models, *SRG1* sequence was replaced with sequence from the *HIS3* gene, with or without a transcription termination sequence. Addition of the terminator allowed initiation of intergenic transcription but stopped it before reaching the *SER3* promoter region, completely relieving *SER3* repression (Figure 12B) (Martens et al. 2004). This result argued against the promoter competition and RNA-mediated models. Most of the *SRG1* sequence is not conserved and can be replaced with heterologous sequence without affecting *SER3* repression. This, along

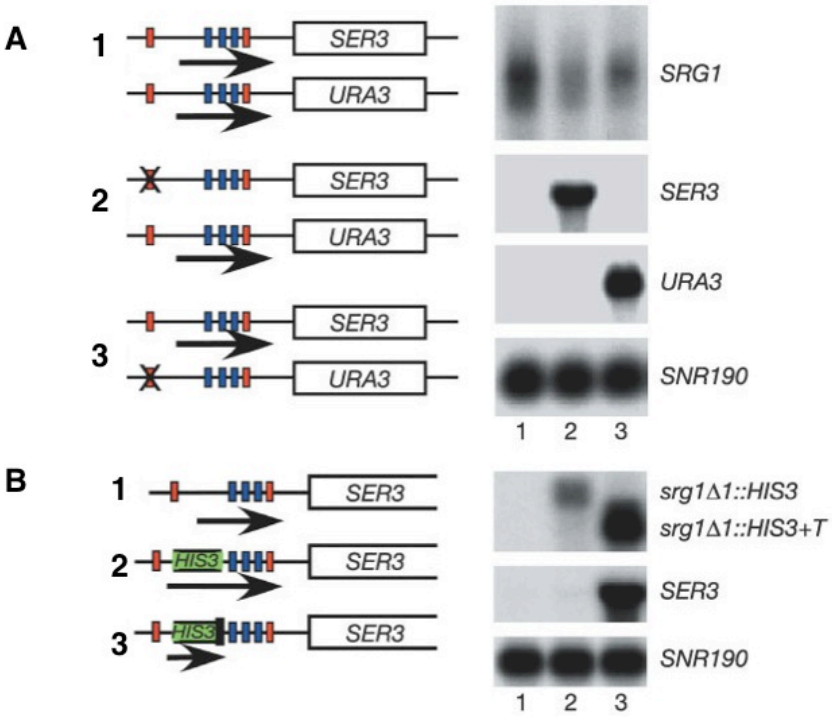

Figure 12: Testing possible models of *SER3* repression.

A) On the left are schematics of the two relevant loci present in each diploid. On the right are Northern analysis results showing *SRG1*, *SER3*, *URA3*, and *SNR190* levels in these strains. The X over the upstream TATA sequence indicates the *srg1-1 TATA* mutant. B) On the left are schematics of the *SER3* locus present in each strain tested. On the right are Northern analysis results showing *SRG1*, *SER3*, and *SNR190* levels in these strains. Figure reprinted by permission from Macmillan Publishers Ltd: Nature (Martens et al. 2004), copyright 2004.

with the fact that *SRG1* cannot act *in trans*, suggests the repression mechanism does not require the ncRNA or any protein produced from intergenic transcription.

The results of the *HIS3* terminator experiment support a transcription interference model whereby transcription must proceed over the *SER3* regulatory regions in order to mediate repression. This mechanism was confirmed by ChIP experiments demonstrating reduced binding of transcription factors due to *SRG1* transcription (Martens et al. 2004). Since the activator(s) of *SER3* is not known, support for this model was obtained by replacing the *SER3* UAS with two binding sites for the well-characterized Gal4 activator and showing that Gal4 binding was inhibited by *SRG1* transcription (Martens et al. 2004).

1.4.3 *SER3* is regulated by serine levels indirectly through the regulation of *SRG1* intergenic transcription

SER3 encodes an enzyme required for serine biosynthesis, and is only expressed in serine starvation conditions (Albers et al. 2003). The serine-dependent regulation of *SER3* is due to the serine-dependent regulation of *SRG1* transcription (Martens et al. 2005). When cells are shifted from serine-rich media to media lacking serine, *SRG1* levels decrease rapidly allowing *SER3* to be maximally expressed after just 15 minutes without serine. This induction of *SER3* is transient, however, as *SRG1* levels begin to increase and *SER3* levels are reduced again after ~90 minutes (Figure 13A). Similarly, as cells are shifted from media lacking serine to serine-rich media, *SRG1* is rapidly induced, repressing *SER3* fully within 15 minutes (Figure 13B). This timely response to serine is entirely dependent on *SRG1*, as *srg1-1* strains lacking intergenic transcription have constitutively derepressed *SER3* under both sets of conditions (Figure 13)

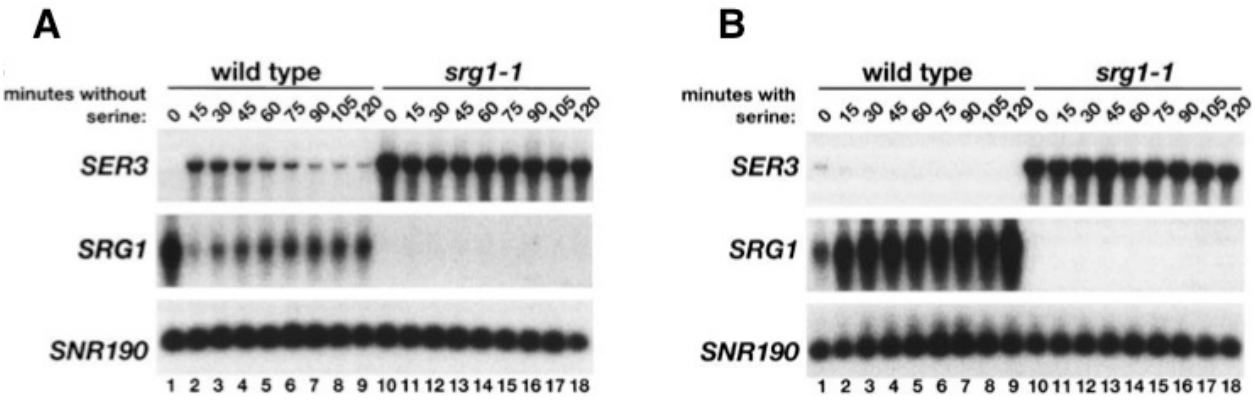

Figure 13: Serine-dependent regulation of *SRG1* and *SER3*.

A) Northern analysis of *SER3, SRG1*, and *SNR190* levels in a wild type and *srg1-1* strain grown in minimal media with 1mM serine before shifting to minimal media lacking serine. B) Northern analysis of *SER3, SRG1*, and *SNR190* levels in a wild type and *srg1-1* strain grown in minimal media lacking serine before shifting to minimal media with 1mM serine. Figure reprinted from (Martens et al. 2005) with permission from Cold Spring Harbor Laboratory Press, copyright 2005.

(Martens et al. 2005). These experiments demonstrate a role for *SRG1* intergenic transcription in regulating the serine-dependent regulation of *SER3*.

The serine-dependent regulation of *SRG1* is mediated by a serine-responsive activator protein Cha4 (Holmberg and Schjerling 1996; Sabet et al. 2003). Cha4 binds a consensus sequence in the *SRG1* promoter and is required for *SRG1* induction. Interestingly, Cha4 seems to be present at the *SRG1* promoter under both serine-rich and serine starvation conditions, indicating that serine regulation occurs at a step after Cha4 binding. The presence of serine regulates the recruitment of two coactivator complexes, SAGA, a histone acetyltransferase complex, and Swi/Snf, a chromatin remodeling complex (Martens et al. 2005). Specific subunits of these complexes are required for full initiation of *SRG1* and repression of *SER3* (Figure 14) (Martens and Winston 2002; Martens et al. 2005). When serine levels are low, Cha4 no longer recruits SAGA and Swi/Snf and *SRG1* is not activated, allowing expression of *SER3* (Martens et al. 2005). It is not known how coactivator recruitment by Cha4 is regulated by serine, but similar mechanisms have been seen for other yeast activator proteins (Sellick and Reece 2005).

The Cha4 activator is also responsible for directly activating another yeast gene, *CHA1*, which encodes an enzyme required for serine catabolism. In response to high serine levels, Cha4 turns on serine catabolism by directly activating *CHA1*, and turns off serine biosynthesis by indirectly repressing *SER3* through the activation of *SRG1*. Under serine starvation conditions, Cha4 no longer recruits SAGA and Swi/Snf shutting off serine catabolism by not activating *CHA1* and turning on serine biosynthesis by down-regulating *SRG1*, relieving repression of *SER3* (Figure 15) (Martens et al. 2005). This is an interesting case in which a single activator can simultaneously activate and repress opposing pathways in response to a single stimulus.

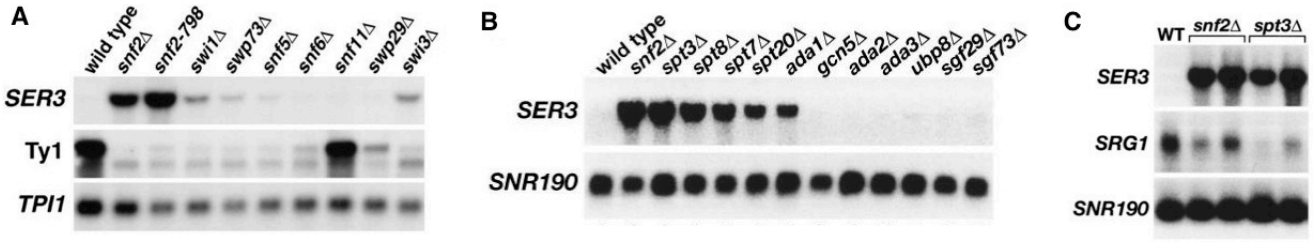

Figure 14: Swi/Snf and SAGA are coactivators of *SRG1* transcription.

A) Northern analysis of *SER3*, *Ty1* (a gene that requires Swi/Snf for activation), and a loading control *TPI1* in strains in which individual Swi/Snf subunits are mutated. B) Northern analysis of *SER3* and a loading control *SNR190* in strains in which individual SAGA subunits are deleted. C) Northern analysis of *SER3*, *SRG1* and a loading control *SNR190* in two replicate strains in which either *SNF2* or *SPT3* are deleted. Figure reprinted from (Martens and Winston 2002; Martens et al. 2005) with permission from Cold Spring Harbor Laboratory Press, copyright 2002 and 2005.

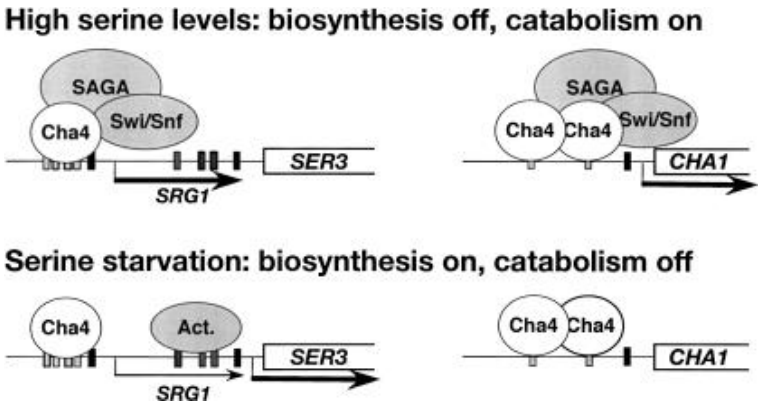

Figure 15: Model for the serine-dependent regulation of *SER3* and *CHA1* by the activator Cha4.

Models of the *SER3* (left) and *CHA1* loci (right) under high serine conditions (top) and serine starvation (bottom). Arrows indicate active transcription. Figure reprinted from (Martens et al. 2005) with permission from Cold Spring Harbor Laboratory Press, copyright 2005.

1.5 THESIS AIMS

When I began my thesis work, there were numerous outstanding questions regarding the mechanism of *SER3* repression by *SRG1* intergenic transcription. For example, while we knew that *SRG1* transcription could interfere with activator binding to the *SER3* promoter, we did not know how this transcription interference was mediated. We also did not know what other factors were involved in this mechanism.

To help address these questions, I performed a genetic screen to identify factors required for *SER3* repression. In this global and unbiased approach, I hoped to further our understanding of the mechanism through identification of its mediators. The screen yielded a number of interesting avenues of investigation. The identification of a histone gene and factors required for histone gene expression led us to test the role of nucleosome positioning and occupancy in *SER3* repression. These studies characterized a repressive chromatin structure, maintained by intergenic transcription that overlaps the *SER3* promoter and contributes to transcription interference. The identification of the Bur2 and Spt4 transcription elongation factors led to investigations of these factors and the Paf1 complex in *SER3* repression. In that study, I identified a role for this pathway of elongation factors in recruiting the Paf1 complex to *SRG1* transcription, where it is required for proper nucleosome structure and repression of *SER3*. These results and other candidates from the screen that may be the focus of future investigations have contributed to our model of *SER3* regulation as well as our understanding of gene regulation in general.

2.0 A GENETIC SCREEN FOR REGULATORS OF *SER3* REPRESSION

The work presented in this chapter is unpublished, but the results of my genetic screen contributed to our published studies (Hainer et al. 2011; Pruneski et al. 2011). This work was aided by an undergraduate Frank Kowalkowski, who helped verify the screen results.

2.1 INTRODUCTION

A major outstanding question is how *SRG1* intergenic transcription is able to prevent transcription factors from accessing the *SER3* promoter. There are many possible mechanisms, or combination of mechanisms, that may contribute to transcription interference. One possibility is that interference is mediated simply by the passage of RNA polymerase II across that region, or perhaps due to transcription elongation factors associated with the polymerase. *SRG1* transcription also terminates near the *SER3* promoter, so the termination mechanism or certain termination factors may be required. Another hypothesis involves alteration of chromatin structure across the *SER3* promoter, either through specific histone modifications or positioning of nucleosomes.

In order to investigate the mechanism of transcription interference, we set out to identify proteins involved in this repression mechanism. In a global and unbiased approach, we designed a genetic screen for mutants that are defective for *SER3* repression using the yeast deletion set, a

collection of *S. cerevisiae* strains that each contain a deletion of a single non-essential gene marked by the *KanMX* drug resistance gene (Winzeler et al. 1999). This screen has advantages over more traditional mutagenesis screens in that each resulting strain should contain only a single mutation, which should simplify the interpretation. Importantly, the deletion in each strain is already known so any candidates affecting *SER3* can be identified immediately. A disadvantage of this type of screen is that it only includes non-essential genes that are present in the deletion set. We are unable to identify essential genes, partial loss of function alleles, or gain of function alleles that can be found in other types of mutagenesis.

To perform the screen, a strain containing a reporter for *SER3* expression was crossed to each of the deletion strains in parallel. A series of selection steps resulted in strains containing both the deletion and the reporter. These strains were then tested on reporter media to determine which mutants affected *SER3* expression. This resulted in a number of candidates that were taken through a series of retest and verification steps to rule out false positives. The result was the identification of 21 genes required for proper repression of endogenous *SER3*. These candidates include a number of factors required for *SRG1* transcription, the *SER3* gene itself, transcription elongation factors, factors required for regulating histone gene expression, and other candidates of various or unknown functions.

2.2 MATERIALS AND METHODS

2.2.1 Yeast strains and media

Most *S. cerevisiae* strains used in this study (Table 1) are isogenic with a *GAL2*$^{+}$ derivative of S288C (Winston et al. 1995). The *MAT***a** deletion collection (Open Biosystems), our wild type control strain OY8 (Martin Schmidt, U. of Pittsburgh), and OY2 are in the BY4741 background (Winzeler et al. 1999). Strains were constructed using standard genetic crosses or by transformation (Ausubel et al. 1991).

The query strain for the genetic screen YP012 was constructed in a series of steps starting with YJ586. The reporter *SER3pr-URA3* was PCR amplified from YJ100 genomic DNA and integrated into YJ586 disrupting the *LYP1* locus to create YP003. The "magic marker" *STE2pr-LEU2* was PCR amplified from OY2 (Charlie Boone, U. of Toronto) genomic DNA and integrated into the *CAN1* locus to create YP006. To generate YP012, the *URA3* gene in the reporter allele was replaced with the *HIS3* gene, which was PCR amplified from YP008 genomic DNA.

Strains were grown in the following media: YPD (1% yeast extract, 2% peptone, 2% glucose), and synthetic complete with 1mM serine (SC+serine) or without serine (SC-serine) (Rose 1991). Reporter plates were SC-his containing 5mM 3AT (3-amino-1,2,4,-triazole, Sigma). Other screen plates were supplemented with 100mg/L canavanine (L-canavanine sulfate salt, Sigma), 200mg/L G418 (Geneticin, Invitrogen), or 100mg/L thialysine (S-(2-aminoethyl)-L-cysteine hydrochloride, Sigma) as needed.

Table 1: *S. cerevisiae* strains used in Chapter 2.

Name	Genotype
FY4	*MAT***a**
FY5	*MATα*
FY2199	*MAT***a** *his3Δ200 lys2-128δ leu2Δ0 ura3Δ0 spt21Δ201::HIS3*
FY2283	*MATα his3Δ200 lys2-128δ ura3Δ0 leu2Δ0 hir1Δ201::KanMX*
FY2441	*MAT***a** *his3Δ200 lys2-128δ leu2Δ0 hir3Δ0::KanMX*
FY2443	*MAT***a** *his3Δ200 lys2-128δ leu2Δ0 hir2Δ0::KanMX*
KY923	*MAT***a** *his3Δ200 lys2-128δ leu2Δ0 spt21Δ201::HIS3*
KY924	*MATα his3Δ200 ura3Δ0 trp1Δ63 spt21Δ201::HIS3*
KY925	*MAT***a** *his3Δ200 leu2Δ0 trp1Δ63 spt21Δ201::HIS3*
OY2	*MAT***a** *can1Δ::STE2pr-LEU2 lyp1Δ his3Δ1 leu2Δ0*
OY8	*MAT***a** *his3Δ1 leu2Δ0 ura3Δ0 met15Δ0*
YJ100	*MATα his3Δ200 leu2Δ0 ura3Δ0 lys2Δ0 met15Δ0 ade8 snf2::LEU2 SER3pr-URA3*
YJ586	*MATα his3Δ200 leu2Δ0 ura3Δ0*
YP003	*MAT***a** *ura3Δ0 leu2Δ0 his3Δ200 lyp1::SRG1pr-URA3*
YP006	*MATα ura3Δ0 leu2Δ0 his3Δ200 lyp1::SER3pr-URA3 can1::STE2pr-LEU2*
YP008	*MAT***a** *ura3Δ0 his3Δ200 lys2Δ0 leu2Δ0 ade8 snf2::leu2 SER3pr-HIS3::CloNAT*
YP012	*MATα ura3Δ0 leu2Δ0 his3Δ200 lyp1::SER3pr-HIS3 can1::STE2pr-LEU2*
YP013	*MATα ura3Δ0 leu2Δ0 his3Δ200 lyp1::SER3pr-HIS3 can1::STE2pr-LEU2*
YP042	*MATα rtt109Δ::KanMX*
YP043	*MAT***a** *rtt109Δ::KanMX ura3Δ0*
YP044	*MAT***a** *rtt109Δ::KanMX leu2Δ0*
YP045	*MATα rtt109Δ::KanMX leu2Δ0*
YP046	*MAT***a** *rtt109Δ::KanMX ura3Δ0 leu2Δ0*
YP047	*MATα rtt109Δ::KanMX ura3Δ0 leu2Δ0*
YP061	*MAT***a** *spt10Δ::KanMX*
YP062	*MATα spt10Δ::KanMX*
YP065	*MAT***a** *spt10Δ::KanMX leu2Δ0*
YP066	*MATα spt10Δ::KanMX leu2Δ0*
YP067	*MAT***a** *spt10Δ::KanMX ura3Δ0 leu2Δ0*
YP092	*MATα ura3Δ0 leu2Δ0*
YP105	*MAT***a** *shp1Δ0::KanMX ura3Δ0*
YP106	*MATα shp1Δ0::KanMX leu2Δ0*
YP107	*MATα shp1Δ0::KanMX ura3Δ0*
YP108	*MAT***a** *shp1Δ0::KanMX ura3Δ0 leu2Δ0*
YP109	*MAT***a** *shp1Δ0::KanMX leu2Δ0*
YP110	*MATα shp1Δ0::KanMX ura3Δ0 leu2Δ0*
YP111	*MAT***a** *rtt106Δ0::KanMX his3Δ200 leu2Δ1*
YP112	*MAT***a** *rtt106Δ0::KanMX*
YP113	*MAT***a** *rtt106Δ0::KanMX leu2Δ1*
YP114	*MATα rtt106Δ0::KanMX his3Δ200 leu2Δ1*
YP115	*MATα rtt106Δ0::KanMX*
YP116	*MATα rtt106Δ0::KanMX leu2Δ1*
YP117	*MATα rtt106Δ0::KanMX his3Δ200*
YP118	*rad27Δ0::KanMX*
YP119	*MAT***a** *rad27Δ0::KanMX ura3Δ0 leu2Δ0*
YP120	*MATα rad27Δ0::KanMX leu2Δ0*
YP121	*MATα rad27Δ0::KanMX ura3Δ0*
YP122	*MATα rad27Δ0::KanMX*
YP123	*MATα rad27Δ0::KanMX ura3Δ0 leu2Δ0*
YP124	*MAT***a** *rad27Δ0::KanMX leu2Δ0*
YP125	*MAT***a** *rad27Δ0::KanMX ura3Δ0*
YP145	*MAT***a** *yel045cΔ0::KanMX*

Table 1 (Cont.)

YP146	*MAT***a** *yel045cΔ0::KanMX*
YP147	*MATα yel045cΔ0::KanMX*
YP148	*MATα yel045cΔ0::KanMX*
YP149	*atp15Δ0::KanMX* *
YP150	*atp15Δ0::KanMX* *
YP151	*atp15Δ0::KanMX* *
YP152	*atp15Δ0::KanMX* *
YP153	*atp15Δ0::KanMX* *
YP154	*atp15Δ0::KanMX* *
YP155	*atp15Δ0::KanMX* *
YP156	*atp15Δ0::KanMX* *
YP157	*hnm1Δ0::KanMX*
YP158	*MATα hnm1Δ0::KanMX ura3Δ0 leu2Δ0*
YP159	*MAT***a** *hnm1Δ0::KanMX leu2Δ0*
YP160	*MAT***a** *hnm1Δ0::KanMX ura3Δ0*
YP161	*MATα hnm1Δ0::KanMX leu2Δ0*
YP162	*MATα hnm1Δ0::KanMX ura3Δ0*
YP163	*hnm1Δ0::KanMX*
YP164	*MAT***a** *hnm1Δ0::KanMX ura3Δ0 leu2Δ0*

* Unable to determine genotype of *atp15Δ::KanMX* strains due to severe growth defect (petite)

2.2.2 Modified Synthetic Genetic Array (SGA) screen

The yeast deletion collection was screened for mutant alleles that derepress *SER3* using a modified version of the SGA technology developed by Charlie Boone at the University of Toronto (Tong et al. 2001; Tong and Boone 2006). Briefly, the YP012 query strain containing a *SER3* reporter construct was mated to each deletion set strain in parallel. Diploids were selected and allowed to sporulate. A series of selection steps enriched for haploid strains containing both the reporter and the *KanMX*-marked deletion. These resulting strains were scored by visual inspection for increased growth on 3AT plates for three consecutive days. The details of the screen are provided in Figure 17 and discussed in Chapter 2.3.2.

2.2.3 Northern Analysis

Total RNA was isolated from cells grown to 1-2x10^7 cells/mL and separated on a 1% formaldehyde-agarose gel as described previously (Ausubel et al. 1991). RNA was transferred to Gene Screen membrane (Perkin-Elmer) and hybridized with radiolabeled probes generated by random-prime labeling of PCR fragments that were amplified from the following genomic sequences: *SRG1* (ChrV: 322258-322559), *SER3* (ChrV: 324059-324307), *HIS3* (ChrXV: 721918-722322) and *SCR1* (ChrV: 441741-442266), which was used as a control for RNA loading.

2.3 RESULTS

2.3.1 Development of a cell growth assay to measure *SER3* expression

In order to screen for yeast gene deletions that derepress *SER3*, I constructed a *SER3* reporter gene that would allow me to measure *SER3* promoter activity using a simple plate assay. To accomplish this, I first integrated a second copy of the *SRG1-SER3* locus disrupting the *LYP1* gene. This achieved two goals; first it left the wild type *SER3* locus intact, which is necessary for proper regulation of the reporter (see 2.3.4.2). Second, it allowed me to select for the presence of the reporter using the drug thialysine, a toxic lysine analog, which cells lacking the lysine permease Lyp1 are resistant (Sychrova and Chevallier 1993). I then replaced the entire *SER3* ORF with the yeast *HIS3* ORF at *lyp1*, putting it under the control of the *SER3* promoter and *SRG1* intergenic transcription. Using *HIS3* as a reporter allows for the selection of growth on SC-His plates. Therefore, *SER3* derepression can be detected by growth of cells in the presence of 3-aminotriazole (3AT), a competitive inhibitor of the His3 enzyme (Klopotowski and Wiater 1965; Struhl and Davis 1977). Addition of increasing amounts of 3AT require increasing amounts of *HIS3* expression in order for the cell to overcome its toxic effects and grow.

Northern analysis confirmed that in the reporter strain, *HIS3* was now repressed in the presence of serine and derepressed in its absence (Figure 16B). I titrated the amount of 3AT that just prevented growth of wild type to optimize the sensitivity of our growth assay for *SER3* repression (Figure 16A). Two reporter strains were patched out and replica-plated to varying concentrations of 3AT along side control strains containing wild type *HIS3* or a *his3*Δ*200* mutant. The *his3*Δ*200* mutant was unable to grow in any of the conditions lacking histidine,

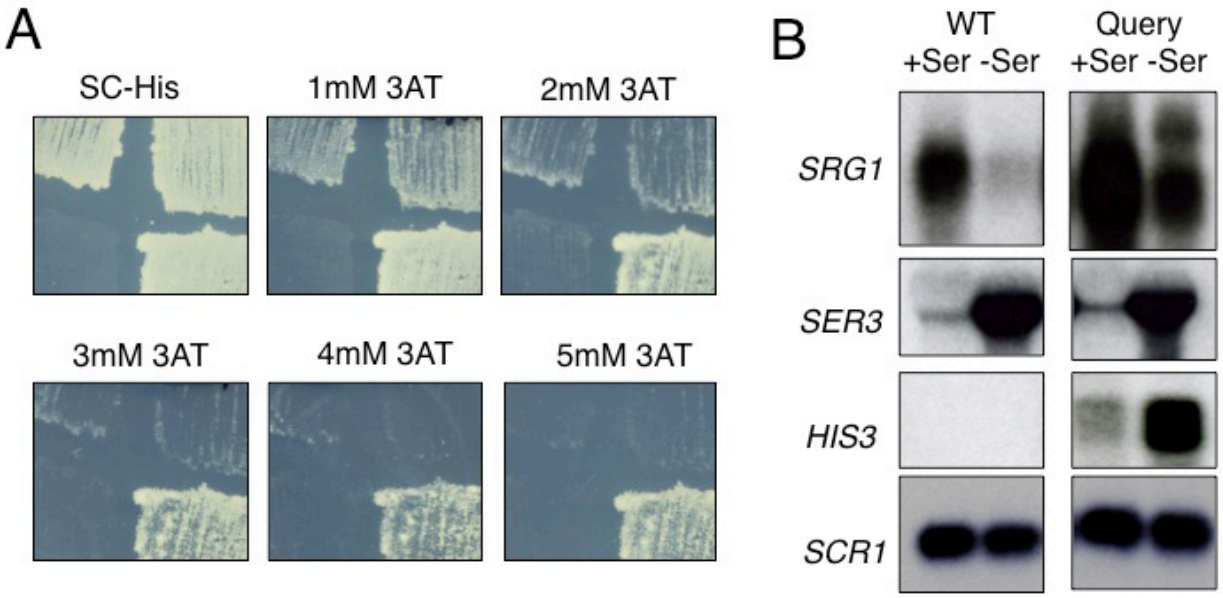

Figure 16: Testing the *SER3pr-HIS3* reporter construct.

A) Strains patched out and replica-plated to plates containing various concentrations of 3AT. Top left and right = two independent query strains (YP012, YP013), bottom left = a *his3Δ200* strain (YP006), and bottom right = a *HIS3* strain (FY3). The repressed reporter can grow in the absence of histidine, but not in the presence of 3AT. 5mM 3AT was chosen as the concentration for the screen. B) Northern analysis of *SRG1, SER3, HIS3,* and *SCR1* levels in the query strain (YP012) and a wild type control (YJ586). The reporter is regulated similar to *SER3* in a control strain and the native *SER3* locus present in the query strain. In the absence of serine, the query strain has higher levels of *SRG1*, but considering there are two copies of *SRG1* in these cells, the signal is not significantly different from the control strain.

while the wild type *HIS3* strain grew in all conditions. The reporter strains grew on SC-his plates suggesting the low level of *HIS3* expression during repression by *SRG1* is sufficient for growth on these conditions, emphasizing the need for 3AT. From this assay, we determine that 5mM 3AT would be used for the screen, as the reporter strain was unable to grow, but the wild type *HIS3* strain still grew well.

2.3.2 Screening the yeast deletion collection using a modified SGA screen

Using a modified SGA method (Tong et al. 2001; Tong and Boone 2006) the yeast deletion set, a collection of 4786 strains each containing a single nonessential open reading frame replaced with the *KanMX* drug resistance cassette, was screened for mutants that derepress *SER3* (Figure 17). First, YP012 was systematically mated to all of the deletion strains on solid YPD media. Mating strains were replica-pinned to media selecting for diploids containing markers from both the query and deletion strains. The diploids were then replica-pinned to enriched sporulation media and incubated for 5 days at 22°C. Sporulated cells were then replica-pinned to a series of selective plates to enrich for only haploid strains of a single mating type. This was accomplished through the use of another reporter present in the query strain, known as the "magic marker." This marker, *STE2pr-LEU2*, consists of the *LEU2* gene under the control of the *MAT***a**-specific promoter of the yeast *STE2* gene. The reporter is integrated at the *CAN1* locus, disrupting the arginine permease rendering the cells resistant to the drug canavanine (Ahmad and Bussey 1986). Strains with this reporter are only able to grow on media lacking leucine if they are *MAT***a** haploids, allowing for genetic selection of haploids during the screen without the usual labor-intensive methods of physically separating the tetrad into four haploid spores. After selecting for haploids containing both the *SER3pr-HIS3* reporter allele and the *KanMX*-marked

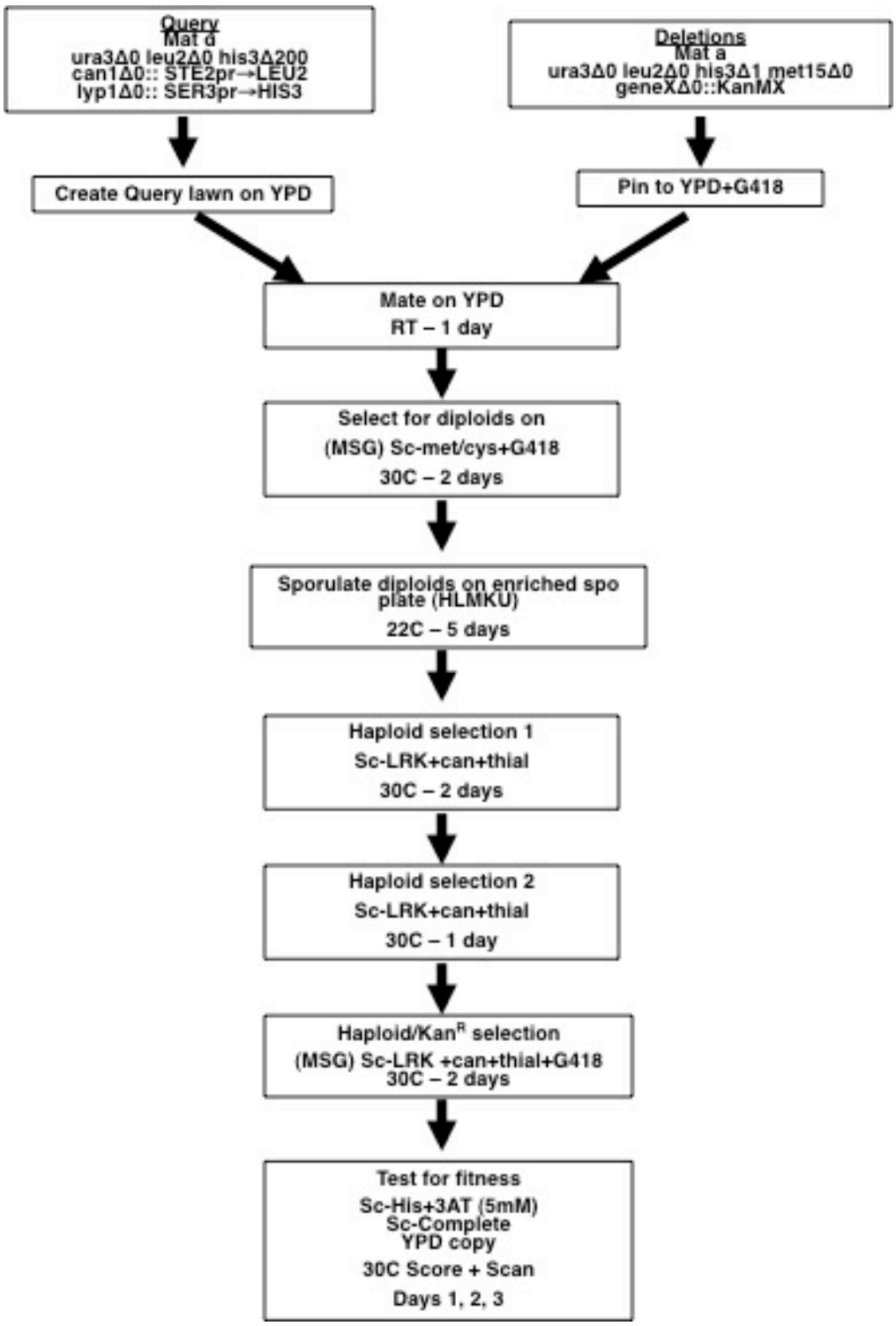

Figure 17: Experimental design of deletion set screen.

Flow chart for the genetic screen indicating the genotypes of the query and deletion set strains and the media and growth conditions used in each step.

deletion allele, these strains were screened for growth on plates lacking histidine and containing 5mM 3AT. *SRG1*-mediated repression of *HIS3* prevented the query strain from growing on these plates, enabling us to screen for mutants that disrupt *SRG1*-mediated repression of *HIS3* and allow these strains to grow in the presence of the drug (Figure 17).

2.3.3 Verification of screen results

After screening the deletion set, my initial results indicated ~210 positive candidates based on their resistance to 3AT. These candidates were taken through a number of verification steps to ensure their effect and eliminate false positives. First, I retested each candidate for 3AT resistance by taking the final strains from the screen, patching them out and replica-plating them to media containing various concentrations of 3AT. I also went back to the original deletion strains, patched them out, and took them through the entire screen by replica-plating. After retesting and rescreening, 73 positive candidates showed increased growth on 3AT. Genetic analyses were also performed on candidates to confirm linkage of the 3AT phenotype to the *KanMX*-marked deletion. Candidates where 3AT resistance was linked to the *KanMX*-marked deletion were then subjected to Northern analyses to measure endogenous *SER3* expression from the *SER3* locus present in the deletion set strains (Table 2). Some candidates that have already been confirmed from previous studies or were in the process of being tested in our strain background were not included in this set of Northern analyses.

Table 2: Endogenous *SER3* levels in deletion set strains.

Gene	Function	*SRG1*	*SER3*
SPT3	SAGA subunit	0.4	109.9
SPT21	Transcriptional regulator of histone genes	0.3	24.4
HTA1	Histone H2A	1.6	16.1
SPT4	Transcription elongation factor	0.9	13.1
SPT7	SAGA Subunit	0.3	12.7
YGL023c	Dubious ORF	0.6	8.3
PEX32	Peroxisomal protein	0.8	8.3
YNL296w	Dubious ORF	1	8.2
RAD27	DNA repair	1.1	7.4
YJL211c	Dubious ORF	1.3	6.3
YJL185c	Uncharacterized ORF	1	5.5
RTT106	TY transposition, heterochromatin, histone gene expression	0.7	4.9
SHP1	Protein ubiquitylation and degradation	0.2	4.5
SPT8	SAGA subunit	0.6	3.2
HPC2	HIR complex, transcriptional corepressor of histone genes	0.7	2.9
ATP15	Mitochondrial ATP synthesis	1.4	2.7
HIR2	HIR complex, transcriptional corepressor of histone genes	0.6	2.4
YEL045c	Dubious ORF	0.3	2.4
HNM1	Choline/ethanolamine transporter	0.8	2.4
SKM1	Serine/threonine protein kinase	0.2	2.2
PIB2	Binds PI3, telomere repression	0.9	2
GIM5	Subunit of prefoldin complex	0.4	2
HIR3	HIR complex, transcriptional corepressor of histone genes	1.3	1.9
BRE2	COMPASS subunit	0.6	1.5
SER1	Serine biosynthesis	0.9	1.4
ETR1	Mitochondrial protein, fatty acid synthesis	1.3	1.4
SNF5	SWI/SNF subunit	1.1	1.4
HTA2	Histone H2A	1.1	1.3
SWD3	COMPASS subunit	0.6	1.2
YHR202w	Dubious ORF	1.5	1.2
RPS25A	Ribosome subunit	0.8	1.2
FUM1	Fumarase, TCA cycle	0.5	1.1
DCN1	Regulates ubiquitin ligase, neddylation	0.9	1.1
SSD1	Cellular integrity, interacts with TOR pathway	0.6	1.1
HUA1	Cytoplasmic zinc finger protein, similar to J-proteins	0.9	1.1
ADD37	ERAD	1.4	1
SER2	Serine biosynthesis	1.4	1
SRO9	Ribosome biogenesis and translation	0.5	1

	Table 2 (cont.)		
MDR1	Protein transport	0.5	1
YUH1	Ubiquitin hydrolase	1	1
RTS1	Regulator of PP2A	0.8	0.9
CKI1	Choline kinase	0.5	0.9
PDR16	Phospholipid transport	0.5	0.9
IZH1	Membrane protein, zinc metabolism	1.3	0.9
PPM1	Regulator of PP2A	0.3	0.8
CAX4	Pyrophosphatase lipid biosynthesis	0.6	0.8
YPR089w	Uncharacterized ORF	0.6	0.8
POR2	Putative mitochondrial porin	0.5	0.8
CHD1	Chromatin remodeling factor, SAGA/SILK complexes	0.6	0.8
PDB1	Mitochondrial pyruvate dehydrogenase	0.2	0.6
HIR1	HIR complex, transcriptional corepressor of histone genes	0.6	0.6
HOF1	SH3 containing, cytokinesis	0.4	0.5
YBR174c	Dubious ORF	1	0.5
ACE2	Transcription factor of G1 genes	2	0.4
PEX2	Peroxisomal protein	1.4	0.4
DON1	Meiosis, spindle pole body formation	1	0.4
PFD1	Prefoldin, chaperonin	0.5	0.3
PCT1	Phosphatidylcholine synthesis	0.8	0.3
YKL118w	Dubious ORF	1.4	0.3

Listed are the results of Northern analyses performed by Frank Kowalkowski on candidate deletion set strains. Not included in this list are some factors previously known to affect *SER3*, such as *CHA4*, *SNF2*, *SWI3*, *SNF5*, *SPT2* and factors I was testing independently, such as *BUR2*, *SPT10* and *RTT109*. The left column is the gene name and the middle column is a brief description of the function of the gene (www.yeastgenome.org). On the right is the quantitation of *SRG1* and *SER3* transcript levels from a single experiment with values made relative to wild type, which has been set to 1. Genes whose deletion caused a two-fold increase in *SER3* levels were deleted in our strain background and retested.

2.3.4 Identification of 21 genes that upregulate endogenous *SER3* levels when deleted

For the factors having at least a two-fold increase of endogenous *SER3* levels by Northern analysis, the deletion strain was tested by PCR to confirm that the correct gene was deleted. We then amplified the deletion allele from the deletion set strain and integrated it into our strain background to reduce potential issues with the genetic background and to make a series of strains to be used in future studies. Northern analyses were then repeated on deletion strains in our strain background, leading to further reduction of false positives. In total, 21 factors met all of these criteria and were shown to derepress endogenous *SER3* at least 2-fold when deleted (Table 3).

2.3.4.1 Factors required for *SRG1* transcription

A number of factors identified by our screen have been previously shown to be required for the initiation of *SRG1* (Table 3, gold). These deletions reduce intergenic transcription, relieving repression of *SER3* and, therefore, serve to validate the screen. Cha4 is the activator of *SRG1* required for recruitment of the Swi/Snf chromatin remodeling and SAGA histone acetyltransferase co-activator complexes (Martens et al. 2005). *SER3* repression is mostly dependent on the Snf2 subunit of Swi/Snf, while Swi3 and Snf5 have a weaker effect (Martens and Winston 2002), consistent with my screen results. Loss of the Spt3, Spt7, and Spt8 subunits of SAGA was also previously shown to result in strong derepression of *SER3* (Martens et al. 2005). The Spt20 and Ada1 subunits were not identified in the screen, but are also required for *SER3* repression and might represent false negatives.

Table 3: 21 genes that derepress endogenous *SER3* when deleted in our strain background.

Gene	Function
CHA4	Activator of *SRG1*
SPT8	SAGA subunit, coactivator of *SRG1*
SPT3	SAGA subunit, coactivator of *SRG1*
SPT7	SAGA Subunit, coactivator of *SRG1*
SNF2	Swi/Snf chromatin remodeling complex, coactivator of *SRG1*
SWI3	Swi/Snf chromatin remodeling complex, coactivator of *SRG1*
SNF5	Swi/Snf chromatin remodeling complex, coactivator of *SRG1*
SER3	Serine biosynthesis
SPT2	HMG-like transcription elongation factor
SPT4	Transcription elongation factor
BUR2	Cyclin for BUR1 CDK, transcription elongation factor
RTT106	Histone chaperone linked to transcription elongation
RTT109	Histone H3 K56 and K9 acetyltransferase
SPT10	Transcriptional regulator of histone genes
SPT21	Transcriptional regulator of histone genes
HTA1	Histone H2A
SHP1	UBX domain-containing protein; involved in degradation of ubiquitylated proteins
RAD27	DNA replication and repair
HNM1	Choline/ethanolamine transporter
ATP15	Subunit of mitochondrial ATP synthase
YEL045c	Dubious ORF

Listed are the 21 factors that derepress endogenous *SER3* when deleted in our strain background and a brief statement of their known function (www.yeastgenome.org). The factors are color-coded by category: gold indicates regulators of *SRG1* transcription, blue indicates a serine biosynthesis gene, purple indicates transcription elongation factors, red indicates factors required for proper histone gene expression, and grey indicates factors with various or unknown functions. Note: endogenous *SER3* levels could not be tested in a *ser3*Δ strain.

2.3.4.2 Deletion of *SER3* derepresses a *SER3* reporter

Interestingly, deletion of the *SER3* gene also derepressed the *SER3* reporter allele (Table 3, blue). The *SER3* reporter was integrated at another genomic location to leave the endogenous *SER3* gene intact. When the query strain was crossed to the deletion set strain lacking *SER3*, this resulted in a strain without a copy of *SER3* and led to derepression of the *SER3* reporter. This result suggests a possible feedback mechanism within the serine biosynthesis pathway affecting *SER3* regulation. Since deletion of *SER1* or *SER2* had little to no effect on *SER3* levels (Table 2) this suggests the feedback may be specific to the *SER3* step of the pathway.

2.3.4.3 Transcription elongation factors

The screen identified a number of factors involved in transcription elongation and transcription through chromatin (Table 3, purple). Spt2 is an HMG-like factor that may play both positive and negative roles in transcription, possibly through the alteration of chromatin structure (Kruger and Herskowitz 1991; Nourani et al. 2006). Spt2 has a previously published role in *SER3* repression (Nourani et al. 2006; Thebault et al. 2011). Spt4 partners with an essential factor Spt5 to form the yeast homolog of the mammalian DSIF transcription elongation complex (Wada et al. 1998). A temperature sensitive allele of *spt5* was shown to have an effect on *SER3* repression (Davis and Ares 2006), but this is the first evidence supporting a role for Spt4 as well. Bur2 acts as the cyclin partner of the essential cyclin-dependent kinase Bur1. Studies of a pathway involving Bur1/Bur2, Spt4/5, and the Paf1 complex led us to our investigation of the function of the Paf1 complex in *SER3* repression, which is discussed in detail in Chapter 4.

2.3.4.4 Factors required for histone gene expression

One of the most striking results of the screen was the number of factors involved in regulation of histone gene expression (Table 3, red). My screen identified one of the genes encoding histone H2A, *HTA1*, indicating reducing levels of this histone affects *SER3* expression. This is consistent with previous microarray experiments that identified *SER3* as a gene upregulated when histone H4 was depleted from cells (Wyrick et al. 1999). Interestingly, deletions of other histone genes were not identified in the screen. This may be because the second copy of a histone gene can often compensate for loss of one copy (Norris and Osley 1987; Moran et al. 1990; Libuda and Winston 2006; Libuda and Winston 2010). It is possible the other deletions have an effect but they may simply be below the limit of detection. Our lab has shown that deletion of one copy each of histone H3 and H4, (*hht1-hhf1)*Δ, leads to a slight derepression of *SER3* (Hainer and Martens 2011a). I also observed similar results in strains lacking one copy each of histone H2A and H2B *(hta2-htb2)*Δ (Chapter 4.3.4). These mutations cause 2-3 fold derepression of *SER3*, an effect that might have been too weak to detect in the screen.

Spt10 and Spt21 were originally isolated as suppressors of a Ty element insertion (Fassler and Winston 1988) and were shown to affect the expression of a number of yeast transcripts (Natsoulis et al. 1991; Yamashita 1993; Natsoulis et al. 1994). Spt10 is a putative histone acetyltransferase and, while its HAT activity has not been confirmed, the HAT domain is required for many of its functions (Neuwald and Landsman 1997; Hess et al. 2004; Eriksson et al. 2005). Spt21 is a cell cycle regulated DNA binding protein responsible for recruiting Spt10 to the genomic locations for some, but not all, of its functions (Hess et al. 2004). It has been shown that Spt10 seems to act mostly at the histone loci and is actually a sequence specific activator of certain histone genes (Dollard et al. 1994; Eriksson et al. 2005). In an *spt10* deletion

there is a global activation of genes, but this seems to be due to its role in activating histone genes. Without Spt10, there is a shortage of histones in the cell, which leads to defective chromatin structure, and then a subsequent increase in the basal transcription level of many genes (Eriksson et al. 2005). I performed Northern analysis *spt10* and *spt21* mutant strains and saw a large derepression of *SER3*, upwards of 25-fold, but no effect on *SRG1* levels (Figure 18). The effect on *SER3* is likely due to the altered chromatin in these strains, further indicating the sensitivity of this system to histone levels and chromatin.

Rtt109 and Rtt106, first isolated for their roles in Ty1 transposition (Scholes et al. 2001), have also been implicated in regulating expression of certain histone genes (Fillingham et al. 2009; Ferreira et al. 2011). Rtt109 is well studied for its role as a histone acetyltransferase targeting histone H3 lysines 4, 9, and 56 (Schneider et al. 2006; Driscoll et al. 2007; Han et al. 2007; Guillemette et al. 2011). The role of Rtt109 in *SER3* expression will be discussed in more detail in Chapter 5. Rtt106 is a histone chaperone that has been functionally linked to heterochromatin silencing, replication-coupled nucleosome deposition, preventing cryptic initiation within genes, and transcription elongation (Huang et al. 2005; Huang et al. 2007; Imbeault et al. 2008). Many of these diverse functions of Rtt106 may be mediated through its role in regulating histone gene expression. Rtt106 maintains a repressive chromatin structure over the regulatory region of certain histone loci, keeping them in an off state until the S-phase of the cell cycle when their expression is needed (Fillingham et al. 2009). This is mediated through recruitment of the repressive chromatin remodeling complex RSC during the G2/M/G1 phases of the cell cycle, which is then switched out for the Swi/Snf co-activator complex during late G1 and S phase (Ferreira et al. 2011).

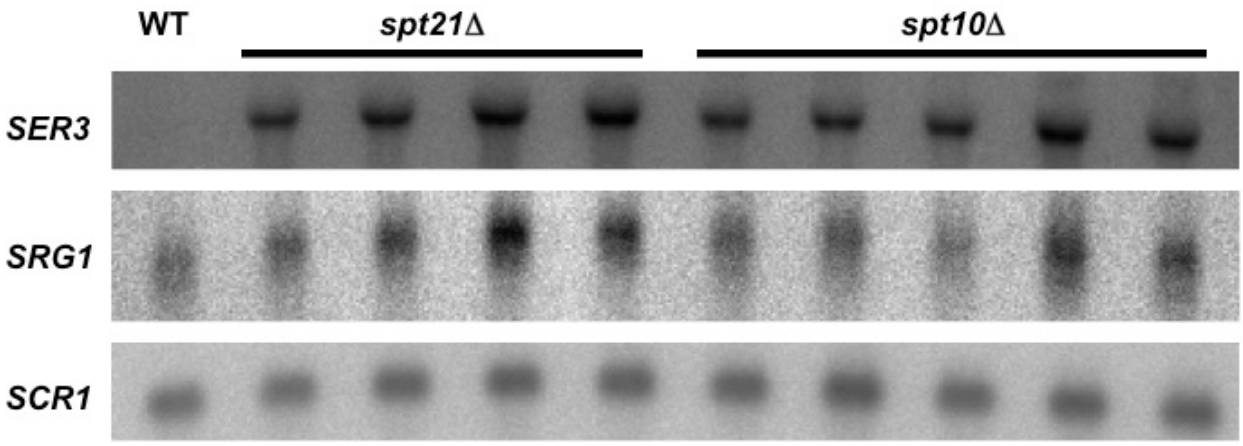

Figure 18: Deletion of *SPT21* and *SPT10* cause strong derepression of *SER3*.

Northern analysis of *SER3*, *SRG1*, and *SCR1* levels in wild type (YP092), *spt21*Δ (FY2199, KY923, KY924, KY925), and *spt10*Δ (YP061, YP062, YP065, YP066, YP067) strains.

It is important to note that not every factor known to alter histone gene expression derepresses *SER3*. The HIR complex, consisting of Hir1, Hir2, Hir3, and Hpc2, is a well-studied repressor of histone genes (Sherwood et al. 1993; Spector et al. 1997), but mutation of the three *HIR* genes had only a modest effect on *SER3* levels by Northern analysis (Figure 19). Similar effects were also seen for deletions of *ASF1*, which plays a similar role to Rtt106 and the HIR complex in histone gene repression (Chapter 5). Taken together, these data suggest that *SER3* is sensitive to histone gene dosage and histone gene expression levels. These observations led us to determine the role of chromatin in *SER3* regulation, as discussed in Chapter 3.

2.3.4.5 Other candidates

Additionally, I identified five factors with no obvious connection to *SER3* expression (Table 3, grey). These factors: Shp1, Rad27, Hnm1, Atp15, and Yel045c play a variety of roles in the cell, without a clear connection to transcription or chromatin function. They may still be playing important and interesting roles in *SER3* regulation, but because of their unclear function and their relatively weak effect on *SER3*, I have not performed follow up experiments on them. Interestingly, many of these factors have severe cellular defects when mutated, such as cell cycle defects (Shp1), genomic instability (Rad27), or loss of mitochondrial function (Atp15) suggesting their effect may be indirect. Here, I briefly summarize their known functions.

Shp1 (Suppressor of High copy PP1) was originally identified as a regulator of phosphoprotein phosphatase 1 (PP1) in yeast (Zhang et al. 1995). It contains a UBA ubiquitin-binding domain that interacts with ubiquitylated proteins, and is involved in degradation of a model substrate. These functions are through its role as a cofactor for an essential AAA ATPase Cdc48, which it binds using its ubiquitin regulatory X (UBX) domain (Schuberth et al. 2004). Cdc48/Shp1 is involved proteasome-dependent protein degradation, stable attachment of

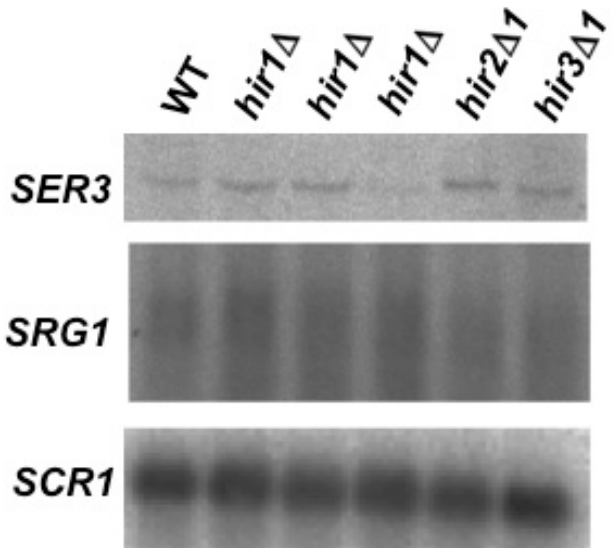

Figure 19: Effect of mutations in the HIR complex on *SER3* repression.

Northern analysis of *SER3*, *SRG1*, and *SCR1* levels in wild type (YJ714), *hir1*Δ (YJ772, YJ773, YJ774), *hir2*Δ*1* (YJ775), and *hir3*Δ*1* (YJ776) strains.

chromosomes to kinetochores during mitosis, and autophagosome biogenesis (Schuberth et al. 2004; Cheng and Chen 2010; Krick et al. 2010).

Rad27 (Fen1) is a multifunctional nuclease involved in many important nuclear processes (Zheng and Shen 2011). It has 5' flap endonuclease activity that is required to remove a 5' overhang of Okazaki fragments produced during lagging strand DNA synthesis (Rossi and Bambara 2006). This activity is also required for removal of another short polynucleotide substrate produced during base excision repair of apurinic/apyrimidinic DNA damage (Wu and Wang 1999). A second 5' to 3' endonuclease activity of Rad27 helps resolve secondary structures formed by repeated sequences which helps prevent the expansion of di- and trinucleotide repeats (Xie et al. 2001). Together these functions point to an important role for Rad27 in maintaining genomic stability.

Hnm1 is a choline and ethanolamine permease required to import these precursor molecules into the cell for production of phosphatidylcholine and phosphatidylethanolamine (Nikawa et al. 1986; Nikawa et al. 1990). Its expression is co-regulated with other genes of the phospholipid biosynthesis pathway and is repressed by the pathway precursors myo-inositol and choline (Li and Brendel 1993). Hnm1 has also been shown to be the target of nitrogen mustard with specific Hnm1 mutations rendering yeast cells hyper-resistant to the toxic compound (Haase and Brendel 1990; Li et al. 1991).

Atp15 is the epsilon subunit of mitochondrial ATP synthase, a large multi-subunit molecular machine essential for ATP synthesis (Guelin et al. 1993). The epsilon subunit forms part of the "central stalk" that connects the membrane bound F_0 proton pump to the soluble F_1 catalytic core (Couoh-Cardel et al. 2010). Mutation of the *ATP15* gene does not result in

inviable cells, but does disable the ATP synthase enzyme. This renders *atp15*Δ cells unable to grow on non-fermentable carbon sources, also known as a petite phenotype (Guelin et al. 1993).

Yel045c was annotated as a potential open reading frame by computational methods and, therefore, was included in the deletion collection but is unlikely to encode a protein based on experimental data. Still, deletion of this genomic region results in certain phenotypes probed in large-scale studies, such as increased drug sensitivity and reduced fitness under particular growth conditions (www.yeastgenome.org).

2.4 CONCLUSIONS

I completed a genetic screen to test deletion of each individual nonessential yeast gene for an effect on *SER3* repression. This yielded a set of 21 candidate genes that have at least a two-fold increase in *SER3* levels when deleted. Among these genes were those that encoded factors previously known to affect *SER3* through their regulation of *SRG1* transcription. Other interesting results included factors required for proper histone gene expression that helped initiate our studies of the role of chromatin structure in *SER3* repression (Chapter 3). I also identified a set of transcription elongation factors that led us to uncover a role for the Paf1 complex (Chapter 4).

While the screen was effective in identifying important regulators of *SER3* repression, there are important limitations of the screen that could be addressed with complementary studies. An obvious drawback is the non-saturating nature of the screen that could only identify deletions of nonessential genes present in the deletion collection. To identify essential genes, I could screen other yeast collections containing temperature-sensitive conditional alleles (Li et al.

2011), tetracycline-repressible alleles that allow dynamic shutoff (Mnaimneh et al. 2004), or hypomorphic alleles that have reduced expression (Breslow et al. 2008). Traditional mutagenesis screens using UV or DNA damaging chemicals such as methyl methanesulfonate (MMS) can also identify alleles of essential genes. These screens have the additional benefit of being able to identify point mutations, conditional alleles, and other subtle mutations that can be more informative than full deletions and useful for future studies.

Another matter of concern is the rate of false positives obtained in the screen. The original screen resulted in 210 candidates, which were very liberally selected to ensure maximum inclusion. Through numerous verification steps, the number of candidates was reduced to 21. These false positives could be due to the sensitivity of the *HIS3* reporter or due to factors that affect *HIS3* expression itself, but not *SER3*. Some deletions may also affect the uptake or metabolism of 3AT, rendering cells resistant to the drug independent of *HIS3* expression.

Perhaps of even more concern than false positive results are false negative results. We know from previous studies that deletion of the *SPT20* and *ADA1* subunits of SAGA derepress *SER3*, although not to the same extent as those that came out of the screen, *SPT3*, *SPT7*, and *SPT8* (Martens et al. 2005). Similarly, in subsequent studies we identified a role for Paf1 and Ctr9 (Chapter 4) but they were also not identified in the screen. These factors may have been missed due to technical errors during the screen, or due to problems with the deletion collection, suggesting other factors with a role in *SER3* regulation may not have been identified in the screen.

One screen result that we have not yet pursued, but we are interested in following up on, is a possible feedback mechanism within the serine biosynthesis pathway that may affect *SER3* regulation. I identified *SER3* in the screen, consistent with previous results that suggest cells

require a copy of *SER3* in order to fully repress a *SER3* reporter. It would be interesting to determine if other enzymes in this pathway: *SER1, SER2,* and *SER33,* are also required for this effect, but preliminary evidence suggests they are not. *SER1* and *SER2* were both candidates pulled out of the screen, but upon retesting, the effects on endogenous *SER3* levels were mild (Table 2). *SER33* was not identified in the screen and previous work has shown no effect of *ser33*Δ on *SER3* regulation (Martens et al. 2004). If indeed this effect is specific to *SER3,* it suggests a role for either the Ser3 enzyme itself, or the step in the pathway it catalyzes, either through the accumulation of the substrate 3-phosphoglycerate or depletion of the product 3-phosphohydroxypyruvate. My hypothesis would be that the feedback mechanism may be detecting something other than serine, since I observe derepression of the *SER3* reporter in rich media containing plenty of serine.

3.0 *SRG1* INTERGENIC TRANSCRIPTION MAINTAINS A REPRESSIVE CHROMATIN STRUCTURE OVER THE *SER3* PROMOTER

The work discussed in this chapter has been adapted from published material (Hainer et al. 2011) and is reprinted, with alterations, by permission from Cold Spring Harbor Laboratory Press, copyright 2011. This project was a collaborative effort involving multiple members of the Martens Lab. I performed the ChIP experiments in Figures 22, 23, and 24, Joe Martens performed the nucleosome scanning experiments in Figures 20, 21, and 23, Sarah Hainer performed Northern analysis in Figures 22 and 25, and Robin Monteverde and Rachel Mitchell constructed strains and contributed to control experiments in Figures 24 and 26.

3.1 INTRODUCTION

The genetic screen described in Chapter 2 identified histone H2A and several regulators of histone gene expression: Spt10, Spt21, Rtt106, and Rtt109. This pointed strongly towards a role for histones and chromatin in *SER3* repression. Other evidence agreed with this hypothesis. DNA microarray experiments revealed that depletion of histone H4 resulted in strong *SER3* derepression (Wyrick et al. 1999). Also, a mutation in *SPT6*, a gene that encodes a protein required to maintain proper chromatin structure over genes during transcription (Kaplan et al.

2003; Cheung et al. 2008), also resulted in *SER3* derepression (Kaplan et al. 2003). These results led us to test for a direct role of nucleosome occupancy and positioning in *SER3* regulation.

In this chapter, we elucidate the mechanism whereby serine-dependent transcription of ncDNA (*SRG1*) in *S. cerevisiae* represses expression of the adjacent *SER3* gene in a histone-dependent manner. We show that *SER3* repression correlates with a broad region of strong micrococcal nuclease (MNase) protection spanning the entire *SRG1* transcription unit, suggesting that nucleosomes are loosely positioned across this region. Surprisingly, conditions that reduce *SRG1* transcription result in dramatically reduced MNase protection at the *SER3* promoter, indicating a loss of nucleosome occupancy. By analyzing mutations in *SPT6* and *SPT16*, two genes that encode subunits of the Spt6/Spn1(Iws1) and FACT elongation complexes, we provide evidence that it is the nucleosomes assembled at the *SER3* promoter by intergenic *SRG1* transcription, not RNA Pol II itself, that interfere with the binding of transcription factors to the *SER3* promoter. While these data highlight specifically the mechanism of *SER3* repression, it is consistent with a general model in which transcription of ncDNA can assemble nucleosomes that occlude DNA from binding by sequence-specific DNA-binding proteins.

3.2 MATERIALS AND METHODS

3.2.1 Yeast strains and media

All *S. cerevisiae* strains used in this study (Table 4) are isogenic with a *GAL2*$^{+}$ derivative of S288C (Winston et al. 1995). Strains were constructed using standard genetic crosses or by transformation (Ausubel et al. 1991). The C-terminus of *RPB1* and *SPT16* were tagged with 13

copies of the c-Myc epitope by PCR-mediated transformation of diploid strains using pFA6a-13myc-KanMX and pFA6a-13myc-HIS3MX, respectively (Longtine et al. 1998). The *spt16-22* and *spt16-23* alleles (Formosa et al. 2001) were integrated into a diploid strain by two-step gene replacement using *Sna*BI-digested pTF142-23 and pTF142-22 plasmids (kindly provided by T. Formosa, University of Utah, Salt Lake City, UT). The *ser3*Δ*UAS* mutation was constructed by replacing 37bp of *SER3* promoter sequence (from -228 to -198; *SER3* ATG=+1) with an *Avr*II restriction site by QuikChange mutagenesis (Agilent Technologies) to yield pRM08 plasmid. The *ser3*Δ*UAS* allele was then integrated into a diploid strain by two-step gene replacement using *Afe*I-digested pRM08. Several strains contain a *KanMX*-marked deletion of the *SER33* gene, which is a paralog of *SER3*. Based on previous studies (Martens and Winston 2002; Martens et al. 2004) and the results presented in this study, the deletion of *SER33* does not affect *SER3* regulation. Strains were grown in the following media as indicated in the figure legends: YPD (1% yeast extract, 2% peptone, 2% glucose), YPgal (1% yeast extract, 2% peptone, 2% galactose), YPraf (1% yeast extract, 2% peptone, 2% raffinose), and synthetic complete with 1mM serine (SC +serine) or without serine (SC-serine) (Rose 1991).

Table 4: *S. cerevisiae* strains used in Chapter 3.

Name	Genotype	Reference/Source
FY4	*MAT*a	(Winston et al. 1995)
FY5	*MATα*	F. Winston
FY111	*MAT*a *his4-914δ lys2-128δ trp1Δ63 ura3-52 spt6-140*	(Hartzog et al. 1998)
FY346	*MAT*a *leu2Δ1 lys2-128δ ura3-52 spt16-197*	(Malone et al. 1991)
FY1221	*MATα his4-914δ lys2-128δ trp1Δ63 ura3-52 spt6-14*	(Hartzog et al. 1998)
FY1350	*MATα leu2Δ0 lys2Δ0 ura3Δ0*	F. Winston
FY1411	*MAT*a *his4-917δ leu2Δ1 lys2-173R2 trp1Δ63 ura3-52 nhp6aΔ::URA3 nhp6bΔ::URA3*	F. Winston
FY2097	*MAT*a *his3Δ200 leu2Δ0 lys2Δ0 ura3Δ0 ser33Δ::KanMX*	(Martens et al. 2004)
FY2099	*MAT*a *his3Δ200 leu2Δ0 lys2Δ0 ura3Δ0 ser33Δ::KanMX ser3-100*	(Martens and Winston 2002)
FY2134	*MAT*a *his4-912δ leu2Δ1 lys2-128δ RPB3-HA1::LEU2 SPT6-FLAG CTR9-9MYC::KanMX*	(Kaplan et al. 2003)
FY2180	*MAT*a *his4-912δ leu2Δ1 lys2-128δ FLAG-spt6-1004*	(Kaplan et al. 2003)
FY2250	*MAT*a *his3Δ200 leu2Δ0 lys2Δ0 ura3Δ0 ser33Δ::KanMX srg1-1*	(Martens et al. 2004)
FY2260	*MAT*a *his3Δ200 leu2Δ0 lys2Δ0 ura3Δ0 ser33Δ::KanMX srg1-1 ser3::GAL7UAS*	(Martens et al. 2004)
FY2425	*MATα his3Δ200 leu2Δ1 lys2-128δ ura3-52 FLAG-spt6-1004*	F. Winston
FY2471	*MAT*a *his3Δ200 leu2Δ0 lys2Δ0 ura3Δ0 srg1-1*	(Martens et al. 2005)
GHY1199	*MATα his4-914δ leu2Δ1 lys2-128δ trp1Δ63 ura3-52 iws1-7-MYC::TRP1*	(Lindstrom et al. 2003)
GHY1200	*MATα his4-914δ leu2Δ1 lys2-128δ trp1Δ63 ura3-52 iws1-13-MYC::TRP1*	(Lindstrom et al. 2003)
KY719	*MAT*a *ura3Δ0*	
KY912	*MAT*a *his3Δ200 leu2Δ1 lys2-128δ ura3-52 set2Δ::HIS3*	K. Arndt
KY934	*MATα his3Δ200 leu2Δ1 trp1Δ63 dot1Δ::HIS3*	K. Arndt
KY938	*MATα his3Δ200 leu2Δ1 trp1Δ63 set1Δ::HIS3*	K. Arndt
KY1235	*MAT*a *his3Δ200 lys2-128δ ura3-52 rco1Δ::HIS3*	K. Arndt
KY1806	*MAT*a *set3Δ::KanMX*	K. Arndt
KY1822	*MAT*a *leu2Δ0 set1Δ::KanMX set2Δ::KanMX*	K. Arndt
YJ275	*MAT*a *his3Δ200 leu2Δ0 lys2Δ0 ura3Δ0 ser33Δ::KanMX ser3-100*	This study
YJ582	*MAT*a *srg1-1*	This study
YJ583	*MAT*a *srg1-1*	This study
YJ585	*MATα leu2Δ0 lys2Δ0 ura3Δ0 srg1-1*	This study
YJ586	*MATα his3Δ200 leu2Δ0 ura3Δ0*	This study
YJ832	*MAT*a *leu2Δ0 ura3Δ0 spt16-22*	This study
YJ833	*MAT*a *ura3Δ0 spt16-23*	This study
YJ841	*MAT*a *his3Δ200 leu2Δ0* or *leu2Δ1 lys2Δ0 ura3Δ0 or ura3-52 RPB1-C13MYC::KanMX spt16-197*	This study
YJ842	*MATα his3Δ200 leu2Δ0* or *leu2Δ1 lys2-128δ ura3Δ0 or ura3-52 RPB1-C13MYC::KanMX spt16-197*	This study
YJ843	*MAT*a *his3Δ200 leu2Δ0* or *leu2Δ1 lys2-128δ ura3Δ0 or ura3-52 RPB1-C13MYC::KanMX spt16-197*	This study
YJ844	*MAT*a *his3Δ200 leu2Δ0* or *leu2Δ1 lys2-128δ ura3Δ0 RPB3-HA::LEU2 spt16-197-C13MYC::HIS3MX*	This study
YJ845	*MAT*a *his3Δ200 leu2Δ0* or *leu2Δ1 lys2-128δ ura3Δ0 RPB3-HA::LEU2 spt16-197-C13MYC::HIS3MX*	This study
YJ846	*MAT*a *his3Δ200 leu2Δ0* or *leu2Δ1 lys2Δ0 ura3Δ0 RPB3-HA::LEU2 spt16-197-C13MYC::HIS3MX*	This study
YJ847	*MATα leu2Δ1 or leu2Δ0 lys2-128δ or lys2Δ0 FLAG-SPT6*	This study

Table 4 (cont.)

YJ850	*MAT*a *leu2Δ1 or leu2Δ0 lys2-128δ ser3::GAL7UAS FLAG-spt6-1004*	This study
YJ855	*MAT*α *leu2Δ1 or leu2Δ0 lys2-128δ ser33Δ::KanMX FLAG-spt6-1004*	This study
YJ859	*MAT*a *leu2Δ1 or leu2Δ0 lys2-128δ ura3-52 or ura3Δ0 spt16-197*	This study
YJ862	*MAT*a *leu2Δ1 or leu2Δ0 lys2-128δ FLAG-spt6-1004*	This study
YJ864	*MAT*a *his3Δ200 his4-912δ leu2Δ0 or leu2Δ1 lys2-128δ ura3Δ0 ser33Δ::KanMX*	This study
YJ867	*MAT*α *leu2Δ0* or *leu2Δ1 lys2Δ0 ura3Δ0 or ura3-52 ser3::GAL7UAS spt16-197*	This study
YJ868	*MAT*α *his3Δ200 leu2Δ0* or *leu2Δ1 lys2-128δ ura3Δ0 or ura3-52 ser3::GALUAS spt16-197*	This study
YJ869	*MAT*α *leu2Δ0* or *leu2Δ1 lys2Δ0 ura3Δ0 or ura3-52 ser3::GAL7UAS spt16-197*	This study
YJ871	*MAT*α *leu2Δ0* or *leu2Δ1 lys2Δ0 or lys2-128δ ura3Δ0 or ura3-52 ser3::GAL7UAS*	This study
YJ872	*MAT*a *his3Δ200 leu2Δ0* or *leu2Δ1 lys2Δ0 or lys2-128δ ura3Δ0 or ura3-52 ser3::GAL7UAS*	This study
YJ873	*MAT*a *his4-912δ leu2Δ0* or *leu2Δ1 lys2Δ0 or lys2-128δ ura3Δ0 ser3::GAL7UAS SPT6-FLAG*	This study
YJ875	*MAT*α *his4-912δ leu2Δ0* or *leu2Δ1 lys2-128δ ura3Δ0 ser3::GAL7UAS FLAG-spt6-1004*	This study
YJ876	*MAT*a *his4-912δ leu2Δ0* or *leu2Δ1 lys2-128δ ser3::GAL7UAS FLAG-spt6-1004*	This study
YJ877	*MAT*a *leu2Δ0* or *leu2Δ1 lys2Δ0 or lys2-128δ ura3Δ0 or ura3-52 RPB1-C13MYC::KanMX FLAG-SPT6*	This study
YJ878	*MATα his3Δ200 leu2Δ0* or *leu2Δ1 lys2Δ0 or lys2-128δ RPB1-C13MYC::KanMX FLAG-SPT6*	This study
YJ879	*MATα his3Δ200 leu2Δ0* or *leu2Δ1 lys2Δ0 or lys2-128δ ura3Δ0 or ura3-52 RPB1-C13MYC::KanMX FLAG-SPT6*	This study
YJ884	*MATα leu2Δ0* or *leu2Δ1 lys2Δ0 or lys2-128δ RPB3-HA:LEU2 RPB1-C13MYC::KanMX FLAG-SPT6*	This study
YJ886	*MAT*a *his3Δ200 leu2Δ0* or *leu2Δ1 lys2-128δ RPB1-C13MYC::KanMX FLAG-spt6-1004*	This study
YJ887	*MATα his3Δ200 leu2Δ0* or *leu2Δ1 lys2-128δ ura3Δ0 or ura3-52 RPB1-C13MYC::KanMX FLAG-spt6-1004*	This study
YJ888	*MAT*a *his3Δ200 leu2Δ0* or *leu2Δ1 lys2Δ0 ura3Δ0 or ura3-52 RPB1-C13MYC::KanMX FLAG-spt6-1004*	This study
YJ892	*MATα his3Δ200 leu2Δ0* or *leu2Δ1 lys2-128δ ura3Δ0 or ura3-52 RPB3-HA::LEU2 RPB1-C13MYC::KanMX FLAG-spt6-1004*	This study
YJ916	*MAT*α *leu2Δ1 or leu2Δ0 lys2-128δ ura3-52 or ura3Δ0 ser33Δ::KanMX spt16-197*	This study
YJ947	*MAT*a *ura3Δ0 ser3ΔUAS*	This study
YJ950	*MATα ura3Δ0 leu2Δ0* or *leu2Δ1*	This study
YJ954	*MATα ura3Δ0 lys2-128δ leu2Δ0* or *leu2Δ1 ser3ΔUAS*	This study
YJ958	*MATα lys2-128δ or LYS2 leu2Δ0* or *leu2Δ1 FLAG-spt6-1004*	This study
YJ962	*MATα lys2-128δ or LYS2 leu2Δ0* or *leu2Δ1 FLAG-spt6-1004 ser3ΔUAS*	This study
YJ966	*MATα ura3Δ0 or ura3-52 lys2-128δ or LYS2 leu2Δ0* or *leu2Δ1 spt16-197*	This study
YJ970	*MATα ura3Δ0 or ura3-52 lys2-128δ or LYS2 leu2Δ0* or *leu2Δ1 spt16-197 ser3ΔUAS*	This study
TF7783-24	*MAT*a *leu2Δ1 trp1Δ63 ura3-52 his4-912δ lys2-128δ spt16-24*	T. Formosa
TF8030-1	*MAT*α *leu2Δ1 trp1Δ63 ura3-52 his4-912δ lys2-128δ spt16-11*	T. Formosa
TF8031-1	*MAT*α *leu2Δ1 trp1Δ63 ura3-52 his4-912δ lys2-128δ pob3-7*	T. Formosa

3.2.2 Nucleosome scanning assay

Nucleosome scanning experiments were performed using a method adapted from those previously described (Whitehouse and Tsukiyama 2006; Brickner et al. 2007; Lee et al. 2007). Cells were grown to 2-3 x 10^7 cells/ml and treated with formaldehyde (2% final concentration) for 30 minutes at 30°C and then glycine (125mM final concentration) for 10 minutes at room temperature. 1.2 x 10^9 formaldehyde-treated cells were harvested by centrifugation, washed with Tris-buffered saline, and then incubated in ZDB buffer (50mM Tris Cl pH 7.5, 1M Sorbitol, 10mM β-mercaptoethanol) containing 1.5mg zymolyase 20T at 30°C for 30 minutes on a rocker platform. Spheroplasts were pelleted by low-speed centrifugation, gently washed with NP buffer (1M sorbitol, 50mM NaCl, 10mM Tris Cl ph 7.4, 5mM MgCl2, 1mM CaCl2, 0.075% NP-40, 1mM β-mercaptoethanol, and 500µM spermidine), and resuspended in 1.8mL NP buffer. Samples were divided into 6 x 300µl aliquots that were then digested with 0, 1, 2.5, 5, 10, 20 units of MNase (Nuclease S7 from Roche) for 45 minutes at 37°C. Digestions were stopped with 75µl Stop buffer (5% SDS, 50mM EDTA) and treated with 100µg proteinase K for 12-16 hours at 65°C. DNA was extracted by phenol/chloroform using PLG-H tubes (5 Prime) and incubated with 50µg RNase A for 1 hour at 37°C. DNA was re-extracted with phenol/chloroform, precipitated with an equal volume of isopropanol, washed with 80% ethanol, and resuspended in 100µl TE. MNase digestions were evaluated by two methods. First, one-fifth of digested DNA was separated by gel electrophoresis. Second, previously characterized *GAL1* promoter sequences (Lohr 1984; Brickner et al. 2007; Floer et al. 2010), one within a positioned nucleosome (*GAL1* NB) and a second adjacent region (*GAL1* NUB) that is rapidly

digested by MNase, were amplified by qPCR from MNase-treated and untreated samples. The MNase concentration that resulted in mostly mononucleosome-sized DNA with a *GAL1* NUB/NB ratio of <15% was subjected to further qPCR using tiled *SER3* primer pairs (Table 4; SER3-1 to SER3-41). For each *SER3* primer set, the amount of protected template was calculated as a ratio between MNase-digested and undigested samples and then normalized to the amount of protected *GAL1* NB template. All nucleosome scanning assays were done in triplicate using at least two independent strains as indicated in the figure legends.

3.2.3 Northern analysis

Northern analysis was performed as previously described (Ausubel et al. 1991) on 20µg of total RNA isolated from cells grown to 1-2 x 10^7 cells/ml. DNA probes were generated by random primed labeling PCR fragments for *SER3* (ChrV:324059-324307), *SRG1* (ChrV:322258-322559), and *SCR1* (ChrV:441741-442266). *SCR1* serves as a loading control since its RNA levels are unaffected by the mutations and growth conditions used in this study.

3.2.4 Chromatin immunoprecipitation

For histone H3, TBP, and Rpb1-C13myc ChIPs, cells were grown in YPD at 30°C to 1-2 x 10^7 cells/ml. For Gal4 ChIPs, cells were grown in YPraf at 30°C to 0.8 x 10^7 cells/ml and then an additional 4 hours at 30°C after addition of 2% galactose. Chromatin preparation and treatment were performed as previously described (Shirra et al. 2005). Gal4, histone H3, TBP, and Rpb1-13myc were immunoprecipitated by incubating sonicated chromatin overnight at 4°C with 1µl anti-GAL4 DBD antibody (sc-577; Santa Cruz), 5µl anti-histone H3 antibody (ab1791; Abcam),

2µl anti-TBP antibody (kind gift from G. Prelich, Albert Einstein College of Medicine), and 4µl anti-c-myc A-14 antibody (sc-789; Santa Cruz), respectively. Dilutions of input and immunoprecipitated DNA were subjected to qPCR. All ChIP signals were normalized to a control: either *GAL1* NB template (histone H3 ChIP), TELVI template located within a telomeric region on chromosome VI (Gal4 ChIP), or No ORF template located within a region of chromosome V that lacks ORFs (Rpb1-C13myc and TBP ChIPs). Details regarding the primers used for qPCR in each ChIP experiment are listed in Table 5.

3.2.5 Quantitative real-time PCR

All qPCR data was obtained using an ABI 7300 or StepOnePlus Real-time PCR system, SYBR green reagents (Fermentas), and the primer sets listed in Table 5. All calculations were performed using Pfaffl methodology for relative quantitation of real-time PCR (Pfaffl 2001).

Table 5: Oligos used in qPCR for ChIP and nucleosome scanning experiments

Name	Forward Primer	Reverse Primer	Position*	Length $	Midpoint &
GAL1 NB	CCCCACAAACCTTCAAATTAACG	CGCTTCGCTGATTAATTACCC	II:278751-278850	100	
GAL1 NUB	CGGATTAGAAGCCGCCGA	ATCTTTATTGTTCGGAGCAGTG	II:278568-278697	130	
SER3-1	CGGTACCAACCAAGTTGACTTAGAC	ATTTCAGCGATGACCAATTCTGCTAC	V:323074-323181	108	447
SER3-2	CGAAGAATCTGGTTTGTATTGGTTG	TTGGAGAAAGGCGAGTTGAAAAC	V:323040-323145	106	412
SER3-3	ACTAGATTAACTTCAAATGTCTTACAACATG	TGGTAGCGTAGTCTAAGTCAAC	V:323009-323108	100	378
SER3-4	GACGTTCATGCTATTGGTATCAGATC	TACCGATACAGAAACAACCAATACAA	V:322979-323078	100	348
SER3-5	TGCCCGAGGAAGAGTTGATC	CAAACCAGATTCTTCGCATGTTGTAA	V:322947-323055	109	320.5
SER3-6	GAAGAGCAAGGTTACCAAGTCGAAT	CTAGTCTTTGATCTGATACCAATAGCATGAAC	V:322907-323013	107	279.5
SER3-7	AAACGTTAATCAAACTGCTATTACAATCTT	GATCTTTTCGATCAACTCTTCCTCGG	V:322876-322975	100	245
SER3-8	GCCTTTCTCAACGGGTGATATG	CAATGAAGATTTATAGAATTCGACTTGGTAAC	V:322837-322948	112	212
SER3-9	ATGCTGTAAAGCACCCAAAAATTT	CGAAGATTGTAATAGCAGTTTGATTAACG	V:322809-322907	99	177.5
SER3-10	CATGAATACCGTTCCACAGCG	ACGTTTTCTAATAGTAAAATCTTCATATCACC	V:322783-322881	99	152
SER3-11	CCCAGGCGCTGTTTGTACTT	AAAGGCTTCAAAATTTTTGGGTG	V:322747-322842	96	114
SER3-12	ACCTTTCAACAAGCTATGAATATGAGC	ACAGCATTCAAGCGCTGTGGA	V:322715-322815	101	84.5
SER3-13	AATGACAAGCATTGACATTAACAACTTAC	CATGAAAGATTGCGTAGGTGAAGTAC	V:322681-322786	106	52
SER3-14	TACAGAACTCTATAAAGAACCACAGAAAAATC	AGCCGCTCATATTCATAGCTTGTTG	V:322643-322745	103	12.5
SER3-16	GGAAGAACCATTTCTAGTTATTTCACTTTT	CATTGCTGTCGATTTTTCTGTGGTTC	V:322585-322684	100	-47
SER3-17	GCAGAGGATAAGGAAATTCTTAAAACTG	GTTCTGTATTTTTACTAAGATAGTTGACAAG	V:322544-322650	107	-84.5
SER3-19	GGATGAAAAAATCAGACAAATATCCAA	CCTTTATATACATAACAGTTTTAAGAATTTCC	V:322485-322586	102	-145
SER3-20	TTAAGAAAATGCAACGCTGCC	GCTCCCTCCTTCCAACAAAG	V:322444-322545	102	-187
SER3-21	GTCCTTGACTTCTACCACGAGAAAA	TTACTCATAACTTGGATATTTGTCTGATTTTTTC	V:322416-322522	107	-212.5
SER3-22	ATTCTTCTCGTTCCCACCTAATTTC	TCAGAAAACCCTGCACGGG	V:322381-322481	101	-250.5
SER3-23	GGAACAACTTCGGTCTCAGCA	TTTCTTAATTTTTTTCTCGTGGTAGAAG	V:322352-322451	100	-280
SER3-24-2	CGATATTTACTCACAAATGGAATTCAAG	GAAGTCAAGGACAATAAATTGCGAA	V:322323-322427	105	-306.5
SER3-25-2	AAACCTAATTTTTTTTGTGGACCCA	AACGAGAAGAATAATTAAAGTGCTGAGAC	V:322294-322392	99	-338.5

Table 5 (cont.)

SER3-26	TAAAAATTTGGTTAAG CAGTTAGGCTG	TCCCCTTGAATTCCATT TGTGAGTAAATAT	V:322250-322354	105	-379.5
SER3-27	GCCAAGCTATGTGCAA ATATCACAAA	TGGGTCCACAAAAAAA ATTAGGTT	V:322223-322318	96	-411
SER3-28	CATTGTTTTAGTTTTTT ACTCACAATCGA	AGGTCCAGCCTAACTG CTTA	V:322179-322281	103	-451.5
SER3-29	AGAAATGCCATTGTTT AATCCTGATT	TTTAATTTGTGATATTT GCACATAGCTTGG	V:322147-322253	107	-481.5
SER3-30-2	TCCCCATTATCTTTGA ATTTTCCTC	CTCGATTGTGAGTAAA AAACTAAAACAATG	V:322094-322207	114	-531
SER3-31	CATCTCCACCTTTCTC CCCAT	ACAATGTAGATAATCA GGATTAAACAATGGC	V:322080-322183	104	-550
SER3-32	GAACTTTCAAATTTAC GATAGGTGGAG	TGGCATTTCTATGGATT TGTTGTTCTCTT	V:322050-322156	107	-578.5
SER3-33	GCGTGATGTTTGGGTG CAAT	GAGGAAAATTCAAAGA TAATGGGGAGAAA	V:322017-322118	102	-614
SER3-34	TGCTGGATTGGATATA TTGATAACGT	ATGTATCTCCACCTATC GTAAATTTGAAAG	V:321978-322082	105	-651
SER3-35	TCCATTTACTAATCAA CTTAACAATGCTG	GTTCCGCTTTTCCGCCA AT	V:321954-322053	100	-678
SER3-36	TAAAACCCTTTTTTGT ACACAATGGA	CAAACATCACGCAACG CTTTTT	V:321922-322028	107	-706.5
SER3-37-2	TATAACAAAATAATCA AGTTAAAACCCT	CGTTATCAATATATCCA ATCCAGCATT	V:321903-322002	100	-724
SER3-38-2	TTCTTTACCTCATTCA ACTGTATAGAACGT	GTTAAGTTGATTAGTAA ATGGAAGAGATTCC	V:321873-321975	103	-762.5
SER3-40-2	GAGACTACACCGTGA AGCAACCT	AACGTTCTATACAGTTG AATGAGGTAAAGA	V:321805-321903	99	-827.5
SER3-41	TGATCAACTATTAAAT TCCGGCAGTA	TTTAGTATAGATTATTT GGTAGCTTCAGG	V:321761-321853	93	-874.5
SER3-3’	TGCAATCGATTCTCAT ACTGTCAAC	TGCCTCAAGCATTCTTC TATCCA	V:324094-324197	104	+1465
GAL7/SER 3	GAAAGGGTCCAAAAA GCGCTCGGA	CCTTTATATACATAACA GTTTTAAGAATTTCC	Details upon request	105	
TELVI	GCGTAACAAAGCCAT AATGCCTCG	CTCGTTAGGATCACGTT CGAATCC	VI:269487-269624	138	
NO ORF	GTGTTTGACCCGAGGG TATG	TAAGGTCCACACCGTC ATCA	V:9797-10013	217	
PMA1 5’ ORF	CTGGTGAATCTTTGGC TGTCG	AAAGTGTTGTCACCGG TAGCG	IV:481864-481975	104	
ADH1	TCAACCAAGTCGTCAA GTCC	CCAAAGCTTCTCTGGTG TCA	XV:159676-159750	99	
GAL1 5’ ORF	GAAAAGTGCCCGAGC ATAAT	CAGGCGATCTAGCAAC AAAA	II:279099-279174	97	
CYC1	TCTTTGGCAGACACTC TGGT	CCCACAACACGTTTTTC TTG	X:526456-526530	96	

*location of amplified product (chromosome: bp-bp) from the *Saccharomyces* Genome Database (http://yeastgenome.org/). $length (bp) of amplified product. &midpoint of amplified product relative to *SER3* ATG plotted in nucleosome scanning graphs.

3.3 RESULTS

3.3.1 Evidence that nucleosomes occupy the *SER3* promoter in repressing conditions

To investigate a possible role for chromatin structure in *SER3* repression, we first determined the positions of nucleosomes across the *SER3* locus in wild-type cells grown in *SER3*-repressing conditions (YPD) by a nucleosome scanning assay (Sekinger et al. 2005; Lee et al. 2007). Briefly, cells are treated with formaldehyde, spheroplasted and then incubated with increasing amounts of MNase to digest non-nucleosomal DNA (see Material and Methods for details). As previously described (Brickner et al. 2007), we monitored MNase digestion of two sequences located in the *GAL1-10* promoter, one within a well-positioned nucleosome (*GAL1* NB) and one within an adjacent MNase-sensitive region (*GAL1* NUB) by qPCR (Data not shown). DNA isolated from the MNase concentration where we observed significant protection of *GAL1* NB relative to *GAL1* NUB was then used to assess MNase protection across *SRG1-SER3*. We performed qPCR with 38 unique primer pairs to amplify overlapping *SRG1-SER3* sequences (Figure 20A) from both MNase-digested and undigested DNA. MNase protection for each of these sequences was quantified as the ratio of template present in MNase-digested DNA over undigested DNA that was then normalized to the amount of MNase-protected *GAL1* NB template. Using this method, we identified peaks of MNase-protection indicating the presence of a positioned nucleosome at the 3' end of *AIM9*, the gene adjacent to *SRG1*, and two at the 5' end of the *SER3* ORF (Figure 20B). We also found a 200-bp MNase-sensitive region (from -750 to -550 with respect to the *SER3* ATG) corresponding to the *SRG1* promoter, indicating a nucleosome-depleted region that is a hallmark of many yeast promoters (Yuan et al. 2005; Albert et al. 2007; Lee et al. 2007). In addition, we identified a broad region of MNase protection that

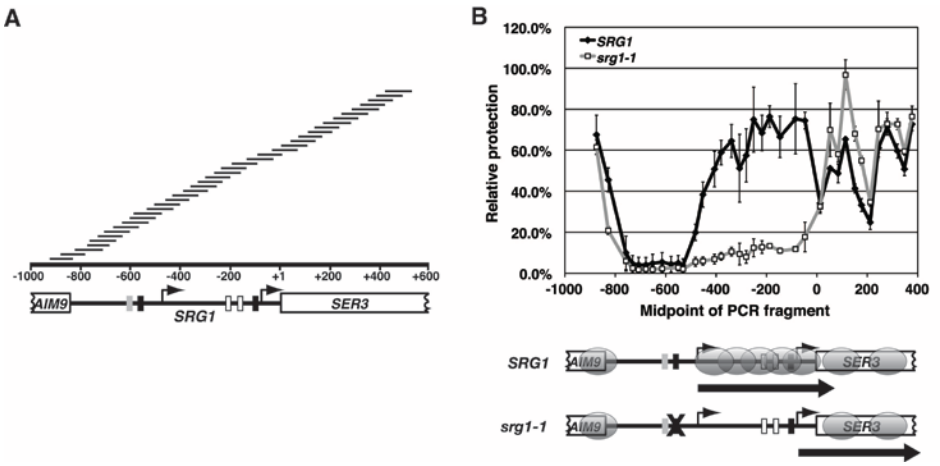

Figure 20: Nucleosome positions and relative occupancy at *SER3* in the presence and absence of *SRG1* transcription.

A) Schematic of *SER3* locus. The arrows at -475 and -75 indicate the TTS of *SRG1* and *SER3*, respectively. Blocks of intergenic sequence identity between *S. cerevisiae* and related yeast strains are marked, including the *SRG1* and *SER3* TATAs (black boxes), sequences required for *SER3* activation (white boxes), and a Cha4 binding site (grey box). The scale represents the distance from the *SER3* translation start (+1). The tiled black bars indicate the DNA fragments amplified by qPCR to quantify nucleosome position and relative occupancy (see Table 5). B) Nucleosome scanning assay was performed on wild-type (FY4, FY2097, FY1350) and *srg1-1* (YJ582, FY2250, YJ585) cells that were grown in YPD medium at 30°C. The relative MNase protection of each *SER3* template was calculated as a ratio to the control *GAL1* NB template. Each point on the graph shows the mean +/-SEM from three independent experiments that are plotted at the midpoint of each PCR product. Below the graph, a diagram of the *SER3* locus indicates the positions of nucleosomes (gray ovals) extrapolated from the MNase protection data. The block arrows indicate the transcription activity of *SRG1* and *SER3* respectively. *srg1-1* strains have a mutated TATA sequence (marked by an X) that inhibits *SRG1* transcription causing *SER3* derepression.

begins at the *SRG1* transcription start site (-475) and extends across the *SER3* promoter to the *SER3* translational start site, a region that defines the *SRG1* transcription unit. This pattern of strong MNase protection implies the presence of nucleosomes that are randomly positioned across the *SRG1* transcription unit. Therefore, the *SER3* promoter lacks the typical nucleosome-depleted region (Yuan et al. 2005; Albert et al. 2007; Lee et al. 2007). These results are consistent with previously reported indirect-labeling experiments (Martens and Winston 2002) and with genome-wide nucleosome positioning experiments (Lee et al. 2007).

To determine if *SRG1* transcription affects the chromatin structure at *SER3,* we repeated the nucleosome scanning assay using *srg1-1* strains that carry a mutation of the *SRG1* TATA sequence. This mutation severely reduces *SRG1* transcription resulting in strong derepression of *SER3* (Martens et al. 2004). In the *srg1-1* cells, MNase protection was reduced specifically over the *SRG1* transcription unit as compared to wild-type cells, indicating a dramatic loss of nucleosome occupancy (Figure 20B). Our results reveal a positive correlation between *SRG1* transcription and nucleosome occupancy across *SRG1*, an unexpected finding given the negative correlation between transcription and nucleosome occupancy generally observed for protein coding genes (Lee et al. 2004; Schwabish and Struhl 2004).

3.3.2 Serine-dependent transcription of *SRG1* intergenic DNA controls nucleosome occupancy of the *SER3* promoter

We have previously shown that *SER3* expression is tightly controlled by the serine-dependent regulation of *SRG1* transcription (Martens et al. 2005). Therefore, we also measured MNase accessibility at *SER3* in wild-type strains that were grown in SC+serine (*SRG1* induced, *SER3* repressed) and then shifted to SC-serine for 25 minutes (*SRG1* repressed, *SER3* induced). Since

the extent of the MNase digestion of the *GAL1* NB region was identical in these different growth conditions (Data not shown), we again normalized all *SER3* data to this region. As expected, for cells grown in serine-rich media, the relative MNase protection across *SRG1-SER3* is nearly identical to that observed for cells grown in YPD (compare wild-type strains in Figures 20B and 21A). When cells were shifted to media lacking serine, we measured a significant decrease in MNase protection over the *SRG1* transcribed region. However, rather than extending across the entire *SRG1* transcription unit as was observed for *srg1-1*, the reduced MNase protection was restricted to a 200bp region that includes sequences that had previously been determined to be required for *SER3* activation (Martens et al. 2004). An MNase-protected region of approximately 350bp, consistent with two closely associated nucleosomes or possibly one nucleosome that adopts multiple positions, remains near the 5' end of *SRG1*. This MNase-protected region begins at a more 5' position, including the *SRG1* transcription start site and possibly the *SRG1* TATA, as compared to the beginning of the broad peak of MNase protection that was measured for cells grown in serine-rich media. Thus, in contrast to the complete loss of nucleosomes across *SRG1* that occurs in the *srg1-1* strains, serine starvation depletes nucleosomes specifically over sequences required for *SER3* activation. Therefore, in response to serine starvation, the *SER3* promoter adopts the typical promoter architecture with +1 and -1 nucleosomes flanking a nucleosome-depleted UAS (Albert et al. 2007; Lee et al. 2007).

To determine if the loss of nucleosome occupancy at the *SER3* promoter is caused by a loss of *SRG1* transcription and is not simply an effect of the resulting increase in *SER3* transcription, we repeated the nucleosome scanning assay using strains that contain a mutation in the *SER3* TATA sequence (*ser3-100*). Although the *ser3-100* mutation strongly inhibits *SER3* activation when cells are shifted from serine-rich to serine starvation media (10-fold decrease in

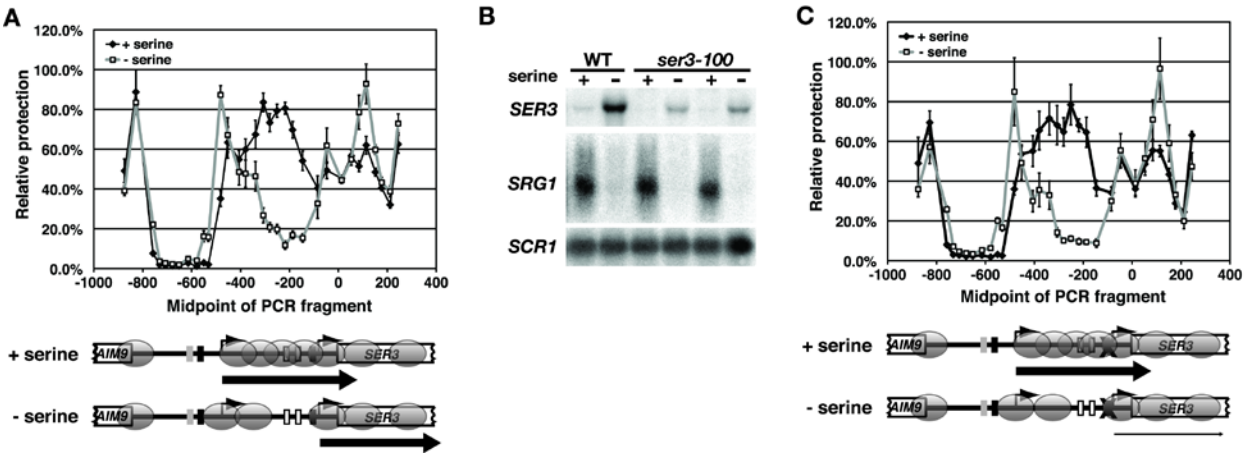

Figure 21: Effect of serine on nucleosome positions and relative occupancy at *SER3*.

A) Nucleosome scanning assay was performed on wild-type cells (FY2097 and FY4) that were grown at 30°C in SC+serine media (+ serine) and then shifted to SC-serine media for 25 minutes (- serine) as described in Figure 20. Each point on the graph shows the mean relative MNase protection +/-SEM from four independent experiments (two for each strain) plotted at the midpoint of each PCR product. Results for amplicons SER3-7 to SER3-41 are shown. B) Northern analysis of *SER3* and *SRG1* was performed on a wild type (FY2097) and two *ser3-100* strains (YJ275 and FY2099) that have a mutated *SER3* TATA. Cells were grown at 30°C in SC+serine (+ serine) and then shifted to SC-serine media for 25 minutes (- serine). *SCR1* serves as a loading control. C) Nucleosome scanning assay was performed on *ser3-100* strains (YJ275 and FY2099) as described in (A).

SER3 mRNA levels; Figure 21B), the changes in MNase protection between these growth conditions were identical to those observed for a wild type (Figure 21; compare panels A and C). Therefore, reduced nucleosome occupancy over the *SER3* promoter is not a consequence of increased *SER3* expression.

3.3.3 FACT and Spt6/Spn1 (Iws1) are required to repress *SER3*

Our results thus far are consistent with two possible mechanisms for transcription interference at *SER3*. In the first possibility, similar to the conventional transcription interference mechanism (Greger et al. 2000), RNA Pol II elongating across *SRG1* competes with transcription factors for binding to the *SER3* promoter. In the second possibility, the nucleosomes maintained over the *SER3* promoter by *SRG1* transcription compete with transcription factor access to the *SER3* promoter. If the latter possibility is true, we reasoned that disrupting nucleosome re-assembly during transcription might cause *SER3* derepression. Several studies have implicated the essential, highly conserved FACT and Spt6/Spn1(Iws1) transcription elongation complexes in transcription-dependent chromatin reassembly (Belotserkovskaya et al. 2003; Kaplan et al. 2003; Mason and Struhl 2003; Cheung et al. 2008; Jamai et al. 2009). Northern analyses were performed on several temperature-sensitive mutants of the Spt6/Spn1(Iws1) and FACT complexes that were grown in YPD at permissive (30°C) and non-permissive (37°C) temperature. Large increases in *SER3* mRNA levels were detected in multiple *spt6* and *spn1(iws1*) mutants at both 30°C and 37°C (Figure 22A). While increases were more modest and variable in the FACT mutants (*spt16*, *pob3*, and *nhp6*), we did find that in at least one mutant, *spt16-197*, a significant increase in *SER3* mRNA levels occurred at 30°C (Figure 22B).

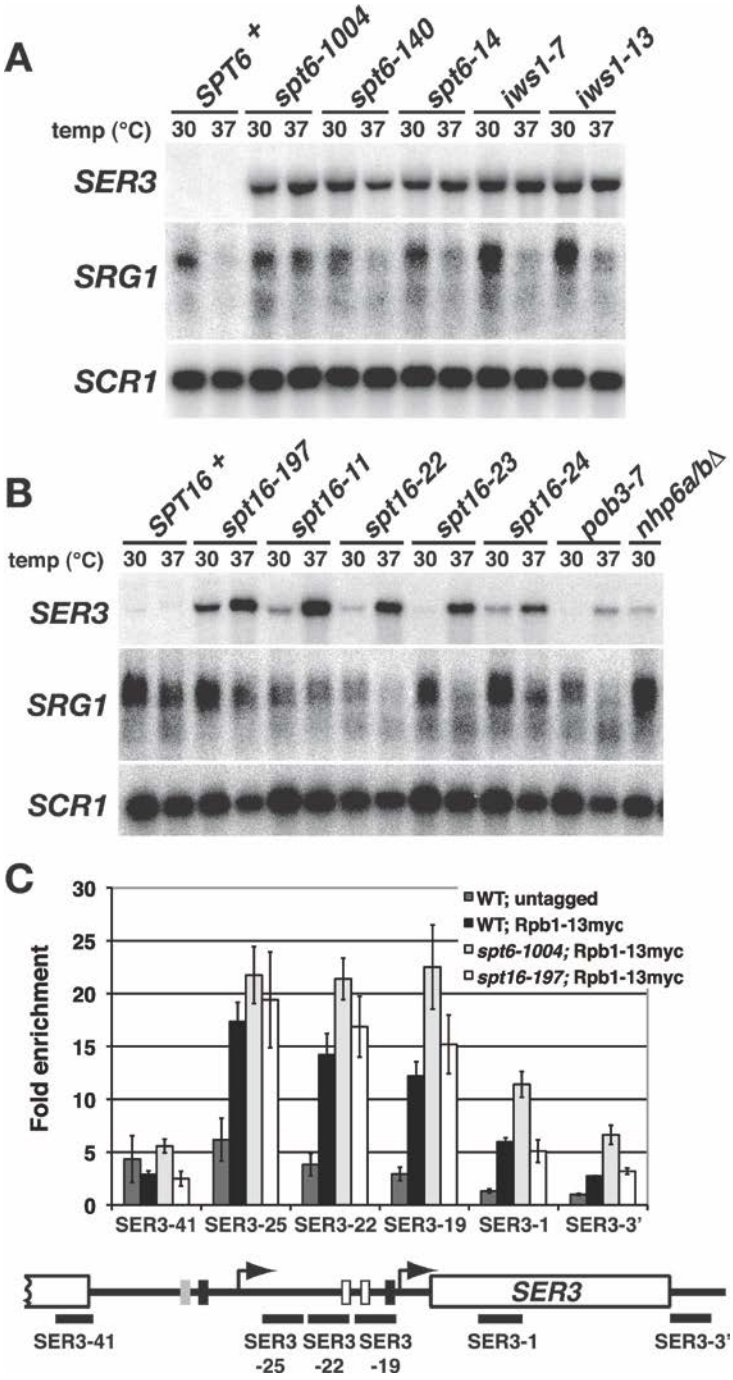
A
SPT6+
spt6-1004
spt6-140
spt6-14
iws1-7
iws1-13
temp (°C) 30 37 30 37 30 37 30 37 30 37 30 37
SER3
SRG1
SCR1
B
SPT16+
spt16-197
spt16-11
spt16-22
spt16-23
spt16-24
pob3-7
nhp6a/bΔ
temp (°C) 30 37 30 37 30 37 30 37 30 37 30 37 30 37 30
SER3
SRG1
SCR1
C
Fold enrichment
30
25
20
15
10
5
0
WT; untagged
WT; Rpb1-13myc
spt6-1004; Rpb1-13myc
spt16-197; Rpb1-13myc
SER3-41 SER3-25 SER3-22 SER3-19 SER3-1 SER3-3'
SER3
SER3-41
SER3 -25
SER3 -22
SER3 -19
SER3-1
SER3-3'

Figure 22: Repression of *SER3* is dependent on Spt6/Spn1(Iws1) and the FACT complex.

A) Northern analysis of *SER3*, *SRG1*, and *SCR1* (loading control) was performed on wild-type (FY4), *spt6-1004* (FY2425), *spt6-140* (FY111), *spt6-14* (FY1221), *iws1-7* (GHY1199), *iws1-13* (GHY1200) strains. Cells were grown in YPD at 30°C to mid-log and then shifted to 37°C for 60 minutes. B) Northern analysis of *SER3*, *SRG1*, and *SCR1* (loading control) was performed on wild-type (FY4), *spt16-197* (FY346), *spt16-11* (TF8030-1), *spt16-22* (YJ832), *spt16-23* (YJ833), *spt16-24* (TF7783-24), *pob3-7* (TF8031-1), *nhp6aΔ::URA3 nhp6bΔ::URA3* (FY1411) strains that were grown in YPD. C) ChIP analysis was performed on chromatin isolated from wild-type (YJ877, YJ878, YJ879, YJ884), *spt6-1004* (YJ886, YJ887, YJ888, YJ892), and *spt16-197* (YJ841, YJ842, YJ843) strains expressing Rpb1-C13myc and untagged control strains (FY4, FY5, YJ586). Rpb1-C13myc was immunoprecipitated with α-myc A14 antibody from chromatin prepared from cells that were grown in YPD at 30°C. The amount of immunoprecipitated DNA was determined by qPCR as a percentage of the input material and expressed as the fold enrichment over a control region of chromosome V that lacks open reading frames (No ORF). Each bar represents the mean +/- SEM from at least three independent experiments. Below the graph is a schematic of *SER3* with black bars corresponding to the regions amplified by qPCR.

Importantly, *SRG1* RNA levels were not significantly reduced in most of the mutant strains as compared to a wild type at 30°C.

I performed chromatin immunoprecipitation (ChIP) experiments to measure RNA Pol II occupancy across the *SRG1/SER3* locus in a wild-type strain, and two of these mutants, *spt6-1004,* and *spt16-197,* that express either untagged Rpb1 (control) or a myc-tagged version of Rpb1 (Rpb1-13myc). The *spt6-1004* and *spt16-197* mutants have both been well characterized and share similar phenotypes characteristic of transcription defects including sensitivity to the nucleotide analog 6-azauracil, suppression of Ty insertions, and cryptic intragenic transcription (Kaplan et al. 2003; Mason and Struhl 2003). Consistent with our Northern data, RNA Pol II strongly associates with the *SRG1* transcription unit (Figure 22C) to similar levels in wild-type, *spt6-1004,* and *spt16-197* cells. Taken together, these results show that *SER3* repression is strongly dependent on both Spt6/Spn1(Iws1) and FACT. When these factors are mutated, *SER3* is derepressed without affecting RNA Pol II levels at *SRG1*. This result argues against a model in which it is the level of active transcription that confers transcription interference.

Beyond the primary sites of *SRG1* transcription termination, I found a 2-fold increase in RNA Pol II occupancy in the *spt6-1004* cells as compared to wild-type cells, which is consistent with our Northern data (Figure 22C). However, I did not detect an increase in RNA Pol II in the *spt16-197* cells. Although surprising given the increase in *SER3* mRNA levels in this mutant, this result may be reconciled if we consider that *SRG1* transcription does not always terminate properly, resulting in the production of a minor read-through that extends to the end of *SER3* (Martens et al. 2004; Thompson and Parker 2007). Importantly, we have found that the level of *SRG1-SER3* read-through product is reduced in both *spt6-1004* and *spt16-197* mutants (S. Hainer; unpublished data), which is likely due to increased initiation at the *SER3* promoter.

Therefore, the expected increase in RNA Pol II occupancy in these mutant strains might be reduced because of the lower read-through of *SRG1* to the end of *SER3*.

3.3.4 Nucleosome occupancy of the *SER3* promoter is reduced in *spt6-1004* and *spt16-197* mutants at permissive temperature

To test whether the level of nucleosomes over *SRG1* affects *SER3* repression, we next performed nucleosome scanning assays to compare MNase accessibility across *SRG1* in wild-type, *spt6-1004,* and *spt16-197* cells that were grown in YPD at 30°C. We again normalized MNase protection of each *SRG1-SER3* region to the *GAL1* NB region, as the MNase accessibility of the *GAL1* control regions was indistinguishable between these strains (Data not shown). Compared to wild-type cells, we measured a significant reduction of MNase protection specifically across the *SRG1* transcribed unit in *spt6-1004* cells (4-fold decrease) and to a slightly lesser extent in *spt16-197* cells (3-fold decrease) (Figure 23A), indicating nucleosome depletion across *SRG1*. These results are strikingly similar to the nucleosome scanning results we obtained for the *srg1-1* mutant (Figure 20B). However, while *SRG1* transcription was greatly reduced in *srg1-1* strains, it remained at wild-type levels in the *spt6-1004* and *spt16-197* mutants.

To complement our MNase experiments, I performed histone H3 ChIP assays in these same strains grown under the same conditions (Figure 23B). In wild-type cells, I detect significant histone H3 occupancy over the *SER3* promoter as compared to the *SRG1* promoter, which is consistent with nucleosomes occupying the *SER3* promoter. Moreover, at least for *spt6-1004* cells, there is a 2- to 3-fold decrease in histone H3 occupancy specifically over the *SER3* promoter that parallels the increase in MNase sensitivity over this region. Curiously, I did not observe a similar decrease in histone H3 occupancy over the *SER3* promoter in *spt16-197* cells.

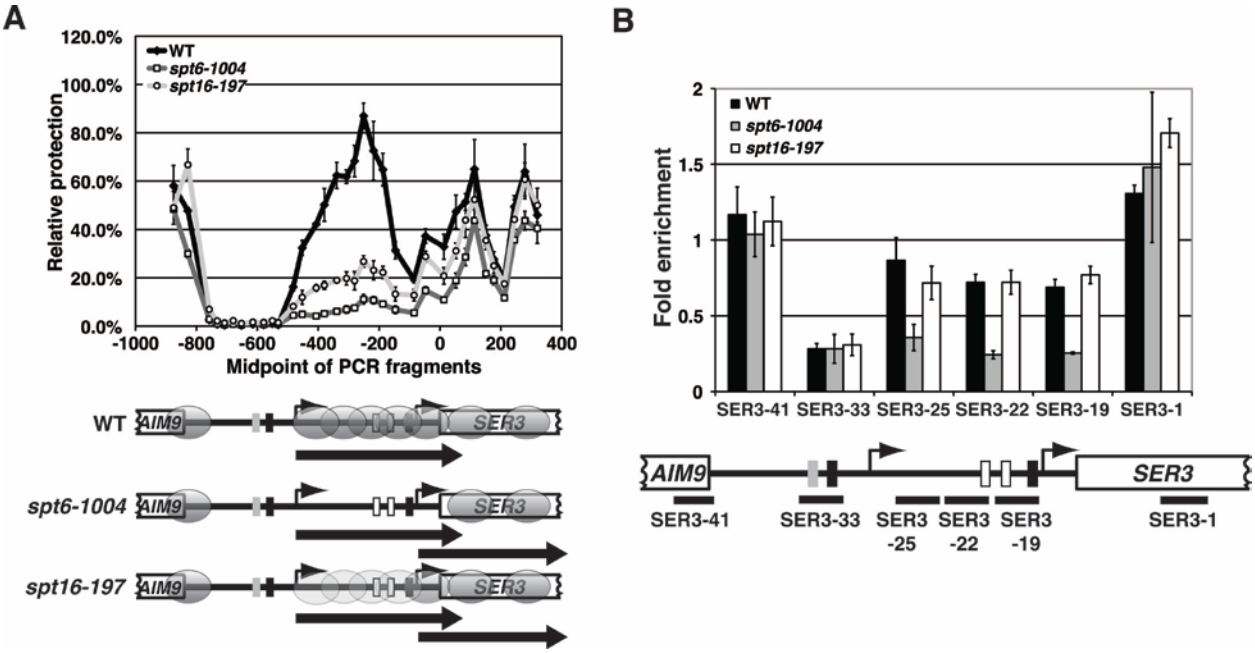

Figure 23: Nucleosome positions and relative occupancy at *SER3* in *spt6-1004* and *spt16-197* mutants.

A) Nucleosome scanning assay was performed on wild-type (FY2134, YJ864, YJ847), *spt6-1004* (FY2180, YJ855, YJ862), and *spt16-197* (FY346, YJ859, YJ916) strains that were grown in YPD at 30°C as described in Figure 20. The light gray ovals over the *SRG1* transcription unit in the *spt16-197* strain reflect that this region is slightly more protected from MNase digestion as compared to the *spt6-1004* strain. B) Histone H3 ChIP was performed on chromatin isolated from wild-type (FY4, FY5, YJ586), *spt6-1004* (YJ886, YJ887, YJ888), and *spt16-197* (YJ844, YJ845, YJ846) cells that were grown in YPD. The amount of immunoprecipitated DNA was determined by qPCR as a percentage of the input material and expressed as the fold enrichment over *GAL1* NB. Each bar represents the mean +/- SEM of at least three independent experiments. Below the graph is a schematic of *SER3* with black bars corresponding to the regions amplified by qPCR.

Since the loss of MNase protection is less pronounced in the *spt16-197* mutants as compared to the *spt6-1004* mutants, it is possible that histone H3 ChIP is not sensitive enough to detect a change in histone occupancy between wild-type and *spt16-197* strains. Alternatively, nucleosomes may only partially reassemble in the *spt16-197* mutant in such a manner that makes them more accessible to MNase without altering histone H3 occupancy. Based on previous studies (Belotserkovskaya et al. 2003; Xin et al. 2009), an intriguing possibility is that reassembly of the H2A/H2B dimers at the *SER3* promoter may be specifically reduced by the *spt16-197* mutation. Taken together, these data support a model whereby FACT and Spt6/Spn1(Iws1) are required for *SRG1* transcription-dependent assembly of nucleosomes that repress *SER3*.

3.3.5 *spt6-1004* and *spt16-197* mutants are defective for transcription interference at *SER3*

To test whether *SRG1* transcription-dependent nucleosomes interfere with transcription factor binding to the *SER3* promoter, we performed ChIP experiments in *spt6-1004* and *spt16-197* mutants. Because sequence-specific activators of *SER3* remain unknown, we first utilized a previously described *ser3::GAL7UAS* allele in which the putative *SER3* UAS is replaced with two binding sites for the Gal4 transcription activator (Martens et al. 2004). We then measured Gal4 occupancy by ChIP in wild-type, *srg1-1*, *spt6-1004*, and *spt16-197* strains that all contain the *ser3::GAL7UAS* allele and were grown in YPgal (Figure 24A). Consistent with our previous data (Martens et al. 2004), Gal4 occupancy at the *SER3* promoter increases 8-fold in the *srg1-1* control strain where *SRG1* is no longer transcribed and the *SER3* promoter is depleted of nucleosomes. In the *spt6-1004* and *spt16-197* strains where *SRG1* is transcribed at wild-type

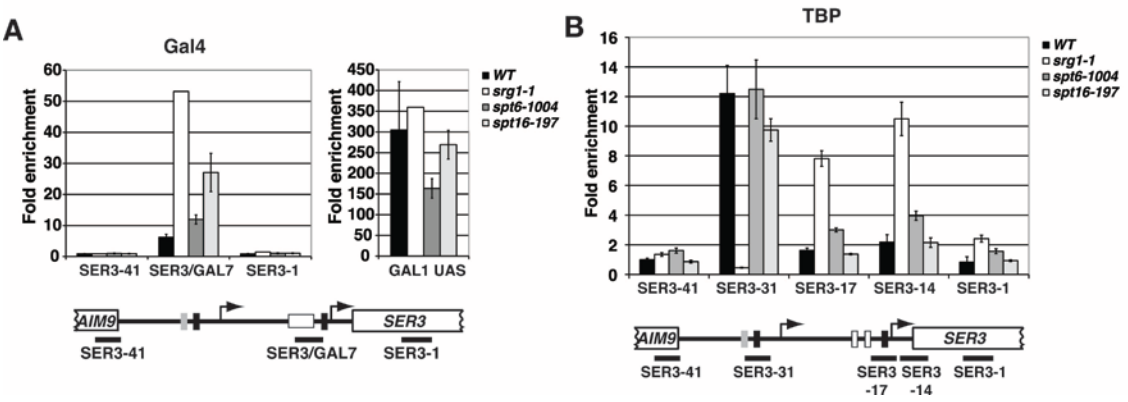

Figure 24: *spt6-1004* and *spt16-197* mutants are defective for transcription interference at *SER3*.

A) Gal4 ChIP was performed on wild-type (YJ871, YJ872, YJ873), *spt6-1004* (YJ875, YJ876, YJ850), *spt16-197* (YJ867, YJ868, YJ869), and positive control *srg1-1* (FY2260) cells that all contain the *ser3::GAL7UAS* allele. Chromatin was prepared from cells grown at 30°C in YPraf to 0.8 x 10^7 cells/ml and then for an additional four hours at 30°C after the addition of 2% galactose. Gal4 ChIP signals were determined by qPCR at the three *SER3* locations (left histogram) and at *GAL1* as a positive control (right histogram). All values were normalized to a control region located near the telomere of chromosome VI (TELVI) and represent the mean +/- SEM. Below the graph is a diagram of the *ser3::GAL7UAS* allele in which the putative *SER3* UAS region was replaced with the *GAL7* UAS region containing two Gal4-binding sites (white box). The black bars indicate the regions of *SER3* amplified by qPCR. B) TBP ChIP was performed on chromatin isolated from wild-type (FY4, FY5, YJ586, KY719), *spt6-1004* (YJ886, YJ887, YJ888, YJ892), *spt16-197* (YJ841, YJ842, YJ843, and YJ844), and positive control *srg1-1* (FY2471, YJ582, YJ583, YJ585) strains that were grown in YPD at 30°C as described in Figure 22C.

levels but nucleosome occupancy at the *SER3* promoter is reduced, Gal4 occupancy at the *SER3* promoter was also increased 2- and 4-fold respectively (Figure 24A, left panel). Based on our *SER3* expression and nucleosome occupancy data (Figures 22A and 23A), the 2-fold increase in Gal4 occupancy at the *SER3* promoter in the *spt6-1004* strains was lower than expected. However, this result is likely related to the fact that we also found reduced Gal4 occupancy at the control *GAL1* promoter in *spt6-1004* cells as compared to wild-type, *srg1-1*, and *spt16-197* cells (Figure 24A, right panel).

I also compared TBP occupancy by ChIP at the *SRG1* and *SER3* TATA sequences in wild-type, *srg1-1*, *spt6-1004*, and *spt16-197* strains that contain the endogenous *SRG1-SER3* locus (Figure 24B). The *SRG1* and *SER3* TATA sequences are both conserved among related yeast strains, bind TBP, and are required for *SRG1* and *SER3* transcription, respectively (Martens and Winston 2002; Martens et al. 2004). At the *SRG1* TATA, there is little difference in TBP occupancy in the *spt6-1004* and *spt16-197* mutants as compared to the wild-type strains, which agrees with our Northern and RNA Pol II ChIP data (see Figure 22). At the *SER3* TATA, TBP occupancy increased 2-fold in *spt6-1004* cells as compared to a 4-fold increase in *srg1-1* control cells, suggesting that the loss of nucleosomes over the *SER3* promoter in the *spt6-1004* mutants either increases TBP binding directly or possibly indirectly by allowing an unknown *SER3* activator protein better access to the *SER3* promoter. Interestingly, I did not observe a significant difference in TBP occupancy in the *spt16-197* mutant. This result may not be surprising considering the increase in *SER3* expression is significantly lower in this mutant as compared to the *spt6-1004* mutant (Figure 22). Therefore, this assay may lack the sensitivity to detect a significant difference in TBP occupancy between wild-type and *spt16-197* cells. From these data, we conclude that transcription interference at *SER3* is dependent, at least in part, on

Spt6 and Spt16. Taken together with results described earlier, our findings suggest that transcription interference of *SER3* is partially mediated by nucleosomes that occupy the *SER3* promoter as a consequence of *SRG1* transcription from intergenic DNA.

3.3.6 Rpd3S and histone methyltransferases are not required for *SER3* repression

Spt6 and Spt16 have previously been shown to suppress transcription initiation from cryptic promoters that are located within protein coding regions (Mason and Struhl 2003; Kaplan et al. 2009). Cryptic intragenic transcription is also suppressed by a cascade of transcription-dependent post-translational histone modifications (Lee and Shilatifard 2007; Li et al. 2007a). During transcription, Set2 methylates lysine 36 of histone H3 thereby marking nucleosomes associated with recently transcribed DNA (Pokholok et al. 2005; Rao et al. 2005). Dimethylated H3 K36 acts as a binding site for the Rpd3S histone deacetylase complex (Youdell et al. 2008). Upon recruitment, Rpd3S deacetylates the reassembled nucleosomes on the amino-terminal tails of histone H3 and H4, which suppresses cryptic intragenic transcription presumably by occluding transcription factor access (Carrozza et al. 2005; Joshi and Struhl 2005; Keogh et al. 2005). Recently, Set1-dependent methylation of H3K4 has also been implicated as a signal for transcription-dependent histone deacetylation by Rpd3S (Pinskaya et al. 2009) and the Set3 complex (Kim and Buratowski 2009). Because of these observations, a likely hypothesis is that Set1 and Set2 may contribute to *SER3* repression by regulating similar histone modifications over the *SER3* promoter in response to *SRG1* transcription. To test this possibility we performed a Northern analysis to measure the effect of deleting the genes encoding the Set1, Set2, and Dot1 histone methyltransferases, the Rco1 subunit of Rpd3S, and the Set3 subunit of the Set3 complex on *SER3* and *SRG1* expression. Deletions of any one of these genes or a *set1*Δ *set2*Δ double

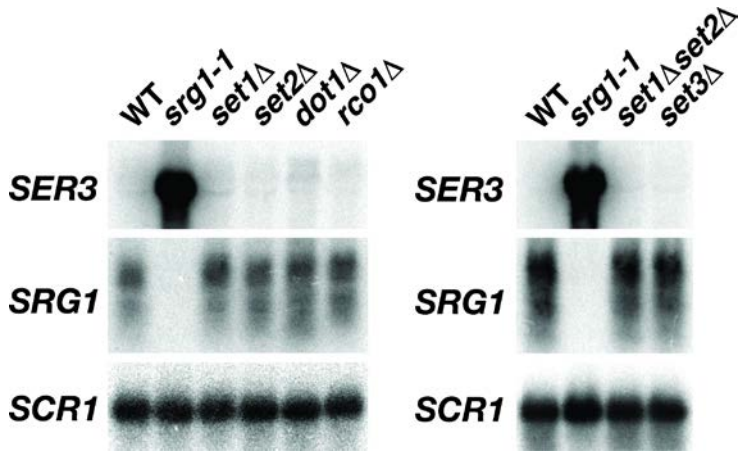

Figure 25: Repression of *SER3* does not require histone methyltransferases or the Rpd3S and Set3C histone deacetylase complexes.

Northern analysis of *SER3*, *SRG1*, and *SCR1* (loading control) was performed on wild-type (YJ586), *srg1-1* (FY2471), *set1*Δ (KY938), *set2*Δ (KY912), *dot1*Δ (KY934), *rco1*Δ (KY1235), *set1*Δ *set2*Δ (KY1822) and *set3*Δ (KY1806) strains that were grown in YPD at 30°C.

deletion has no effect on *SER3* or *SRG1* mRNA levels (Figure 25). Moreover, mutations of histone H3 lysine 4, lysine 36, or lysine 79 also have little to no effect on *SER3* repression (Hainer and Martens 2011a). Therefore, our results suggest that the relative contribution of these histone reassembly mechanisms may vary at different loci throughout the genome.

3.4 CONCLUSIONS

In this work, we provide evidence that intergenic transcription represses adjacent gene transcription by assembling a repressive chromatin structure, rather than by the act of transcription. First, we showed that *SRG1* intergenic transcription is not only required for repression of the adjacent *SER3* gene, but is also required to maintain MNase protection of the *SER3* promoter. Second, we determined that changes in the MNase protection of the *SER3* promoter are caused by changes in *SRG1* transcription and not an effect of the changes to *SER3* transcription. Third, we found that cells expressing mutant versions of the Spt6 and Spt16 elongation factors derepress *SER3* and reduce MNase protection across the *SER3* promoter without altering *SRG1* RNA levels or RNA Pol II occupancy across *SRG1*. These results clearly implicate the nucleosomes assembled on the *SER3* promoter as the key factor in *SER3* repression. Finally, we found that Spt6 and Spt16 are required to inhibit transcription factor binding to the *SER3* promoter, which suggests that the nucleosomes assembled at the *SER3* promoter by these factors interfere with the binding of transcription factors to their sites on DNA.

Taken together with our previous studies (Martens and Winston 2002; Martens et al. 2004; Martens et al. 2005), we propose the following model for *SER3* regulation. When cells are

grown in serine-rich medium, the Cha4 DNA binding protein recruits the Swi/Snf and SAGA complexes resulting in the induction of *SRG1* transcription. RNA Pol II transcribes *SRG1* across the *SER3* promoter, disassembling nucleosomes in its path and then reassembling them in its wake by a mechanism that involves both Spt6 and Spt16. *SRG1* transcription is thus required to maintain nucleosomes across the *SER3* promoter, interfering with transcription factor binding. When cells are then transferred to serine starvation conditions, Cha4 no longer recruits Swi/Snf and SAGA, resulting in decreased *SRG1* transcription. Without intergenic transcription to maintain them, nucleosomes are depleted over the *SER3* UAS allowing transcription factors, either an as yet unknown site-specific DNA binding activator or possibly TBP and RNA Pol II, to bind and activate *SER3*. Two positioned nucleosomes remain at the 5' end of *SRG1* where they are likely to inhibit *SRG1* transcription.

In addition to its role in nucleosome assembly during transcription, Spt6 has also been reported to reassemble nucleosomes at the promoters of *PHO5* and several other yeast genes during repression (Adkins and Tyler 2006). Therefore, an alternative model for *SER3* repression is that Spt6, and possibly Spt16, reassemble nucleosomes over the *SER3* promoter independently of *SRG1* transcription. Thus, mutations in these factors may bypass the normal role for *SRG1* transcription, which is to interfere with the recruitment of chromatin remodeling factors needed to displace the repressive nucleosomes at the *SER3* promoter. A prediction of this model is that the increased levels of *SER3* expression observed in the *spt6-1004* and *spt16-197* mutants would no longer be dependent on sequence-specific activators to recruit chromatin remodeling factors, analogous to what has been observed for *PHO5* (Adkins and Tyler 2006). To test this alternative model, we first identified a 37-bp sequence within the *SER3* promoter (from -192 to -228; *SER3* ATG=+1) that when deleted, is required for *SER3* activation in response to serine starvation

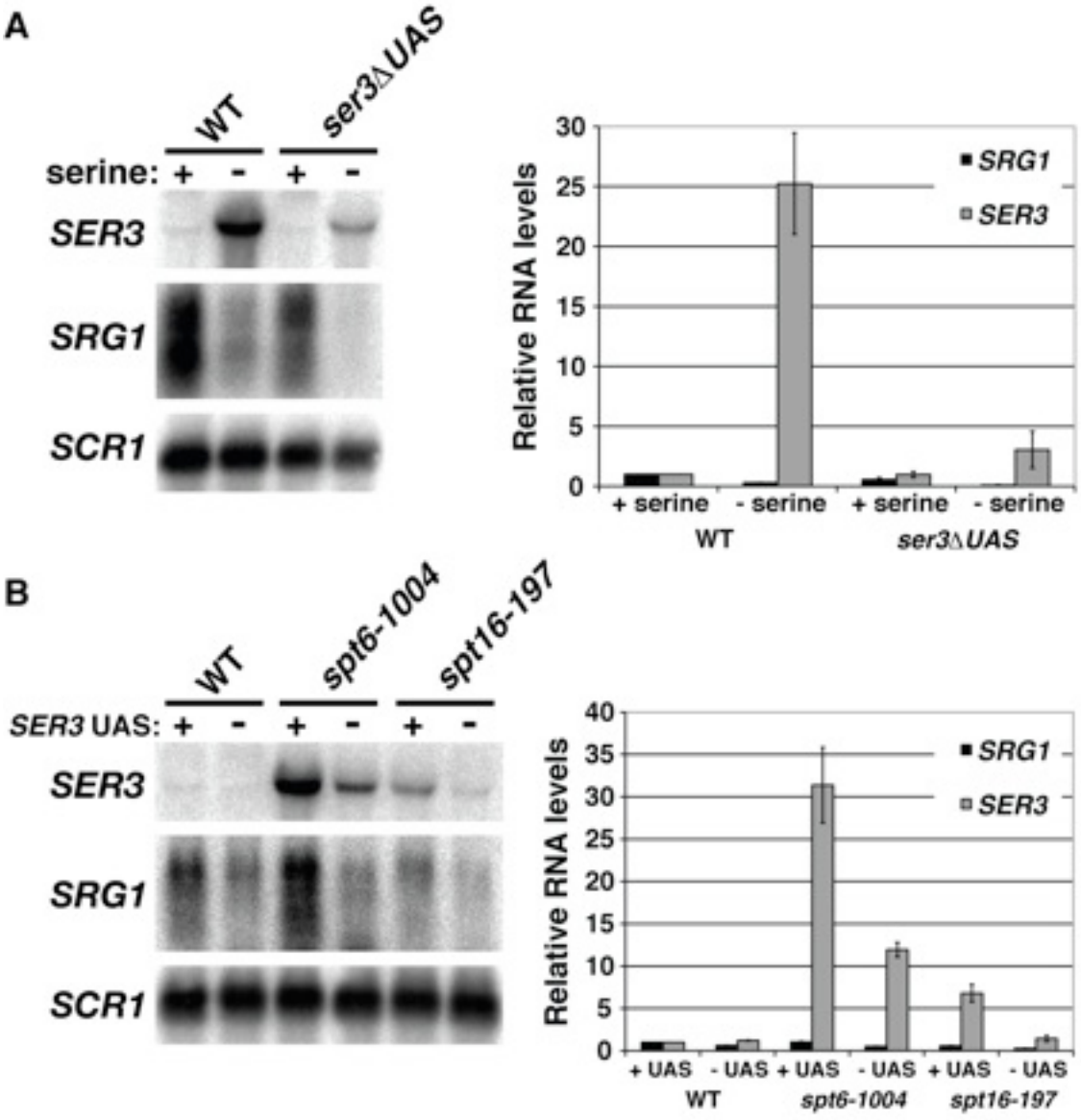
A
WT
ser3ΔUAS
serine: + - + -
SER3
SRG1
SCR1
Relative RNA levels
30
25
20
15
10
5
0
SRG1
SER3
+ serine
- serine
+ serine
- serine
WT
ser3ΔUAS
B
WT
spt6-1004
spt16-197
SER3 UAS: + - + - + -
SER3
SRG1
SCR1
Relative RNA levels
40
35
30
25
20
15
10
5
0
SRG1
SER3
+ UAS
- UAS
+ UAS
- UAS
+ UAS
- UAS
WT
spt6-1004
spt16-197

Figure 26: Identification of promoter sequence required for *SER3* activation.

A) Northern analysis (left panel) of *SER3*, *SRG1*, and *SCR1* (loading control) was performed on wild-type (KY719) and *ser3ΔUAS* (YJ947) strains that were grown at 30°C in SC+serine media (+ serine) and then shifted to SC-serine for 25 minutes (- serine). The *ser3*Δ*UAS* allele replaces a 37bp of the *SER3* promoter (–228 to -198; *SER3* ATG=+1) with an *Avr*II restriction enzyme site. The bar graph (right panel) summarizes *SER3* and *SRG1* RNA levels (normalized to *SCR1*) of three independent experiments. Each bar represents the mean +/- SEM of either *SRG1* or *SER3* RNA levels relative to wild-type cells that were grown in serine-rich media, which was arbitrarily set to 1. B) *SER3* activation in *spt6-1004* and *spt16-197* is impaired in the absence of *SER3* UAS. Northern analysis (left panel) of *SER3*, *SRG1*, and *SCR1* (loading control) was performed on wild-type, *spt6-1004*, and *spt16-197* strains expressing either wild-type *SER3* (YJ950, YJ958, YJ966) or the *ser3*Δ*UAS* allele (YJ954, YJ962, YJ970) that were grown in YPD at 30°C. The bar graph (right panel) summarizes *SER3* and *SRG1* RNA levels (normalized to *SCR1*) of four independent experiments. Each bar represents the mean +/- SEM of either *SRG1* or *SER3* RNA levels relative to wild type cells that were grown in serine-rich media, which was arbitrarily set to 1.

(Figure 26A). When this sequence was deleted in the *spt6-1004* and *spt16-197* strains, *SER3* mRNA levels were reduced as compared to similar strains expressing wild-type *SER3* (Figure 26B). Also, nucleosomes were still lost in the *ser3*Δ*UAS* mutant after shifting to low serine (S. Hainer, unpublished). Therefore, *spt6-1004* and *spt16-197* mutations do not bypass the requirement of the *SER3 UAS* for *SER3* activation, which argues against this alternative model.

Although MNase accessibility has been extensively used to predict nucleosome occupancy in eukaryotic organisms (for examples see (Yuan et al. 2005; Lee et al. 2007)), we cannot rule out the possibility that DNA-binding proteins may contribute to the protection of the *SER3* promoter from MNase digestion in serine-rich conditions. However, our observation that MNase protection over the *SER3* promoter was reduced in *spt6-1004* and *spt16-197* mutants without affecting RNA Pol II occupancy suggests that at least RNA Pol II and its associated factors do not affect MNase digestion.

If *SRG1* transcription from intergenic DNA is required to maintain nucleosomes over the *SER3* UAS, then from where might these nucleosomes originate? An intriguing source of these nucleosomes would be those positioned over the *SRG1* transcription start site and TATA (Figure 24), which likely inhibit *SRG1* transcription in the absence of serine. Based on this study and our previous work (Martens and Winston 2002; Martens et al. 2004; Martens et al. 2005), Swi/Snf, when recruited to the *SRG1* promoter in response to serine, may slide these nucleosomes toward *SER3*, to facilitate pre-initiation complex assembly and *SRG1* transcription. Once RNA Pol II begins to transcribe *SRG1*, the nucleosomes originally moved by Swi/Snf are disassembled to allow passage of RNA Pol II and then reassembled behind RNA Pol II by Spt6 and Spt16. Therefore, the activities of Swi/Snf, Spt6/Spn1, and FACT may combine to establish and maintain nucleosomes over the *SER3* promoter, which interfere with transcription factor

binding to this region. This scenario would also explain the difference in nucleosome occupancy at the 5' end of *SRG1* observed for wild-type cells grown in the serine starvation media as compared to *srg1-1* cells grown in serine-rich media, two conditions in which *SER3* is strongly derepressed (Figures 20 and 21A). In contrast to wild-type cells grown in serine starvation medium where it is no longer recruited, Swi/Snf is presumably still recruited by Cha4 in the *srg1-1* (*SRG1* TATA mutant) cells that are grown in serine-rich media. Thus, Swi/Snf can remodel the nucleosomes at the 5' end of *SRG1*; however, these nucleosomes cannot be maintained in the absence of *SRG1* transcription.

In addition to the nucleosome reassembly activity of Spt6/Spn1 and FACT, it has been well documented that a cascade of transcription-dependent post-translational modifications of histones found within nucleosomes over protein-coding genes contribute to the repression of intragenic transcription initiation (Lee and Shilatifard 2007; Li et al. 2007a). However, our studies show that *SER3* repression appears to be independent of at least some of these marks, including Set1-mediated methylation of histone H3 K4, Set2-mediated methylation of K36, and the removal of histone H3 and H4 acetylation by the Rpd3S and Set3C histone deacetylase complexes. Although we cannot rule out the possibility that other post-translational histone modifications may be involved, our results indicate a difference in the requirement of transcription-dependent post-translational histone modifications between *SER3* repression by *SRG1* transcription and repression of cryptic intragenic transcription. This difference may be related to the fact that *SRG1* is a relatively short transcription unit (~400 base pairs) that is highly transcribed. It has been recently reported that cryptic intragenic transcription preferentially occurs at lowly transcribed genes (Li et al. 2007b; Cheung et al. 2008; Lickwar et al. 2009). Therefore, it is possible that highly transcribed *SRG1* may not be dependent on H3K36

methylation and subsequent histone deacetylation for protection from intragenic transcription because of the frequent passage of RNA Pol II. Alternatively, short, highly transcribed genes may never establish this histone mark since histone H3 K36 methylation predominates towards the 3' ends of transcribed genes (Pokholok et al. 2005). In support of this possibility, genome-wide analyses of K36 methylation indicate little K36 trimethylation at *SRG1* (Pokholok et al. 2005).

In contrast to the characteristic transcription-dependent depletion of nucleosomes seen at protein-coding genes (Yuan et al. 2005; Lee et al. 2007), we show transcription-dependent assembly of nucleosomes across intergenic *SRG1*. How does one account for this apparent contradiction between nucleosome occupancy and transcription? Several recent studies have indicated that DNA sequence can either favor or refract nucleosome formation thereby influencing genome-wide nucleosome positioning (Yuan et al. 2005; Ioshikhes et al. 2006; Segal et al. 2006; Peckham et al. 2007; Field et al. 2008; Kaplan et al. 2009). As has been proposed for yeast genes containing nucleosome-depleted promoter regions (Segal and Widom 2009), one possibility is that the underlying DNA sequence of the *SER3* promoter may normally disfavor nucleosome formation to facilitate transcription factor binding. Therefore, by reassembling nucleosomes after each passage of RNA Pol II, *SRG1* transcription effectively maintains nucleosomes over DNA that is normally refractory to nucleosomes. Several observations support this possibility. First, the *SER3* UAS region that is nucleosome-depleted in the absence of *SRG1* transcription contains poly(dA:dT) tracts; a sequence motif that resists bending and thus disfavors nucleosome formation (Segal and Widom 2009). Second, the *SER3* UAS sequence is predicted to have a low nucleosome-forming potential by an algorithm developed using comparative genomics (Ioshikhes et al. 2006). Finally, the *SER3* UAS sequence failed to form a

stable nucleosome in a genome-wide *in vitro* nucleosome reconstitution assay (Kaplan et al. 2009).

In *Saccharomyces cerevisiae*, cells respond to changes in serine availability by rapidly inducing or repressing transcription of *SER3*. This response involves a dynamic competition between nucleosomes and transcription factors that is controlled by the transcription of *SRG1* from intergenic ncDNA. Our findings raise the intriguing possibility that widespread transcription of ncDNA may impact genome-wide chromatin architecture. In doing so, transcription of ncDNA may influence not only gene expression, but also other cellular processes that are dependent on protein-DNA interactions.

4.0 THE ROLE OF THE PAF1 TRANSCRIPTION ELONGATION COMPLEX IN *SER3* REPRESSION

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4.1 INTRODUCTION

I identified three factors with important roles in transcription elongation as candidates required for *SER3* repression: Spt2, Bur2, and Spt4. I hypothesized that these elongation factors could affect *SER3* in a variety of ways. By associating with RNA Polymerase II during *SRG1* transcription, they could directly interfere with activator binding. Alternatively, they could affect the rate or processivity of Pol II, allowing for more efficient binding of transcription factors to the *SER3* promoter. Another possibility is that these factors may alter the chromatin structure during transcription, which we have demonstrated, is important for *SER3* repression. Indeed, Spt2 is required for maintaining nucleosomes over the *SER3* promoter (Thebault et al. 2011), similar to Spt6 and Spt16.

In this chapter, we set out to determine how Spt4 and Bur2 affect *SER3* expression. Spt4 partners with the essential factor Spt5 to form the yeast homolog of the mammalian DSIF complex (yDSIF) (Wada et al. 1998). yDSIF associates with Pol II over actively transcribed genes, has numerous physical and genetic interactions with other transcription factors, and facilitates elongation through chromatin (Hartzog et al. 1998; Squazzo et al. 2002; Rondon et al. 2003; Simic et al. 2003). Bur2 acts as a cyclin to activate the essential cyclin-dependent kinase Bur1, forming a partial functional homolog of mammalian P-TEFb (Yao et al. 2000; Wood and Shilatifard 2006). The Bur1/2 complex plays a variety of roles in transcription through the phosphorylation of substrates including the CTD of the RNA Pol II subunit Rpb1 (Murray et al. 2001; Qiu et al. 2009), the ubiquitin conjugating enzyme Rad6 (Wood et al. 2005), and the C-terminal repeat region of Spt5 (Liu et al. 2009; Zhou et al. 2009). Phosphorylation by Bur1/2 activates the Spt5 protein, which promotes the recruitment of the Paf1 complex to chromatin (Liu et al. 2009; Zhou et al. 2009).

The Paf1 complex is a conserved, multi-subunit complex that plays a number of important roles in the transcription cycle (Jaehning 2010). In higher eukaryotes, the Paf1 complex has important roles in embryonic development (Tenney et al. 2006; Akanuma et al. 2007; Wang et al. 2008), maintenance of stem cell fate (Ding et al. 2009), and tumorigenesis (Moniaux et al. 2006; Chaudhary et al. 2007; Lin et al. 2008). In *S. cerevisiae*, the Paf1 complex is comprised of five subunits, Paf1, Ctr9, Rtf1, Cdc73, and Leo1 (Shi et al. 1997; Krogan et al. 2002; Mueller and Jaehning 2002; Squazzo et al. 2002) that co-localize with RNA Pol II across transcribed genes exiting near the polyadenylation sites (Krogan et al. 2002; Pokholok et al. 2002; Kim et al. 2004; Mayer et al. 2010). During elongation, the Paf1 complex has been shown to promote histone modifications (Krogan et al. 2003; Ng et al. 2003a; Wood et al. 2003; Chu et

al. 2006), alter the phosphorylation state of the RNA Pol II carboxy-terminal domain (CTD) (Mueller et al. 2004; Nordick et al. 2008), and facilitate proper transcription termination (Mueller et al. 2004; Kaplan et al. 2005; Penheiter et al. 2005; Sheldon et al. 2005; Tomson et al. 2011). Co-localization of the Paf1 complex with RNA Pol II is dependent on the Bur1/Bur2 and Spt4/Spt5 transcription elongation factors (Laribee et al. 2005; Qiu et al. 2006; Liu et al. 2009; Zhou et al. 2009). Other factors, such as Spt6, FACT (composed of Spt16 and Pob3), and Ccr4-Not may also contribute to the recruitment of the Paf1 complex, but their roles are not clearly defined (Kaplan et al. 2005; Pavri et al. 2006; Mulder et al. 2007).

One of the primary functions of the Paf1 complex is to promote histone modifications associated with active transcription (Jaehning 2010). In yeast, the Paf1 complex promotes monoubiquitylation of histone H2B at lysine 123 (K123ub) by the ubiquitin conjugase Rad6 and ubiquitin ligase Bre1 (Ng et al. 2003a; Wood et al. 2003). Ubiquitylation of H2B is required for subsequent methylation of histone H3 at lysine 4 (K4me) and lysine 79 (K79me) by the Set1 and Dot1 methyltransferases, respectively (Ng et al. 2002; Sun and Allis 2002; Krogan et al. 2003; Ng et al. 2003a; Wood et al. 2003; Shahbazian et al. 2005). These modifications are predominantly dependent on the Rtf1 subunit of the Paf1 complex, specifically involving a central region of the protein termed HMD for histone modification domain (Warner et al. 2007; Tomson et al. 2011). Furthermore, the Paf1 and Ctr9 subunits are required for trimethylation of histone H3 at lysine 36 (K36me3) by the Set2 methyltransferase (Chu et al. 2007). Together, these modifications control histone acetylation across transcribed genes through the recruitment of histone deacetylases (Carrozza et al. 2005; Joshi and Struhl 2005; Keogh et al. 2005; Youdell et al. 2008; Kim and Buratowski 2009; Pinskaya et al. 2009).

Other connections of the Paf1 complex to chromatin have also been described. In yeast, Paf1 and Ctr9 were shown to affect the rate of induction of *GAL* genes by promoting nucleosome eviction from the *GAL1-10* promoter during activation (Marton and Desiderio 2008). The *Drosophila* Paf1 complex has been shown to facilitate recruitment of the Spt6 and FACT histone chaperones during transcription (Adelman et al. 2006). These studies establish the importance of the Paf1 complex in transcription through chromatin, but little is known about how the complex mediates this function.

In this chapter, I examine the role of the elongation factors Bur2, Spt4, and the Paf1 complex in *SRG1* transcription-dependent repression of *SER3*. I find *SER3* repression to be primarily dependent on the Paf1 and Ctr9 subunits of the Paf1 complex and provide genetic evidence suggesting that these factors act in a previously described pathway with the Bur1/Bur2 kinase/cyclin and the Spt4/Spt5 transcription elongation complex. My results indicate that while the Paf1 complex co-localizes with *SRG1* transcription, its absence did not reduce *SRG1* transcript levels, and its role in *SER3* repression is largely independent of its ability to orchestrate covalent histone modifications. Rather, I find that Paf1 and Ctr9 promote both nucleosome occupancy over actively transcribing *SRG1* and normal recruitment of Spt6 and Spt16 to this region. These results suggest that the Paf1 and Ctr9 subunits of the Paf1 complex repress *SER3* by facilitating *SRG1* transcription-dependent nucleosome occupancy of the *SER3* promoter, possibly by stabilizing the association of Spt6 and Spt16.

4.2 MATERIALS AND METHODS

4.2.1 Yeast strains and media

Saccharomyces cerevisiae strains used in this study are derivatives of a *GAL2*$^{+}$ strain of S288C (Winston et al. 1995) and are listed in Table 6. All experiments were performed on multiple independent strains, but, generally, only one representative strain of each is listed in the figure legends and strain table. Strains were created using standard genetic crosses or by transformation (Ausubel et al. 1991). Gene replacements of *PAF1*, *RTF1*, *CTR9*, *LEO1*, *CDC73*, *RKR1*, *SET2*, *RAD6*, *BRE1*, *BUR2*, and *HTA2-HTB2* with *KanMX*, *HIS3*, or *URA3* have been previously described (Braun et al. 2007; Chu et al. 2007; Crisucci and Arndt 2011; Tomson et al. 2011). Other alleles that have been previously described include: *spt4*Δ*1::URA3* (Swanson and Winston 1992), *spt4*Δ*2::HIS3* (Basrai et al. 1996), *spt5-194* (Winston et al. 1984), *bur2*Δ*2::URA3 (Chu et al. 2006)*, *HTA1-htb1*$_{K123R}$ (Tomson et al. 2011), and *rtf1Δ100::URA3* (Stolinski et al. 1997), *set2-1-261* (Youdell et al. 2008) and the epitope-tagged versions of *PAF1*, *CTR9*, *LEO1*, *CDC73*, *RTF1*, *SPT6*, and *RPB3* (Squazzo et al. 2002; Kaplan et al. 2003; Warner et al. 2007). Yeast cells were grown at 30°C in YPD media containing 1% yeast extract, 2% peptone, and 2% glucose or synthetic complete media lacking uracil (Sc-URA) (Rose 1991).

Table 6: *S. cerevisiae* strains used in Chapter 4.

Name*	Genotype
FY4	*MAT***a**
FY5	*MATα*
KY399	*MATα rtf1Δ100::URA3 leu2Δ1 ura3-52 trp1Δ63*
KY628	*MAT***a** *chd1Δ::URA3 his4-192δ leu2Δ1 ura3-52*
KY629	*MATα chd1Δ::URA3 his4-192δ trp1Δ63 ura3-52 lys2-128δ*
KY630	*MAT***a** *chd1Δ::URA3 lys2-128δ leu2Δ1 ura3-52*
KY631	*MATα chd1Δ::URA3 his4-192δ his3Δ200 trp1Δ63 ura3-52 lys2-128δ*
KY716	*MATα his3Δ200 lys2-128δ ura3-52 spt5-194*
KY735	*MATα his4-912δ lys2-128δ leu2Δ1 ura3-52 arg4-12 spt4Δ1::URA3*
KY785	*MAT***a** *his4-912δ lys2-128δ leu2Δ(0* or *1) trp1Δ63 CTR9-6xMYC::LEU2 LEO1-3xHA::HIS3 SPT5-FLAG*
KY786	*MAT***a** *his4-912δ lys2-128δ leu2Δ(0* or *1) ura3-52 trp1Δ63 CTR9-6xMYC::LEU2 3xHA-CDC73::URA3 SPT5-FLAG*
KY1349	*MAT***a** *bur2Δ2::URA3 his4-912∂ lys2-128∂ suc2::UAS (-1900/-390) ura3-52 trp1Δ63*
KY1451	*MATα paf1Δ::KanMX bur2Δ::KanMX*
KY1467	*MATα set2Δ::HIS3 his3Δ200 lys2-173R2 leu2Δ1 ura3-52 trp1-63 ade8*
KY1700	*MATα paf1Δ::KanMX*
KY1703	*MAT***a** *rtf1Δ::KanMX*
KY1706	*MATα cdc73Δ::KanMX*
KY1712	*MATα rad6Δ::KanMX*
KY1713	*MAT***a** *bre1Δ::KanMX*
KY1721	*MATα 3xHA-PAF1*
KY1805	*MATα leo1Δ::KanMX*
KY1865	*MATα set2-1-261::13MYC::KanMX his3Δ200 leu2Δ0 ura3Δ0*
KY2082	*MATα ura3-52 lys2-128δ leu2Δ1 trp1Δ63 3xHA-RTF1*
KY2167	*MATα HTA1-htb1*$_{K123R}$ *(hta2-htb2)Δ::KanMX ura3Δ0*
KY2170	*MAT***a** *ctr9Δ::KanMX leu2Δ1*
KY2172	*MATα (hta2-htb2)Δ::KanMX ura3Δ0*
MBY21	*MAT***a** *rkr1Δ::KanMX his3Δ200 leu2Δ1*
MBY24	*MAT***a** *rkr1Δ::KanMX lys2-128δ leu2Δ1 ura3-52*
MBY30	*MAT***a** *rkr1Δ::KanMX ura3-52*
MBY32	*MATα rkr1Δ::KanMX lys2-128δ ura3-52*
YJ586	*MATα ura3Δ0 leu2Δ0 his3Δ200*
YJ759	*MAT***a** *ctr9Δ::KanMX leu2Δ0 ura3Δ0 his3Δ0 lys2Δ0*
YJ771	*MATα ura3Δ0*
YJ786	*MATα paf1Δ::KanMX ura3Δ0 his3Δ200 lys2Δ0*
YJ882	*MATα ura3-52* or *ura3Δ0 lys2Δ0* or *lys2-128δ his3Δ200 leu2Δ0* or *leu2Δ1 RPB3-3HA::LEU2 SPT6-FLAG*
YJ1013	*MAT***a** *ura3-52* or *ura3Δ0 lys2Δ0* or *lys2-128δ leu2Δ0* or *leu2Δ1 RPB3-3HA::LEU2 SPT6-FLAG rtf1Δ::KanMX*
YJ1014	*MAT***a** *ura3-52 or ura3Δ0 lys2Δ0* or *lys2-128δ leu2Δ0* or *leu2Δ1 his3Δ200 RPB3-3HA::LEU2 SPT6-FLAG rtf1Δ::KanMX*
YJ1016	*MAT***a** *ura3-52* or *ura3Δ0 lys2Δ0* or *lys2-128δ leu2Δ0* or *leu2Δ1 his3Δ200 RPB3-3HA::LEU2 SPT6-FLAG ctr9Δ::KanMX*
YJ1030	*MAT***a** *his4-192δ trp1Δ63* (*ura3-52 or URA3) lys2-128δ leu2Δ1 SPT6-FLAG RPB3-3HA::LEU2 paf1Δ::URA3*
YJ1031	*MATα his4-192δ trp1Δ63* (*ura3-52* or *URA3) lys2-128δ leu2Δ1 SPT6-FLAG RPB3-3HA::LEU2 paf1Δ::URA3*
YJ1087	*MAT***a** *ura3Δ0 pRS416*

* FY and KY/MBY strains were kindly provided by Fred Winston and Karen Arndt, respectively

4.2.2 Plasmids

pRS316-HA-PAF1 is a *URA3*-marked, *CEN/ARS* plasmid expressing an HA-epitope tagged version of *PAF1*. pAP10 is a *URA3*-marked, *CEN/ARS* plasmid expressing *CTR9*. Both plasmids were kind gifts from K. Arndt (University of Pittsburgh). pRS416 is a *URA3*-marked *CEN/ARS* plasmid (Brachmann et al. 1998).

4.2.3 Northern analysis

Total RNA was isolated from cells grown to 1-2x10^7 cells/mL and separated on a 1% formaldehyde-agarose gel as described previously (Ausubel et al. 1991). RNA was transferred to Gene Screen membrane (Perkin-Elmer) and hybridized with radiolabeled probes generated by random-prime labeling of PCR fragments that were amplified from the following genomic sequences: *SRG1* (ChrV: 322258-322559), *SER3* (ChrV: 324059-324307), and *SCR1* (ChrV: 441741-442266), which was used as a control for RNA loading.

4.2.4 Chromatin immunoprecipitation

Cells were grown in YPD at 30°C to a density of 1-2x10^7 cells/mL and then treated with 1% formaldehyde for 20 min. Chromatin was isolated and sonicated as previously described (Shirra et al. 2005) and then incubated with antibodies overnight at 4°C. Anti-Flag M2 agarose (30µL, Sigma, A2220) was used to immunoprecipitate Spt6-FLAG. Anti-HA (1µL, Santa Cruz Biotechnology, sc-7392) was used to immunoprecipitate HA-Paf1, HA-Rtf1, Leo1-HA, HA-Cdc73, and Rpb3-HA. Anti-Myc (1µL, Santa Cruz Biotechnology, sc-789), anti-Spt16 (1µL,

kind gift from T. Formosa, University of Utah), and anti-histone H3 (5µL, Abcam, ab1791) were used to immunoprecipitate Ctr9-MYC, Spt16 and histone H3, respectively. Primary antibody-protein conjugates were isolated by incubating with 30µL Protein A or Protein G-coupled sepharose beads (GE Healthcare) at 4°C for 2-3 hours. After purifying DNA through PCR purification columns (Qiagen), the amount of immunoprecipitated (IP) DNA relative to input DNA was determined by qPCR and then normalized to a control region on chromosome V that lacks open reading frames (No ORF) that has been previously described (Komarnitsky et al. 2000).

4.2.5 Nucleosome scanning assay

Nucleosome scanning assays were performed as previously described (Chapter 3 Materials and Methods) (Hainer et al. 2011). Briefly, cells were grown in YPD to 2-3x10^7 cells/mL at 30°C and then treated with 2% formaldehyde followed by 300mM glycine. 1.2x10^9 cells were spheroplasted with Zymolyase 20T (Seikagaku Biobusiness), divided into six aliquots, which were then incubated with increasing concentrations of micrococcal nuclease (MNase, Nuclease S7, Roche). DNA was extracted, treated with RNase A, and subjected to gel electrophoresis and qPCR to determine extent of MNase digestion. Well-characterized regions of the *GAL1* promoter, one bound by a nucleosome (NB) and another nucleosome-free (NUB) were used as controls (Lohr 1984; Brickner et al. 2007; Floer et al. 2010). The samples in which the concentration of MNase yielded mostly mononucleosome-sized fragments and the NUB/NB ratio was less than 15% were then subjected to further qPCR analyses using primer pairs that amplify ~100bp fragments that tile the *SER3* locus. The amount of amplification for each *SER3*

primer pair (*SER3*-7 to *SER3*-41) in the digested sample was made relative to the undigested sample and normalized to the *GAL1* NB region.

4.2.6 Quantitative real-time PCR

Results of nucleosome scanning and ChIP assays were analyzed using ABI 7300 or StepOnePlus real-time PCR systems and SYBR green reagents (Fermentas). Primer sets used in ChIP and nucleosome scanning experiments are listed in Table 5. Quantitation of real-time PCR results was performed using the Pfaffl method (Pfaffl 2001).

4.2.7 Western blot analysis

Whole cell extracts (WCE) were prepared from cells grown in YPD at 30°C to 1-2 x 10^7 cells/mL using trichloroacetic acid as previously described (Cox et al. 1997; Zheng et al. 2010). Equal volumes of WCE were separated by 10% acrylamide SDS-PAGE, transferred to nitrocellulose (Whatman), and immunoblotted with anti-FLAG antibody (Sigma, F3165) or anti-Spt16 antibody (kind gift from T. Formosa, University of Utah). After incubation with anti-mouse (FLAG) or anti-rabbit (Spt16) HRP-conjugated secondary antibody (GE Healthcare), the immunoreactive proteins were visualized by enhanced chemiluminescence detection (Perkin-Elmer) using a Kodak Image Station 440CF. Blots were then stripped and re-probed with anti-G6PDH antibody (Sigma, A9521) as a loading control. Quantitation of Spt6-FLAG and Spt16 protein levels was determined by measuring the signal intensities using the Kodak 1D 3.6 software. Spt6-FLAG and Spt16 signals were made relative to signal from the G6PDH loading control and normalized to wild-type signal, which was set to 1.

4.3 RESULTS

4.3.1 Spt4, Spt5, and Bur2 are required to repress *SER3* transcription through a pathway involving Paf1

In order to determine a role for the Spt4/5 and Bur2 transcription elongation factors in *SER3* repression, I measured *SER3* transcript levels in strains lacking functional copies of Spt4, Spt5, Bur2, and the Paf1 subunit of the Paf1 complex (Figure 27). In agreement with the results from my genetic screen, both *spt4*Δ and *bur2*Δ mutants strongly derepress *SER3*, with the *spt4*Δ mutant being slightly more defective in *SER3* repression. A temperature sensitive mutation of the essential *SPT5* gene, *spt5-194*, not only derepresses *SER3* at a non-permissive temperature (39°C; data not shown), as had been previously shown (Davis and Ares 2006), but also at a permissive temperature (30°C; Figure 27). Strains lacking Paf1 exhibited increased *SER3* transcript levels similar to those observed in the *bur2*Δ and *spt5-194* strains. This result agrees with previous microarray data showing increased *SER3* levels in a *paf1*Δ strain (Penheiter et al. 2005). Consistent with these factors working in the same pathway, a *paf1*Δ *bur2*Δ double mutant derepressed *SER3* to a level that is equivalent to either *paf1*Δ or *bur2*Δ single mutants (Figure 27). Importantly, *SRG1* transcript levels are not dramatically reduced, indicating that *SER3* repression in these mutants cannot be explained solely by reduced *SRG1* transcription. Taken together, these results show that Paf1 is required for *SER3* repression, likely involving its recruitment to *SRG1* by Bur1/2 and Spt4/5 as has been seen at other transcribed regions (Liu et al. 2009; Zhou et al. 2009).

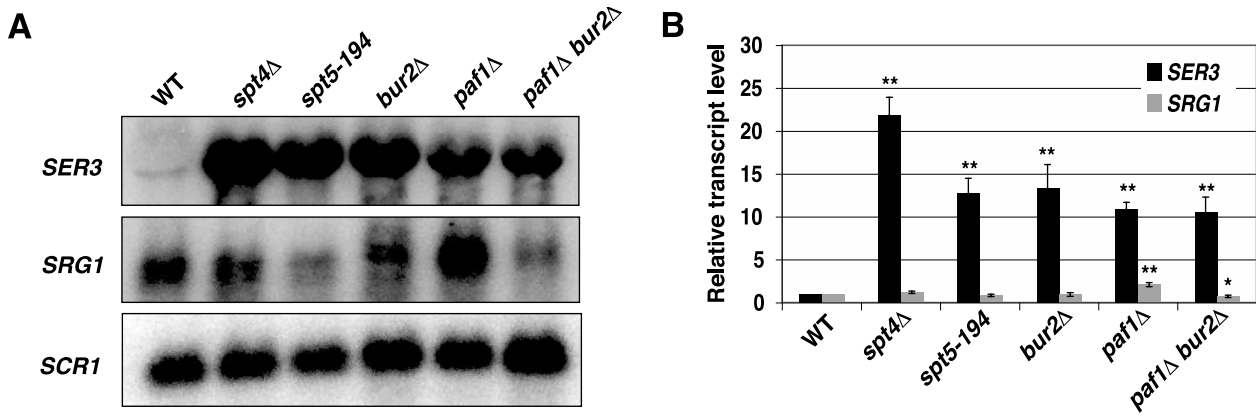

Figure 27: Spt4, Spt5, Bur2, and Paf1 are required for *SER3* repression.

A) Representative Northern analysis of *SER3*, *SRG1*, and *SCR1* (loading control) RNA levels in wild-type (FY4), *spt4*Δ (KY735), *spt5-194* (KY716), *bur2*Δ (KY1349), *paf1*Δ (KY1700), and *paf1*Δ *bur2*Δ (KY1451) strains. B) Quantitation of results from a minimum of four biological replicates. The values shown are the mean *SER3* (black) and *SRG1* (gray) transcript levels that have been normalized to the *SCR1* loading control and made relative to the wild-type strains. Error bars indicate the standard error from the mean (SEM) and asterisks indicate statistical significance determined by pairwise comparisons between wild type and mutant using a two-tailed students t-test (* $P < 0.05$, ** $P < 0.01$).

4.3.2 *SER3* repression by the Paf1 complex depends primarily on the Paf1 and Ctr9 subunits

To further investigate the requirement for Paf1 complex subunits in *SER3* repression, I examined *SER3* and *SRG1* transcript levels from mutant strains that each lack one of the five subunits of the Paf1 complex (Figure 28). These experiments revealed strong derepression of *SER3* (8- to 10-fold) in *paf1*Δ and *ctr9*Δ mutants as compared to wild-type strains. Complementation of these strains with plasmid-borne copies of wild type *PAF1* and *CTR9,* respectively, restores *SER3* repression (Figure 28C and 28D). More modest defects in *SER3* repression (2- to 3-fold increases in *SER3* transcript levels) were seen in *leo1*Δ, *cdc73*Δ, and *rtf1*Δ strains (Figure 28B). Whereas the Paf1 complex has been shown to facilitate transcription elongation (Rondon et al. 2004; Pavri et al. 2006; Chen et al. 2009; Jaehning 2010; Kim et al. 2010; Tous et al. 2011), it is important to note that the *paf1*Δ and *ctr9*Δ strains did not exhibit reduced *SRG1* RNA levels. Rather, *SRG1* levels are increased 2-fold in these mutants. However, these increases in *SRG1* RNA levels are unlikely to impact *SER3* levels as *cdc73*Δ strains also exhibit higher *SRG1* levels, but show only a modest increase in *SER3* levels. *SRG1* levels show some variability in *leo1*Δ and *rtf1*Δ strains, but when multiple experiments are averaged, there is no difference from wild-type levels (Figure 28B). We conclude from these data that Paf1 complex-mediated repression of *SER3* occurs primarily through the activities of the Paf1 and Ctr9 subunits by a mechanism that does not involve the control of *SRG1* transcript levels.

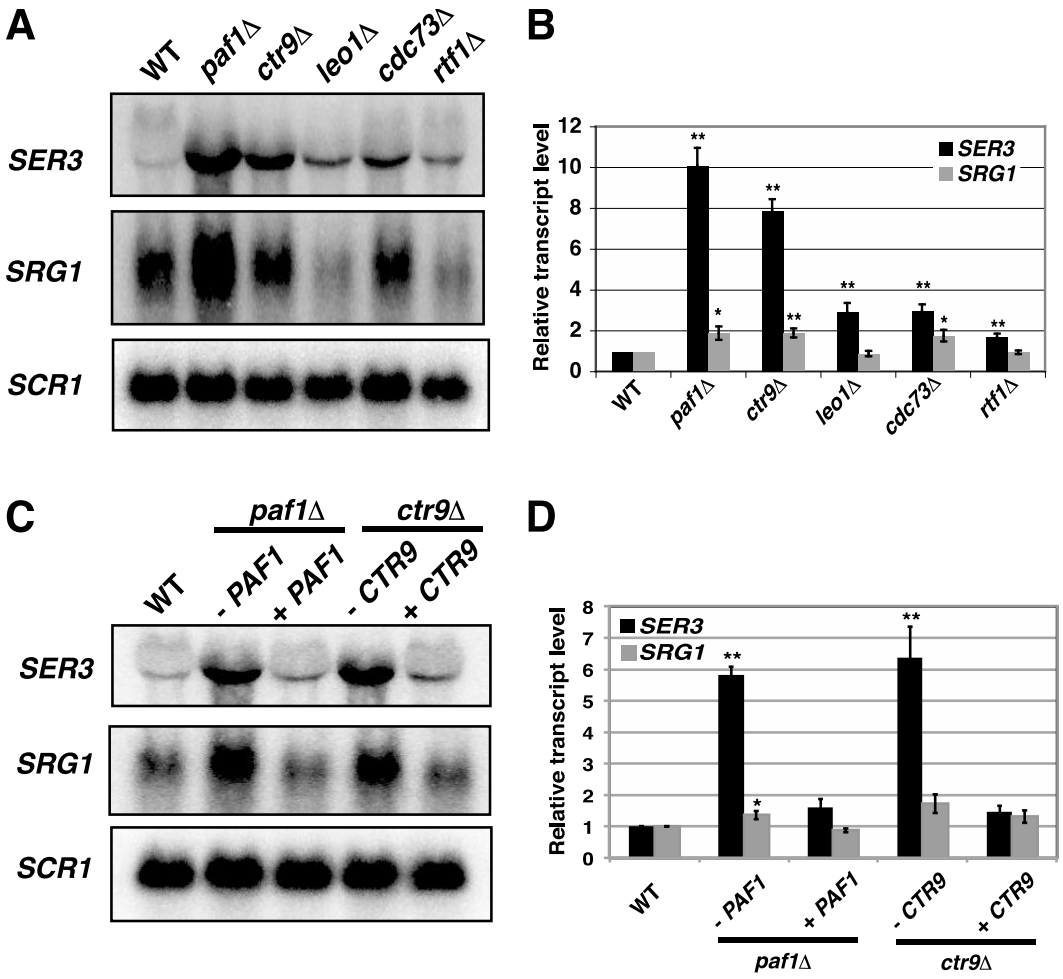

Figure 28: The Paf1 complex repression of *SER3* is primarily mediated by Paf1 and Ctr9.

A) Representative Northern analysis of *SER3*, *SRG1*, and *SCR1* (loading control) RNA levels in wild-type (FY5), *paf1*Δ (KY1700), *ctr9*Δ (KY2170), *leo1*Δ (KY1805), *cdc73*Δ (KY1706), and *rtf1*Δ (KY1703) strains. B) Quantitation of results from a minimum of seven biological replicates. The values shown are the mean *SER3* (black) and *SRG1* (gray) transcript levels that have been normalized to the *SCR1* loading control and made relative to the wild-type strains. Error bars indicate SEM and asterisks indicate statistical significance compare to wild type (* P <0.05, ** P <0.01). C) Representative Northern analysis of *SER3*, *SRG1*, and *SCR1* (loading control) RNA levels. The wild-type strain (YJ1087) has been transformed with a control plasmid

(pRS416). The *paf1*Δ strain (YJ786) has been transformed with either pRS416 (-*PAF1)* or pRS316-HA-PAF1 (+*PAF1*). The *ctr9*Δ (YJ759) strain has been transformed with either pRS416 (-*CTR9)* or pAP10 (+*CTR9*). D) Quantitation of results from a minimum of three biological replicates. The values shown are the mean *SER3* (black) and *SRG1* (gray) transcript levels that have been normalized to the *SCR1* loading control and made relative to the wild-type strains. Error bars indicate SEM and asterisks indicate statistical significance compared to wild type (* $P < 0.05$, ** $P < 0.01$).

4.3.3 *SER3* repression is largely independent of known Paf1 complex-dependent histone modifications

Several studies have indicated a role for the Paf1 complex in mediating transcription-dependent post-translational modifications of histone proteins. These include H2B K123ub by the Rad6 ubiquitin conjugase and Bre1 ubiquitin ligase and subsequent methylation of H3 K4 and K79 by the Set1 and Dot1 histone methyltransferases and the methylation of K36 by Set2 (Ng et al. 2002; Wood et al. 2003; Shahbazian et al. 2005; Chu et al. 2007). Previously, we have shown by Northern analysis that either the deletion of the methyltransferases responsible for methylation of K4, K36 and K79 of histone H3 (Chapter 3.3.6) or the mutation of these lysine residues to alanines has little to no effect on *SER3* repression (Hainer and Martens 2011a; Hainer et al. 2011). K36 methylation by Set2 is largely regulated by the Paf1 and Ctr9 subunits of the Paf1 complex, which are required for the transition from dimethylation to trimethylation. Therefore, in *paf1*Δ or *ctr9*Δ strains only trimethylation is lost while mono and dimethylation remain largely intact (Chu et al. 2007). To mimic this effect and test for a role for K36 trimethylation specifically, I tested a *SET2* truncation allele, *set2-1-261,* that lacks trimethylation activity but retains mono and dimethylation activity (Youdell et al. 2008). This strain shows no derepression of *SER3* further suggesting a role for the Paf1 complex outside of regulating histone methylation (Figure 29).

Despite there being no role for the downstream methylation marks, it is possible that the upstream H2B K123ub does regulate *SER3* repression. Therefore, we assayed the effect of histone H2B K123ub on *SER3* repression. Northern analyses revealed only modest increases in *SER3* expression in *rad6*Δ (2-fold) and *bre1*Δ (1.5-fold) mutants (Figure 30). Similarly, a conservative mutation that replaces H2B lysine 123 with arginine, also results in less than a

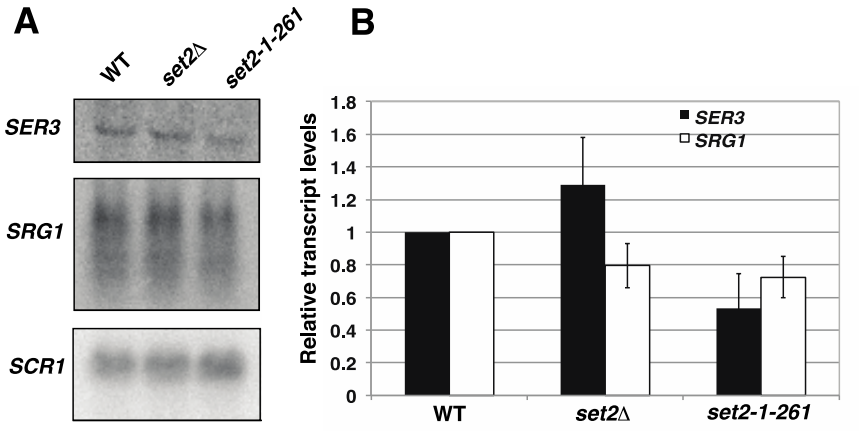

Figure 29: Specific loss of H3 K36 trimethylation has no effect on *SER3* levels.

A) Representative Northern analysis of *SER3*, *SRG1*, and *SCR1* (loading control) RNA levels in wild-type (YJ771), *set2*Δ (KY1467), and *set2-1-261* (KY1865) strains. B) Quantitation of results from a minimum of 4 biological replicates. The values shown are the mean *SER3* (black) and *SRG1* (white) transcript levels that have been normalized to the *SCR1* loading control and made relative to the wild-type strains. Error bars indicate SEM.

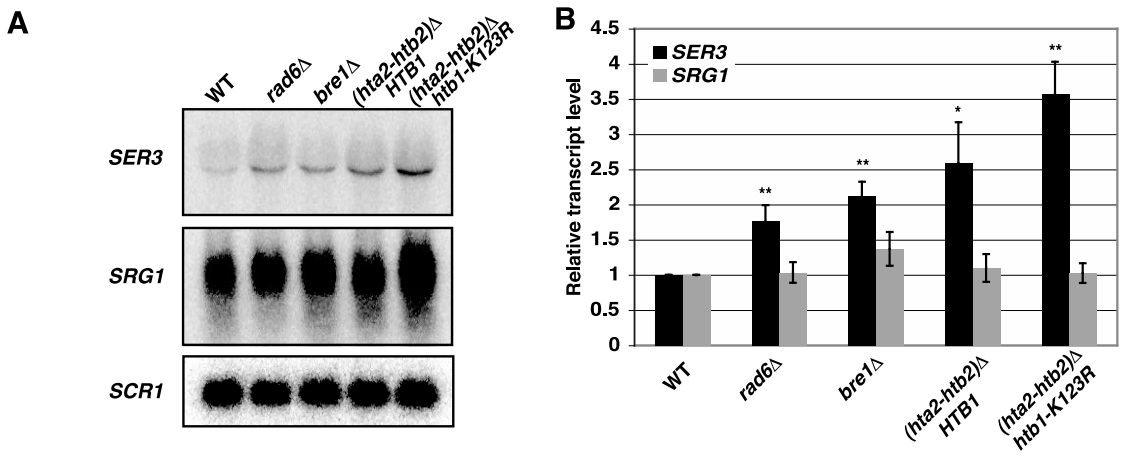

Figure 30: *SER3* repression is largely independent of histone H2B monoubiquitylation.

A) Representative Northern analysis of *SER3*, *SRG1*, and *SCR1* (loading control) RNA levels in wild-type (FY5) *rad6*Δ (KY1712), *bre1*Δ (KY1713), (*hta2-htb2*)Δ (KY2172), and (*hta2-htb2*)Δ *htb1-K123R* (KY2167) strains grown in YPD at 30°C. B) Quantitation of results from a minimum of three biological replicates. The values shown are the mean *SER3* (black) and *SRG1* (gray) transcript levels that have been normalized to the *SCR1* loading control and made relative to the wild-type strains. Error bars indicate SEM and asterisks indicate statistical significance compared to wild type (* $P < 0.05$, ** $P < 0.01$).

2-fold increase in *SER3* levels when compared to the relevant control strain lacking one copy of the histone H2A and histone H2B genes (compare *(hta2-htb2)*Δ strains expressing *HTB1* or *htb1-K123R*). These results are consistent with the minor defect in *SER3* repression that was observed for cells lacking Rtf1 (Figure 28), which has been previously shown to be the subunit primarily required for this modification (Ng et al. 2003a; Wood et al. 2003; Warner et al. 2007; Tomson et al. 2011). Taken together, the role of the Paf1 complex in *SER3* repression seems to be largely independent of its known roles in regulating histone modifications.

4.3.4 Some known functions of Rtf1 have little effect on *SER3* repression

It is clear that the effect of the Paf1 complex on *SER3* repression is largely mediated by Paf1 and Ctr9, while other subunits such as Rtf1 have little effect. Therefore, many of the known functions of the Paf1 complex that act through Rtf1, such as the histone modifications (Figures 29, 30), are unlikely to have much effect, but I tested some of them directly. Rtf1 is required for recruitment of Chd1, an ATP-dependent chromatin remodeling factor (Tran et al. 2000; Simic et al. 2003; Warner et al. 2007). Given the importance of chromatin structure in *SER3* repression, I tested if this remodeler affected *SER3* levels by altering chromatin structure (Figure 31A). Strains lacking *CHD1* had only a modest 2-fold effect on *SER3* levels by Northern analysis, consistent with the limited role of Rtf1, suggesting it is not important for *SER3* repression.

Another factor I tested was Rkr1, which is synthetically lethal with *rtf1*Δ when mutated (Costa and Arndt 2000; Braun et al. 2007). Rkr1 was shown to be a ubiquitin ligase with phenotypes and genetic interactions that suggested a role in chromatin and transcription (Braun et al. 2007). More recently, Rkr1 (Ltn1) was found to play an important role in the degradation of non-stop proteins, aberrant polypeptides produced through the mutation or read-through of

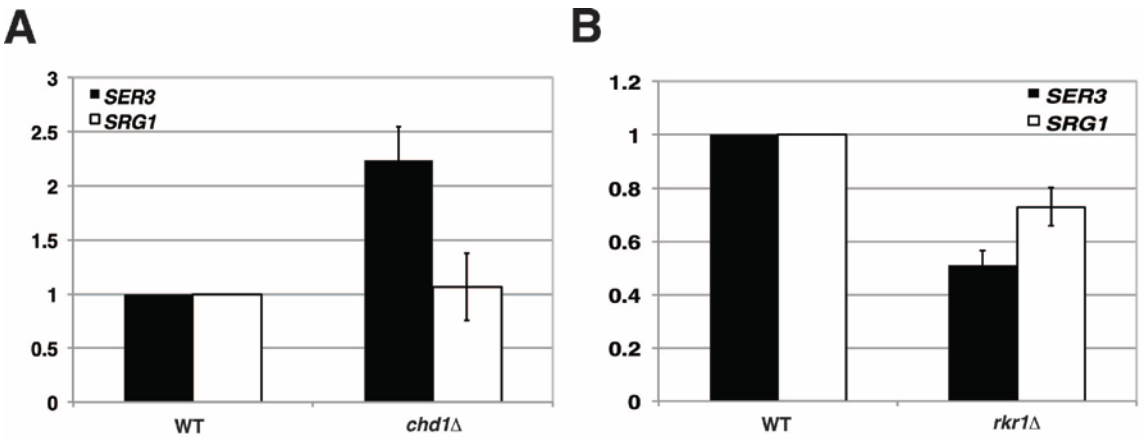

Figure 31: Effect of *chd1*Δ and *rkr1*Δ on *SER3* expression.

A) Quantitation of Northern analysis results showing relative *SER3* (Black) and *SRG1* (White) levels in WT (FY3) and *chd1*Δ (KY628, KY629, KY630, KY631) strains. B) Quantitation of Northern analysis results showing relative *SER3* (Black) and *SRG1* (White) levels in WT (YJ586) and *rkr1*Δ (MBY21, MBY24, MBY30, MBY32) strains. Error bars represent SEM for 4 independent strains.

normal translational stop codons (Bengtson and Joazeiro 2010). This new function would suggest the previously characterized roles of Rkr1 in transcription might be indirect effects of altered protein synthesis. Regardless, *rkr1*Δ strains have no increase of *SER3* levels by Northern analysis, suggesting Rkr1 is not involved in this mechanism (Figure 31B).

4.3.5 The Paf1 complex localizes to the *SER3* promoter when *SRG1* is transcribed

To test for a possible direct role in *SER3* repression, I performed ChIP experiments to determine if the Paf1 complex is physically associated with the *SER3* promoter when *SER3* is repressed. Cells expressing previously described epitope-tagged derivatives of Paf1, Rtf1, Leo1, Cdc73, and Ctr9 (Squazzo et al. 2002; Warner et al. 2007) were grown in serine-rich media. Chromatin was isolated from these cells after crosslinking with formaldehyde and then subjected to immunoprecipitation with antibodies that recognize the epitope tags. I detected strong occupancy of all Paf1 complex subunits specifically at the *SER3* promoter (Figure 32). This is likely a consequence of its co-localization with RNA Pol II over actively transcribed *SRG1*, similar to what has been previously described at other actively transcribed genes (Krogan et al. 2002; Pokholok et al. 2002; Squazzo et al. 2002). These results suggest that the Paf1 complex may directly contribute to the mechanism by which *SRG1* transcription represses *SER3* transcription.

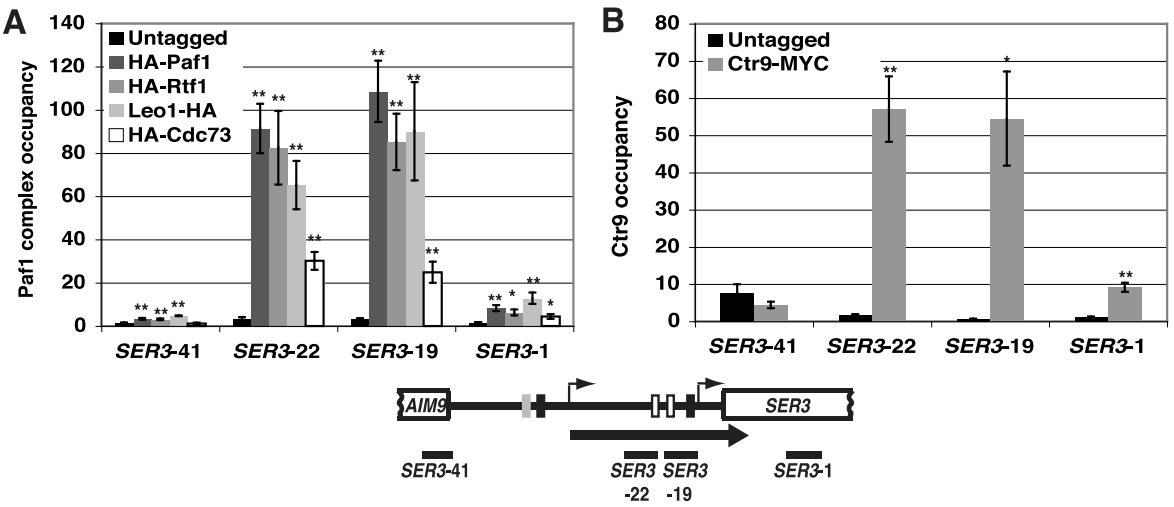

Figure 32: The Paf1 complex localizes with *SRG1* transcription during *SER3* repression.

A) ChIP analysis of HA-tagged Paf1 complex subunits at *SRG1* (*SER3*-22 and *SER3*-19) and the flanking *AIM9* (*SER3*-41) and *SER3* (*SER3*-1) genes from untagged (FY4), *3xHA-PAF1* (KY1721), *3xHA-RTF1* (KY2082), *LEO1-3xHA* (KY785), and *3xHA-CDC73* (KY786) strains grown in YPD at 30°C. B) ChIP analysis of Ctr9-Myc from untagged (KY399) and *CTR9-6xMYC* (KY785) strains grown in YPD at 30°C. The relative occupancies of these factors were calculated using qPCR as described in Materials and Methods. Each value represents the mean +/- SEM of three biological replicates and asterisks indicate statistical significance compared to the untagged control (* $P < 0.05$, ** $P < 0.01$). Below the graphs is a schematic of the *SRG1/SER3* locus with the arrows indicating the transcription start sites of *SRG1* and *SER3.* The gray box represents the Cha4 binding site, black boxes indicate TATA sequences, and white boxes are sequences required for *SER3* activation. The block arrow indicates *SRG1* transcription and the horizontal black bars mark the location of the DNA fragments amplified by qPCR.

4.3.6 Paf1 and Ctr9 are required for nucleosome occupancy over the *SER3* promoter

We have demonstrated an important role for chromatin structure in the repression of *SER3* (Chapter 3) (Hainer et al. 2011). Under conditions in which *SRG1* is transcribed and *SER3* is repressed, the *SER3* promoter is occupied by randomly positioned nucleosomes that prevent transcription factors from binding to this region. When *SRG1* is down-regulated, the *SER3* promoter region becomes nucleosome free, allowing transcription factors to bind and induce *SER3* expression (Hainer et al. 2011). These results support a promoter occlusion model whereby intergenic *SRG1* transcription deposits and maintains nucleosomes over the *SER3* promoter to mediate repression. To test whether the Paf1 complex contributes to *SER3* repression by affecting *SRG1* transcription-dependent nucleosome occupancy of the *SER3* promoter, I first performed nucleosome scanning assays on mutant strains that each lack one of the five subunits of the Paf1 complex (Figure 33A). Micrococcal nuclease (MNase) protection across *SER3* was determined by qPCR and normalized to the protection of a well-positioned nucleosome in the *GAL1* promoter whose digestion by MNase is unaffected in these mutants (data not shown). In wild-type cells (black line), I observe a broad peak of protection over the *SRG1* transcribed unit that overlaps the *SER3* promoter as we have previously reported (Hainer et al. 2011). Strikingly, the effects of these mutants on MNase protection across the *SRG1* transcription unit are consistent with their effects on *SER3* expression. For *paf1*Δ and *ctr9*Δ strains that exhibit strong *SER3* derepression, MNase protection across *SRG1* is significantly reduced. For *leo1*Δ, *rtf1*Δ and *cdc73*Δ mutants that more modestly derepress *SER3*, I observed more modest decreases in MNase protection across this region.

I also performed histone H3 ChIP assays on *paf1*Δ, *ctr9*Δ and *rtf1*Δ strains (Figure 33B). Consistent with my nucleosome scanning results, I detected reduced histone H3 occupancy

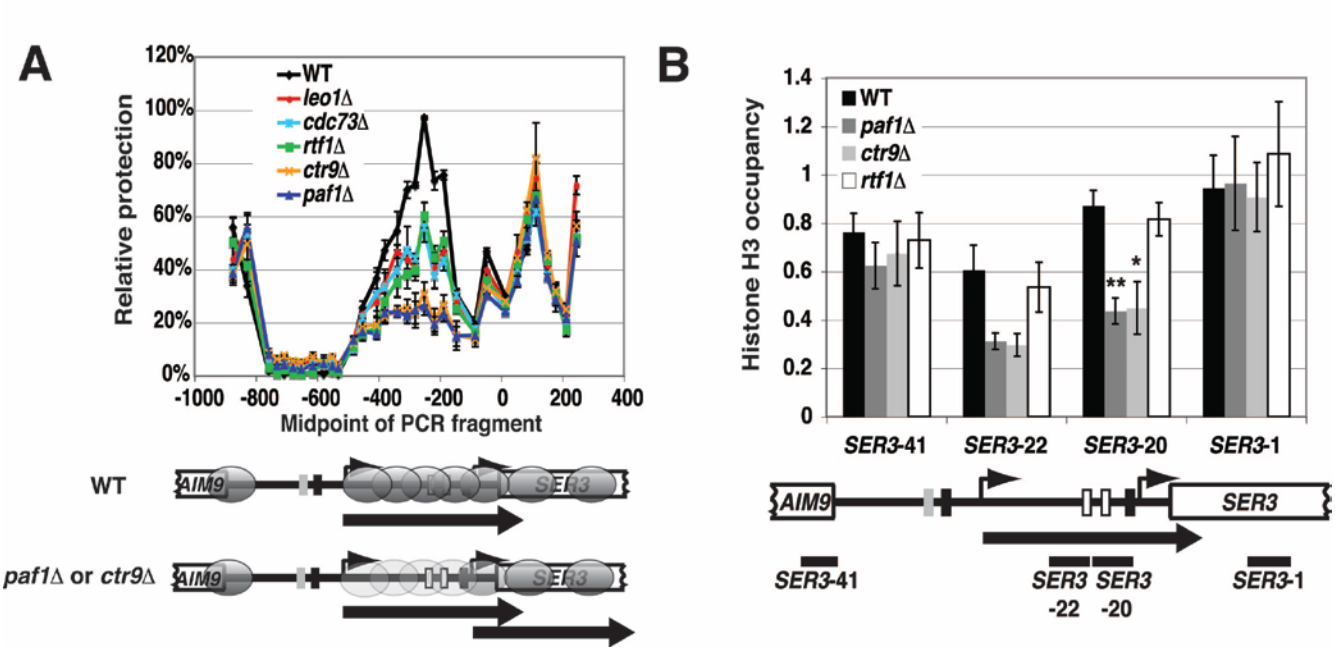

Figure 33: Paf1 and Ctr9 are required for nucleosome occupancy over the *SER3* promoter.

A) Nucleosome scanning assays were performed on wild-type (FY4), *leo1*Δ (KY1805), *cdc73*Δ (KY1706), *rtf1*Δ (KY1703), *ctr9*Δ (KY2170), and *paf1*Δ (KY1700) strains grown in YPD at 30°C. MNase protection across the *SER3* locus was calculated relative to a positioned nucleosome within the *GAL1* promoter using qPCR. The mean +/- SEM from three biological replicates is plotted at the midpoint for each PCR product. Shown below the graph is a diagram of the *SER3* locus comparing the positions of nucleosomes (gray ovals) extrapolated from the MNase protection data between wild-type and *ctr9*Δ or *paf1*Δ strains. The light gray ovals are indicative of reduced nucleosome occupancy compared to the darker ovals depicted for wild-type strains. B) ChIP analysis of histone H3 from wild-type (FY4), *paf1*Δ (YJ1030), *ctr9*Δ (YJ1016), and *rtf1*Δ (YJ1014) strains grown in YPD at 30°C. The relative occupancies of these factors were calculated as described in Materials and Methods. Each value represents the mean +/- SEM of at least three biological replicates and asterisks indicate statistical significance compared to

wild type (* P <0.05, ** P <0.01). The P-values for the decrease in H3 occupancy over primer set *SER3*-22 in *paf1*Δ and *ctr9*Δ strains were 0.07 and 0.06 respectively. Below the graphs is a schematic of the *SRG1/SER3* locus with the arrow indicating *SRG1* transcription and the black bars indicating the location of the DNA fragments amplified by qPCR.

specifically over the *SRG1* transcribed region in both *paf1*Δ and *ctr9*Δ strains, but not *rtf1*Δ strains, when compared to wild-type strains (Figure 33B, compare amplicons *SER3*-20 and *SER3*-22 to *SER3*-1 and *SER3*-41). Taken together, my nucleosome scanning and histone H3 ChIP results indicate that the Paf1 complex, primarily through the activities of the Paf1 and Ctr9 subunits, contributes to *SER3* repression by promoting *SRG1* transcription-dependent nucleosome occupancy across the *SER3* promoter.

4.3.7 Spt16 co-localization with RNA Pol II across *SRG1* is reduced in a *paf1*Δ strain

We have shown that the transcription-dependent nucleosome assembly mediating *SER3* repression requires the essential histone chaperones Spt6 and Spt16. Impairment of either factor reduces nucleosome occupancy over the *SER3* promoter and strongly derepresses *SER3*, even though *SRG1* transcription is maintained (Chapters 3.3.3 and 3.3.4) (Hainer et al. 2011). Given the importance of these factors in *SER3* repression and that a previous study in *Drosophila* provided evidence to support a role for the Paf1 complex in recruiting Spt6 and FACT to actively transcribed genes (Adelman et al. 2006), I performed ChIP assays to compare the occupancy of Spt6, Spt16 and the Rpb3 subunit of RNA Pol II at actively transcribed *SRG1* between wild-type, *paf1*Δ and *rtf1*Δ strains (Figure 34). Consistent with my Northern data, I found equivalently high levels of Rpb3 associating with actively transcribed *SRG1* that overlaps the *SER3* promoter (Figure 34C; amplicons *SER3*-19, 22) in all three strains. In wild-type cells, I detected strong Spt6 and Spt16 occupancy that co-localizes with Rpb3 across the *SRG1* transcription unit (Figure 34A and 34B). Whereas the occupancy of these factors was not significantly affected in an *rtf1*Δ mutant, I observed a 4- to 5-fold reduction in Spt16 occupancy over this region in a *paf1*Δ mutant while Spt6 occupancy was more moderately reduced (less than 2-fold). Notably, the

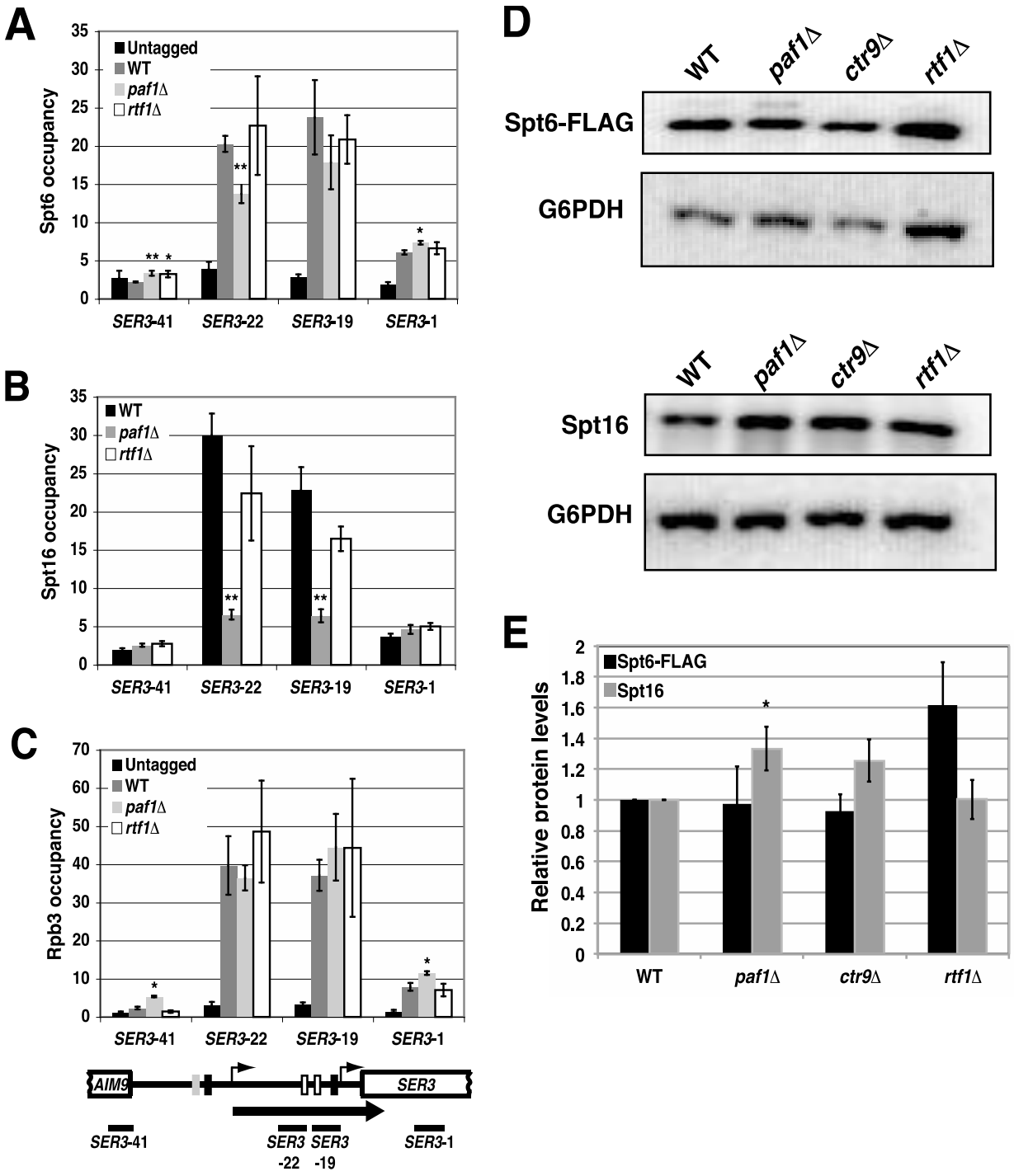
A
Spt6 occupancy
Untagged
WT
paf1Δ
rtf1Δ
SER3-41
SER3-22
SER3-19
SER3-1
B
Spt16 occupancy
C
Rpb3 occupancy
AIM9
SER3
D
WT
paf1Δ
ctr9Δ
rtf1Δ
Spt6-FLAG
G6PDH
Spt16
E
Relative protein levels
Spt6-FLAG
Spt16

Figure 34: Paf1 is required for Spt6 and Spt16 co-localization with actively transcribed *SRG1*.

ChIP of Spt6-FLAG (A) Spt16 (B) and Rpb3-HA (C) from untagged (FY4) strains and wild-type (YJ882), *paf1*Δ (YJ1031), and *rtf1*Δ (YJ1013) strains that all express epitope-tagged versions of Spt6 (*SPT6-FLAG*) and Rpb3 (*RPB3-3xHA*). The relative occupancies of these factors were calculated using qPCR as described in Materials and Methods. Each value represents the mean +/- SEM of three biological replicates and asterisks indicate statistical significance compared to wild type (* $P < 0.05$, ** $P < 0.01$). Below the graphs is a schematic of the *SRG1/SER3* locus with the arrow indicating *SRG1* transcription and the black bars indicating the location of the DNA fragments amplified by qPCR. D) Western analysis of Spt6 and Spt16. The wild-type, *paf1*Δ, *ctr9*Δ, and *rtf1*Δ strains described in panel A were subjected to Western blotting to compare Spt6 (top panel) and Spt16 (bottom panel) protein levels between these strains. Representative immunoblots are shown. These blots were reprobed with an antibody to G6PDH as a loading control. E) Quantitation of Western analyses from a minimum of four biological replicates. The values shown are the mean Spt6-FLAG (black) and Spt16 (gray) protein levels that have been normalized to the G6PDH loading control and made relative to the wild-type strains. Error bars indicate SEM and asterisks indicate statistical significance compared to wild type (* $P < 0.05$, ** $P < 0.01$).

decreased association of these factors is not due to a reduction in Spt6 or Spt16 expression levels as determined by Western analysis of whole cell extracts prepared from wild-type and Paf1 complex mutant strains (Figure 34D, E). Taken together, these data show that Paf1 is required for Spt16 occupancy and, to a lesser extent, Spt6 occupancy over actively transcribed *SRG1*, which overlaps the *SER3* promoter.

4.3.8 The effect of Paf1 complex mutants at other transcribed yeast genes

Since the Paf1 complex co-localizes with Pol II over all actively transcribed genes (Krogan et al. 2002; Pokholok et al. 2002; Kim et al. 2004; Mayer et al. 2010), I performed ChIP assays to determine if the occupancy of histone H3, Spt6, and Spt16 at other transcribed genes is dependent on Paf1 as I have observed for *SRG1*. The Paf1 complex, Spt6, Spt16, and Rpb3 are present at high levels within the open reading frames of the highly transcribed *PMA1* and *ADH1* genes compared to background levels of association with the lowly transcribed *GAL1* and *CYC1* genes (Figure 35A-E). Similar to our results for *SRG1*, occupancy of Spt6 and Spt16 was reduced over *PMA1* and *ADH1* in *paf1*Δ mutants but not *rtf1*Δ mutants (Figure 35C-D). However, in contrast to what I observed at *SRG1*, Rpb3 occupancy was modestly reduced over *PMA1* and *ADH1* in *paf1*Δ strains (Figure 35E). Since interactions with RNA Pol II may contribute to the recruitment of Spt6 and Spt16 to actively transcribed genes (Mason and Struhl 2003; Diebold et al. 2010; Sun et al. 2010), I recalculated Spt6 and Spt16 occupancy relative to Rpb3 occupancy (Figure 35G-H). When made relative to Rpb3 occupancy, a reduction in Spt16 occupancy in *paf1*Δ cells remains evident over these two highly transcribed genes, although the difference is less significant and is now similar to what I observe for *rtf1*Δ cells. Additionally, the reduction in Spt6 occupancy that was observed in *paf1*Δ cells is no longer evident.

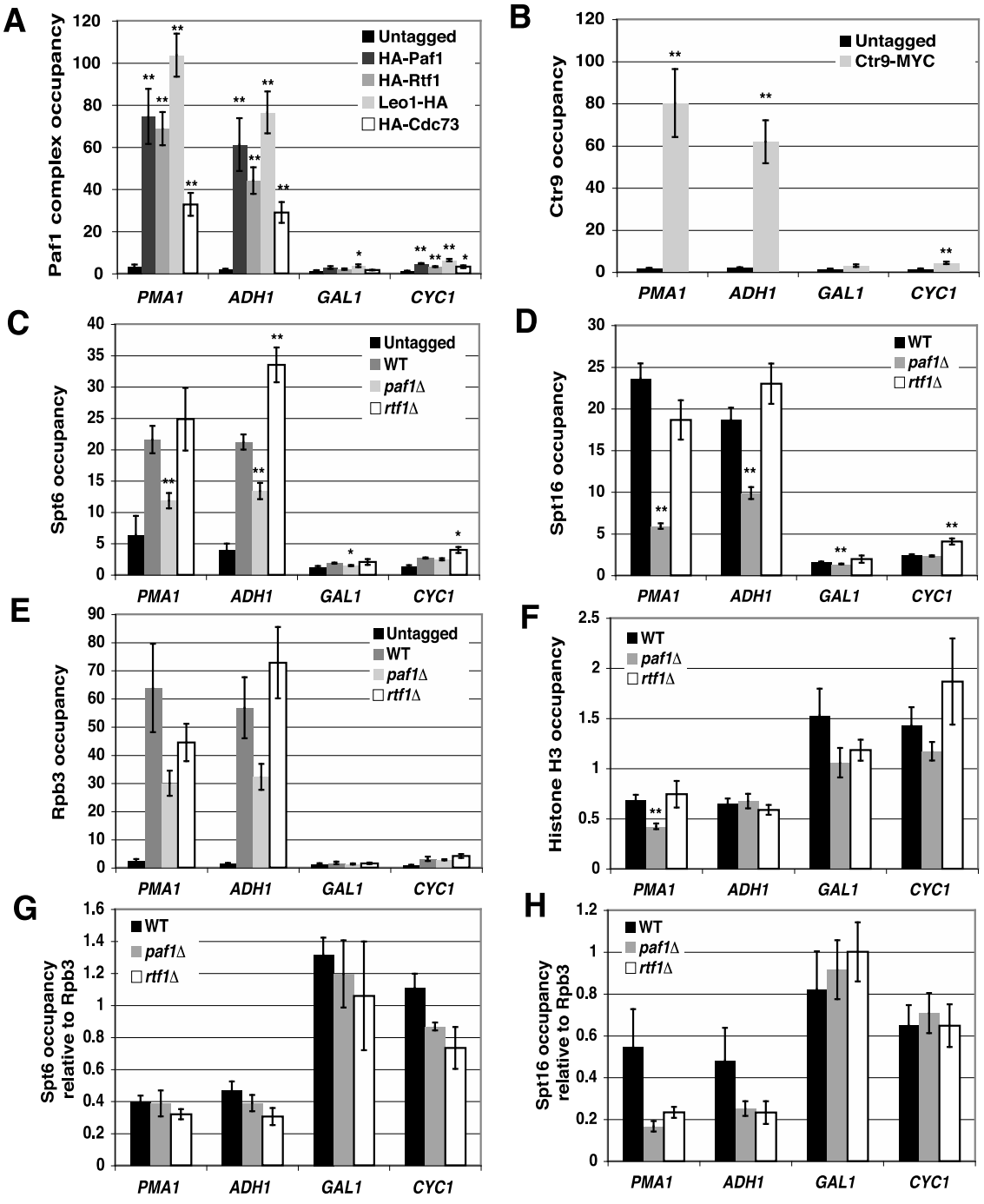
A
Paf1 complex occupancy
Untagged
HA-Paf1
HA-Rtf1
Leo1-HA
HA-Cdc73
PMA1
ADH1
GAL1
CYC1
B
Ctr9 occupancy
Untagged
Ctr9-MYC
C
Spt6 occupancy
Untagged
WT
paf1Δ
rtf1Δ
D
Spt16 occupancy
WT
paf1Δ
rtf1Δ
E
Rpb3 occupancy
F
Histone H3 occupancy
G
Spt6 occupancy relative to Rpb3
H
Spt16 occupancy relative to Rpb3

Figure 35: Effect of Paf1 complex mutants on Spt6, Spt16, Pol II, and histone H3 association with actively transcribed genes.

The relative occupancy of HA-Paf1, HA-Rtf1, Leo1-HA, HA-Cdc73 (A), and Ctr9-MYC (B) within the coding sequence of two highly transcribed genes, *PMA1* and *ADH1*, and two lowly transcribed genes, *GAL1* and *CYC1*, was determined by qPCR using the immunoprecipitated chromatin assayed in Figure 32. The relative occupancy of Spt6-FLAG (C), Spt16 (D), Rpb3-HA (E) at these four genes was determined by qPCR using the immunoprecipitated chromatin assayed in Figure 34. The relative occupancy of histone H3 (F) was determined by qPCR using the immunoprecipitated chromatin assayed in Figure 33B. The occupancy of each of these factors at these genes was normalized to their occupancy at a control region on chromosome V that contains no open reading frames (No ORF). Each value represents the mean +/- SEM for at least three biological replicates and asterisks indicate statistical significance (* $P < 0.05$, ** $P < 0.01$). (G) Spt6-FLAG and (H) Spt16 ChIP data from wild-type, *paf1*Δ, and *rtf1*Δ strains (C, D) was normalized to Rpb3-HA ChIP data (E).

Interestingly, I found that histone H3 occupancy was significantly reduced at *PMA1* in *paf1*Δ mutants, however I did not detect any change in histone H3 occupancy at *ADH1* (Figure 35F). Taken together, these data indicate that the requirement of Paf1 and Ctr9 for transcription-dependent nucleosome occupancy and recruitment of Spt6 and Spt16 at *SRG1* is not universal for all highly transcribed genes.

4.4 CONCLUSIONS

In this study, I sought to further our understanding of the repression of *SER3* by *SRG1* intergenic transcription. I uncovered a role for the multifunctional Paf1 transcription elongation complex in *SER3* repression. I showed that the Paf1 complex co-localizes with Pol II during *SRG1* transcription and provided genetic data indicating that the Paf1 complex functions in a previously characterized pathway with Bur1/2 and Spt4/5. My data indicate that *SER3* repression requires the Paf1 and Ctr9 subunits to promote *SRG1* transcription-dependent nucleosome occupancy across the *SER3* promoter, possibly by facilitating the association of the histone chaperones Spt6 and Spt16.

My nucleosome scanning and histone ChIP experiments provide evidence that Paf1 and Ctr9 repress *SER3* by facilitating *SRG1* transcription-dependent nucleosome occupancy of the *SER3* promoter (Figure 33). Previous studies have indicated that a primary function of the Paf1 complex is to establish several histone modifications that are important for chromatin dynamics at actively transcribed genes. However, these marks do not appear to play a major role in *SER3* repression by *SRG1*. First, we have shown that both an *rtf1*Δ mutant and mutations that prevent Rtf1-dependent monoubiquitylation of histone H2B at K123 - either an arginine substitution of

lysine 123 or deletion of the *RAD6* or *BRE1* genes responsible for this mark - only weakly derepress *SER3* compared to *paf1*Δ and *ctr9*Δ mutants (Figures 28, 30). Second, we determined that a parallel set of mutations preventing the subsequent methylation of histone H3 K4 and K79, have no effect on *SER3* repression (Figure 25) (Hainer and Martens 2011a; Hainer et al. 2011). Finally, we had previously shown that *SER3* repression is also unaffected by mutations that prevent methylation of histone H3 K36 (Figures 25, 29) (Hainer and Martens 2011a; Hainer et al. 2011); a modification that is dependent on Paf1, Ctr9, and to a lesser extent Cdc73 (Chu et al. 2007).

The Paf1 complex has also been shown to play a role in regulating phosphorylation events occurring in the heptapeptide repeats in the carboxy-terminal domain (CTD) of RNA Pol II and transcription termination (Mueller et al. 2004; Kaplan et al. 2005; Penheiter et al. 2005; Sheldon et al. 2005; Nordick et al. 2008; Tomson et al. 2011). Although I cannot absolutely rule out a role for these Paf1 complex-dependent activities in regulating *SER3* repression, my data suggest that these activities are unlikely to account for the role of the Paf1 complex in maintaining *SRG1* transcription-dependent nucleosome occupancy of the *SER3* promoter. First, there are distinct differences in the subunit requirements for these activities. Whereas nucleosome occupancy of the *SER3* promoter is primarily dependent on Paf1 and Ctr9, additional subunits of this complex are required for the phosphorylation of the serines at position 2 within the heptapeptide repeats (Ser2P) and proper transcription termination (Mueller et al. 2004; Kaplan et al. 2005; Penheiter et al. 2005; Sheldon et al. 2005; Nordick et al. 2008; Tomson et al. 2011). Second, the termination of *SRG1* has been mapped to two distinct sites, 75bp 5’ and 25bp 3’of the *SER3* translation start site, along with a minor read-through product to the end of *SER3* (Thompson and Parker 2007). In Paf1 complex subunits deletions, I do not observe any

increase in this read-through product as might be expected if the Paf1 complex was affecting *SRG1* termination (data not shown). Taken together, my studies of *SER3* repression suggest a role for the Paf1 complex in controlling transcription-coupled nucleosome occupancy that is primarily dependent on the Paf1 and Ctr9 subunits and independent of previously characterized activities of this complex.

Interestingly, a possible role for the Paf1 complex in regulating chromatin dynamics during transcription, independent of its roles in promoting histone modifications, was recently proposed by Kim et al. based on an *in vitro* transcription system where the human Paf1complex was shown to stimulate elongation through nucleosomes (Kim et al. 2010). My studies of *SER3* regulation suggest this role may be specific to the Paf1 and Ctr9 subunits, involving a more direct role for these two factors in promoting nucleosome occupancy during transcription. One study previously implicated the Paf1 complex in altering nucleosome occupancy during gene induction. However, rather than promoting nucleosome occupancy, this study showed that Paf1 and Ctr9 were required for efficient histone eviction at the *GAL1-10* promoter during the induction of these genes in response to galactose (Marton and Desiderio 2008). Several studies have also linked efficient induction of *GAL1-10* to H2B K123ub by Rad6/Bre1 (Henry et al. 2003; Kao et al. 2004; Xiao et al. 2005), suggesting that the effect of the Paf1 complex on *GAL1-10* induction is likely through its role in promoting histone modifications.

How might Paf1 and Ctr9 promote *SRG1* transcription-dependent nucleosome occupancy at the *SER3* promoter? One possibility is that Paf1 regulates the histone chaperones Spt6 and/or Spt16, which are required for the maintenance of nucleosomes over the *SER3* promoter (Hainer et al. 2011). In yeast, the Paf1 complex has genetic interactions with Spt6 (Kaplan et al. 2005) and both physical and genetic interactions with Spt16 (Squazzo et al. 2002; Pavri et al. 2006).

The Paf1 complex has also been shown in *Drosophila* to be required for full recruitment of Spt6 and the FACT subunit SSRP1 during transcription (Adelman et al. 2006). These connections led me to examine whether the recruitment of these factors is affected in Paf1 complex mutants in yeast (Figure 34). ChIP experiments revealed that Spt16 occupancy over *SRG1* is strongly dependent on Paf1 but not Rtf1, which correlates with the effect that each of these factors have on *SER3* repression. Western analyses and RNA Pol II ChIP data indicate that this reduction in Spt16 occupancy in *paf1*Δ cells is not caused by a reduction in Spt16 protein levels or by a reduction in RNA Pol II levels across *SRG1* (Figure 34). Taken together, my results support a role for Paf1 and Ctr9 in promoting FACT occupancy across actively transcribed *SRG1*. In contrast to Spt16, I found Spt6 occupancy at *SRG1* to be only modestly dependent on Paf1. Therefore, while Paf1 and Ctr9 may play a prominent role in Spt16 occupancy at *SRG1*, other factors are likely to contribute significantly to Spt6 occupancy of this region. Spt6 is known to associate with elongating Pol II through a direct interaction with Pol II CTD containing Ser2-P (Diebold et al. 2010; Sun et al. 2010). Although it is unlikely to be part of the Paf1-dependent pathway that represses *SER3* as I discussed earlier, this mark may contribute to Spt6 occupancy at *SRG1* in a Paf1-independent pathway. Interestingly, Thebault et al. recently reported that Spt6 occupancy at *SRG1* is also partially dependent on Spt2, an HMG-like transcription elongation factor (Thebault et al. 2011). Although I cannot rule out the possibility that Spt2 contributes to Spt6 occupancy in a pathway with Paf1, Spt2 may also facilitate Spt6 recruitment independently of Paf1. Overall, my results are consistent with those observed in *Drosophila*, in which depletion of Paf1, and to a lesser extent, Rtf1, led to reduced association of Spt6 and FACT over an actively transcribed gene, without affecting Pol II association or global protein levels (Adelman et al. 2006). Interestingly, localization of the Paf1 complex to actively transcribed

genes has also been shown to be partially dependent on Spt6 and Spt16 (Kaplan et al. 2005; Pavri et al. 2006). Therefore, it is possible that recruitment of Spt6, Spt16, and the Paf1 complex is interdependent, where the disruption of one of these factors results in reduced association of the others.

Cells lacking Paf1 not only reduce the association of Spt6 and Spt16 across the *SRG1* transcription unit, but also reduce nucleosome occupancy over this region that overlaps the *SER3* promoter. Because both Spt6 and Spt16 interact with histones (Bortvin and Winston 1996; Formosa et al. 2001; Belotserkovskaya et al. 2003) and have been implicated in restoring nucleosome occupancy after the passage of RNA Pol II at transcribed genes (Belotserkovskaya et al. 2003; Kaplan et al. 2003; Mason and Struhl 2003; Cheung et al. 2008; Jamai et al. 2009), it is also possible that the loss of these factors over *SRG1* is a consequence, rather than a cause, of reduced nucleosomes over this region in the *paf1*Δ strains. In this case, the Paf1 complex may be required for the transcription-dependent nucleosome reassembly activity of Spt6 and Spt16 rather than recruitment of these factors. Interestingly, Spt2 is also required to promote nucleosome occupancy across the *SRG1* transcription unit to repress *SER3* transcription (Nourani et al. 2006; Thebault et al. 2011). In addition, Spt2 has been genetically linked to Paf1 and Ctr9 and its co-localization with RNA Pol II across actively transcribed genes is dependent on Paf1, albeit weakly (Nourani et al. 2006). It will be interesting to decipher the functional interplay between the Paf1 complex, Spt6, FACT, and Spt2 in promoting *SRG1* transcription-dependent nucleosome occupancy and *SER3* repression.

In addition to the well-characterized role of the Paf1 complex in promoting transcription, whole genome expression analyses indicated that the Paf1 complex also functions as a negative regulator of transcription (Penheiter et al. 2005). My finding that the Paf1 complex indirectly

represses *SER3* expression by positively regulating the chromatin dynamics associated with *SRG1* intergenic transcription across the *SER3* promoter has provided one of the first insights into understanding how this complex negatively regulates transcription. A negative regulatory role for the Paf1 complex has also been recently characterized at the yeast gene *ARG1* (Crisucci and Arndt 2011). In this case, Paf1 complex members were found associating with both the *ARG1* promoter and ORF during repressing conditions. Similar to *SER3* repression, *ARG1* repression is most strongly dependent on the Paf1 and Ctr9 subunits, however Rtf1 and Rtf1-regulated histone modifications seem to have a greater effect at *ARG1* than at *SER3* (Crisucci and Arndt 2011). Although the mechanistic role of Paf1 and Ctr9 in *ARG1* repression has yet to be defined, it is interesting to note that transcription antisense to *ARG1* has been detected at this locus (David et al. 2006; Xu et al. 2009). Additional experiments are required to determine if *SER3* and *ARG1* repression occur by a common mechanism involving Paf1-mediated chromatin dynamics during transcription of ncDNA.

In addition to *SRG1*, I assayed the effect of deleting *PAF1* on histone H3, Spt6, and Spt16 occupancy over the transcribed regions of two other highly transcribed genes, *PMA1* and *ADH1* (Figure 35). Similar to my results for *SRG1*, I detected reduced Spt6 and Spt16 occupancy in cells lacking Paf1. However, in contrast to what I observed at *SRG1*, RNA Pol II levels at these genes were also slightly reduced in *paf1*Δ cells. Gene-specific differences in RNA Pol II occupancy have been previously reported for *paf1*Δ mutants (Mueller et al. 2004). When normalized to RNA Pol II levels, there is no longer a reduction in Spt6 occupancy, while Spt16 occupancy is reduced to similar levels in both *paf1*Δ and *rtf1*Δ mutants (Figure 35G, H). Furthermore, histone H3 occupancy was unaffected at *ADH1* and only moderately reduced at *PMA1* in *paf1*Δ cells. These studies suggest that there are likely to be additional factors that

influence the role of the Paf1 complex in regulating transcription-coupled nucleosome assembly at specific genes. One factor that may contribute to these gene-specific differences is the properties of the transcribed DNA. *SRG1* is transcribed across the promoter region of *SER3*. In general, promoter regions tend to be comprised of sequences that are refractory to nucleosome formation, whereas the sequence of ORFs generally do not contain these properties (Segal and Widom 2009). This would explain the inherent instability of nucleosomes over the *SER3* promoter in the absence of *SRG1* transcription. This characteristic of the *SRG1* transcription unit makes it unique compared to the transcription of most protein-coding genes and may have allowed me to uncover this new role for the Paf1 complex that may not be readily detectable at other transcribed regions of the genome.

In conclusion, my analysis of *SER3* repression by intergenic *SRG1* transcription supports a previously uncharacterized role for the Paf1 complex in promoting transcription-dependent nucleosome occupancy. This activity is primarily dependent on the Paf1 and Ctr9 subunits, possibly mediated by their requirement for the recruitment and/or activity of the Spt6 and Spt16 histone chaperones. Additional studies will be necessary to elucidate the precise mechanism by which Paf1 and Ctr9 regulate chromatin dynamics during *SRG1* transcription, to determine how broadly these two factors function in a similar manner at other transcribed regions of the genome, and to determine the characteristic of a transcription unit that makes it susceptible to this new activity of the Paf1 complex.

5.0 THE ROLE OF THE RTT109 HISTONE ACETYLTRANSFERASE IN *SER3* REPRESSION

5.1 INTRODUCTION

Rtt109 was one of the factors identified by my genetic screen as a potential repressor of *SER3* transcription. This was an exciting result because this little known factor had recently been discovered to be a histone acetyltransferase (HAT) targeting histone H3 lysine 56 (K56) (Schneider et al. 2006; Driscoll et al. 2007; Han et al. 2007). Since this discovery, a large amount of work has been done characterizing the HAT activity, binding partners, and functions of Rtt109.

The first evidence of HAT activity for Rtt109 was a proteomic screen that showed it was required for global K56 acetylation (Schneider et al. 2006). Other *in vitro* and *in vivo* studies confirmed this HAT activity and showed that Rtt109 was the only HAT required for K56 acetylation (Driscoll et al. 2007; Han et al. 2007). Rtt109 has also been shown to have activity at H3 K4, K9, and K27, but these residues can also be acetylated by another HAT, Gcn5 (Fillingham et al. 2008; Burgess et al. 2010; Guillemette et al. 2011). For these acetylation marks, Rtt109 and Gcn5 function redundantly, and removal of both factors is required to lose global K4, K9, or K27 acetylation.

Rtt109 HAT activity transfers an acetyl group from acetyl-CoA to the amine group of the histone lysine. Interestingly, acetylation by Rtt109 is targeted to histone proteins before incorporation into chromatin (Kuo et al. 1996; Masumoto et al. 2005; Tsubota et al. 2007). Crystal structures of Rtt109 revealed interesting insights into the biology of this HAT. While Rtt109 shares little sequence homology with other HATs, and therefore was not identified as a HAT by early computational methods, it shares considerable structural homology with another HAT, p300/CBP (Berndsen et al. 2008; Lin and Yuan 2008; Tang et al. 2008). p300/CBP is the metazoan HAT responsible for K56 acetylation suggesting this mechanism is conserved in the enzyme structure, though not in the primary sequence (Tang et al. 2008). Rtt109 also contains a lysine residue, K290, near its active site, which undergoes autoacetylation, a step required for the acetylation of histone H3 (Stavropoulos et al. 2008; Albaugh et al. 2011).

An interesting feature of Rtt109 is its requirement for a binding partner, either Asf1 or Vps75, for its HAT activity. Asf1 and Vps75 both act as histone chaperones, binding to H3-H4 dimers and tetramers, respectively (English et al. 2006; Bowman et al. 2011). They both stimulate Rtt109 by increasing its ability to bind its histone targets, but they act in different ways. Vps75 forms a tight complex with Rtt109 and is only required for acetylation of K9, K27, and likely K4 (Guillemette et al. 2011; Keck and Pemberton 2011; Tang et al. 2011). Asf1 has a more transient interaction with Rtt109 and is mainly required for acetylation of K56 (Driscoll et al. 2007; Han et al. 2007; Fillingham et al. 2008).

All of the known functions of Rtt109 appear to be through acetylation of one or more of its targeted residues on histone H3, with K56 acetylation being the best characterized. K56 acetylation occurs on newly synthesized histones as they get incorporated during S phase (Ozdemir et al. 2006). This mark and, consequently, Rtt109 and Asf1 are required for proper

DNA replication, DNA damage response, and cell cycle progression. Loss of K56 acetylation leads to increased DNA damage, mutations, cell cycle arrest, and reduced lifespan suggesting it plays important roles in promoting genomic stability (Driscoll et al. 2007; Han et al. 2007; Feser et al. 2010). Rtt109 and K56 acetylation also have roles in chromatin dynamics outside of S phase. K56 acetylation has been implicated in global histone exchange during transcription as well as over promoter regions and has specific roles in nucleosome exchange during the induction of the *PHO5* and *GAL1* genes (Rufiange et al. 2007; Williams et al. 2008; Durairaj et al. 2010). Rtt109, Asf1, and Vps75 are also required for proper regulation of certain histone genes (Fillingham et al. 2009).

In this chapter, I investigated the role of Rtt109 in *SER3* repression. Strains lacking *RTT109* have variable and inconsistent defects in *SER3* repression that may be due to indirect effects. While Rtt109, Asf1, and Vps75 are all weakly associated with *SRG1* transcription, these histone chaperone partners and the acetylation targets of Rtt109 are only mildly required for *SER3* repression compared to other factors that repress *SER3*, such as Snf2, Spt6, and Paf1. Not surprisingly, Rtt109 had little to no effect on the chromatin structure over the *SER3* promoter. Overall, Rtt109 and Asf1 may play a minor role in *SER3* repression, possibly through their role in histone exchange, but Rtt109 likely has additional indirect effects through maintaining genomic stability and proper regulation of histone genes.

5.2 MATERIALS AND METHODS

5.2.1 Yeast strains and media

Most *S. cerevisiae* strains used in this study (Table 7) are isogenic with a *GAL2*$^{+}$ derivative of S288C (Winston et al. 1995). Strains were constructed using standard genetic crosses or by transformation (Ausubel et al. 1991). Strains containing C-terminal TAP-tagged versions of Rtt109, Asf1, and Vps75 were obtained from the TAP collection (Open Biosystems) (Ghaemmaghami et al. 2003). Histone H3 K9R and K56R mutant strains were obtained from Jef Boeke (Johns Hopkins University) (Dai et al. 2008). Strains were grown in the following media: YPD (1% yeast extract, 2% peptone, 2% glucose), and synthetic complete lacking uracil (SC-ura) (Rose 1991). Growth assays were performed by spotting serially diluted cultures onto SC-ura and YPD containing 200mM Hydroxyurea (USB) and growing at 30ºC for 3 days.

Table 7: *S. cerevisiae* strains used in Chapter 5.

Name	Genotype
FY4	*MAT***a**
FY5	*MATα*
YP042	*MATα rtt109Δ::KanMX*
YP043	*MAT***a** *rtt109Δ::KanMX ura3Δ0*
YP044	*MAT***a** *rtt109Δ::KanMX leu2Δ0*
YP045	*MATα rtt109Δ::KanMX leu2Δ0*
YP046	*MAT***a** *rtt109Δ::KanMX ura3Δ0 leu2Δ0*
YP047	*MATα rtt109Δ::KanMX ura3Δ0 leu2Δ0*
YP048	*asf1Δ::KanMX*
YP049	*MATα asf1Δ::KanMX*
YP050	*MAT***a** *asf1Δ::KanMX ura3Δ0*
YP051	*MATα asf1Δ::KanMX ura3Δ0*
YP052	*MAT***a** *asf1Δ::KanMX leu2Δ0*
YP053	*MAT***a** *asf1Δ::KanMX ura3Δ0 leu2Δ0*
YP054	*MAT***a** *vps75Δ::KanMX*
YP055	*MATα vps75Δ::KanMX*
YP056	*MAT***a** *vps75Δ::KanMX ura3Δ0*
YP057	*MATα vps75Δ::KanMX ura3Δ0*
YP058	*MAT***a** *vps75Δ::KanMX leu2Δ0*
YP059	*MATα vps75Δ::KanMX leu2Δ0*
YP060	*MAT***a** *vps75Δ::KanMX ura3Δ0 leu2Δ0*
YP069	*asf1Δ::KanMX vps75Δ::KanMX*
YP070	*MAT***a** *asf1Δ::KanMX vps75Δ::KanMX ura3Δ0*
YP071	*MAT***a** *asf1Δ::KanMX vps75Δ::KanMX ura3Δ0 leu2Δ0*
YP072	*MAT***a** *asf1Δ::KanMX vps75Δ::KanMX ura3Δ0 leu2Δ0*
YP274	*ura3Δ0 pRS416*
YP275	*ura3Δ0 pRS416*
YP276	*ura3Δ0 pRS416*
YP277	*ura3Δ0 pRS416*
YP278	*rtt109Δ::KanMX ura3Δ0 pRS416*
YP279	*rtt109Δ::KanMX ura3Δ0 pRS416*
YP280	*rtt109Δ::KanMX ura3Δ0 pRS416*
YP281	*rtt109Δ::KanMX ura3Δ0 pRS416*
YP282	*ura3Δ0 pRS416::FLAG-RTT109*
YP283	*ura3Δ0 pRS416::FLAG-RTT109*
YP284	*ura3Δ0 pRS416::FLAG-RTT109*
YP285	*ura3Δ0 pRS416::FLAG-RTT109*
YP286	*rtt109Δ::KanMX ura3Δ0 pRS416::FLAG-RTT109*
YP287	*rtt109Δ::KanMX ura3Δ0 pRS416::FLAG-RTT109*
YP288	*rtt109Δ::KanMX ura3Δ0 pRS416::FLAG-RTT109*
YP289	*rtt109Δ::KanMX ura3Δ0 pRS416::FLAG-RTT109*
YP290	*ura3Δ0 pRS416::FLAG-rtt109::D89A*
YP291	*ura3Δ0 pRS416::FLAG-rtt109::D89A*
YP292	*ura3Δ0 pRS416::FLAG-rtt109::D89A*
YP293	*ura3Δ0 pRS416::FLAG-rtt109::D89A*
YP294	*rtt109Δ::KanMX ura3Δ0 pRS416::FLAG-rtt109::D89A*
YP295	*rtt109Δ::KanMX ura3Δ0 pRS416::FLAG-rtt109::D89A*
YP296	*rtt109Δ::KanMX ura3Δ0 pRS416::FLAG-rtt109::D89A*
YP297	*rtt109Δ::KanMX ura3Δ0 pRS416::FLAG-rtt109::D89A*
YP298	*ura3Δ0 pRS416::FLAG-rtt109::DD287-288AA*
YP299	*ura3Δ0 pRS416::FLAG-rtt109::DD287-288AA*
YP300	*ura3Δ0 pRS416::FLAG-rtt109::DD287-288AA*

Table 7 (cont.)

YP301	*ura3Δ0 pRS416::FLAG-rtt109::DD287-288AA*
YP302	*rtt109Δ::KanMX ura3Δ0 pRS416::FLAG-rtt109::DD287-288AA*
YP303	*rtt109Δ::KanMX ura3Δ0 pRS416::FLAG-rtt109::DD287-288AA*
YP304	*rtt109Δ::KanMX ura3Δ0 pRS416::FLAG-rtt109::DD287-288AA*
YP305	*rtt109Δ::KanMX ura3Δ0 pRS416::FLAG-rtt109::DD287-288AA*
YP306	*MATα asf1Δ::KanMX ura3Δ0*
YP307	*MATα asf1Δ::KanMX ura3Δ0*
YP308	*MATα asf1Δ::KanMX ura3Δ0*
YP309	*MAT***a** *asf1Δ::KanMX ura3Δ0*
YP310	*MAT***a** *vps75Δ::KanMX ura3Δ0*
YP311	*MATα vps75Δ::KanMX ura3Δ0*
YP312	*MAT***a** *vps75Δ::KanMX ura3Δ0*
YP313	*MAT***a** *vps75Δ::KanMX ura3Δ0*
OY8	*MAT***a** *his3Δ1 leu2Δ0 ura3Δ0 met15Δ0*
Rtt109-TAP	*MAT***a** *his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 RTT109-TAP::HIS3MX6*
Asf1-TAP	*MAT***a** *his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 ASF1-TAP::HIS3MX6*
Vps75-TAP	*MAT***a** *his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 VPS75-TAP::HIS3MX6*
H3 WT	*MAT***a** *his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 hht1-hhf1::NatMX4 hht2-hhf2::HHT2-HHF2::URA3 can1::MFA1pr-HIS3*
H3 K9R	*MAT***a** *his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 hht1-hhf1::NatMX4 hht2-hhf2::hht2-K9R-HHF2::URA3 can1::MFA1pr-HIS3*
H3 K56R	*MAT***a** *his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 hht1-hhf1::NatMX4 hht2-hhf2::hht2-K56R-HHF2::URA3 can1::MFA1pr-HIS3*

5.2.2 Northern Analysis

Total RNA was isolated from cells grown to 1-2x10^7 cells/mL and separated on a 1% formaldehyde-agarose gel as described previously (Ausubel et al. 1991). RNA was transferred to Gene Screen membrane (Perkin-Elmer) and hybridized with radiolabeled probes generated by random-primed labeling of PCR fragments that were amplified from the following genomic sequences: *SRG1* (ChrV: 322258-322559), *SER3* (ChrV: 324059-324307), and *SCR1* (ChrV: 441741-442266), which was used as a control for RNA loading.

5.2.3 Plasmids

pRS416 is a *URA3*-marked *CEN/ARS* plasmid (Brachmann et al. 1998). pRTT109, D89A, and DD287-288AA, are derivatives of pRS416, obtained from Zhiguo Zhang (Mayo Clinic College of Medicine) that express wild type or catalytically dead versions of Rtt109 with a FLAG epitope tag at the C-terminus. Previous work demonstrated the proteins are properly folded and expressed from these constructs (Han et al. 2007).

5.2.4 Chromatin Immunoprecipitation

Cells were grown in YPD at 30°C to a density of 1-2x10^7 cells/mL and then treated with 1% formaldehyde for 20 min. Chromatin was isolated and sonicated as previously described (Shirra et al. 2005) and then incubated with antibodies overnight at 4°C. Anti-protein A antibody (1µL, Sigma, P3775) was used to immunoprecipitate TAP-tagged proteins. Primary antibody-protein conjugates were isolated by incubating with 30µL Protein A-coupled sepharose beads (GE

Healthcare) at 4°C for 2-3 hours. After purifying DNA through PCR purification columns (Qiagen), the amount of immunoprecipitated (IP) DNA relative to input DNA was determined by qPCR and then normalized to a control region on chromosome V that lacks open reading frames (No ORF) that has been previously described (Komarnitsky et al. 2000).

5.2.5 Nucleosome Scanning Assay

Nucleosome scanning assays were performed as previously described (Chapter 3 Materials and Methods) (Hainer et al. 2011). Briefly, cells were grown in YPD to 2-3x10^7 cells/mL at 30°C and then treated with 2% formaldehyde followed by 300mM glycine. 1.2x10^9 cells were spheroplasted with Zymolyase 20T (Seikagaku Biobusiness), divided into six aliquots, which were then incubated with increasing concentrations of micrococcal nuclease (MNase, Nuclease S7, Roche). DNA was extracted, treated with RNase A, and subjected to gel electrophoresis and qPCR to determine extent of MNase digestion. Well-characterized regions of the *GAL1* promoter, one bound by a nucleosome (NB) and another nucleosome-free (NUB) were used as controls (Lohr 1984; Brickner et al. 2007; Floer et al. 2010). The samples in which the concentration of MNase yielded mostly mononucleosome-sized fragments and the NUB/NB ratio was less than 15% were then subjected to further qPCR analyses using primer pairs that amplify ~100bp fragments that tile the *SER3* locus. The amount of amplification for each *SER3* primer pair (*SER3*-7 to *SER3*-41) in the digested sample was made relative to the undigested sample and normalized to the *GAL1* NB region.

5.2.6 Quantitative real-time PCR

Results of nucleosome scanning and ChIP assays were analyzed using ABI 7300 or StepOnePlus real-time PCR systems and SYBR green reagents (Fermentas). Primer sets used in ChIP and nucleosome scanning experiments are listed in Table 5 and have been previously described (Hainer and Martens 2011a; Hainer et al. 2011). Quantitation of real-time PCR results was performed using the Pfaffl method (Pfaffl 2001).

5.3 RESULTS

5.3.1 Initial results suggested a role for Rtt109 in *SER3* repression independent of its previously known functions

To test whether Rtt109 is required to repress endogenous *SER3*, I deleted the gene and the genes of its known partners *ASF1* and *VPS75* in our strain background. Initial Northern analysis of these strains revealed a relatively strong derepression of *SER3* (~8 fold) in *rtt109*Δ strains with only a minor effect (~2-fold) in *asf1*Δ strains and no effect in *vps75*Δ strains (Figure 36). This result was surprising since all of the known functions of Rtt109 require at least one of these factors (Driscoll et al. 2007; Han et al. 2007; Williams et al. 2008; Durairaj et al. 2010). Moreover, the two histone chaperones did not seem to be acting redundantly since deleting both genes showed similar derepression of *SER3* as *asf1*Δ strains.

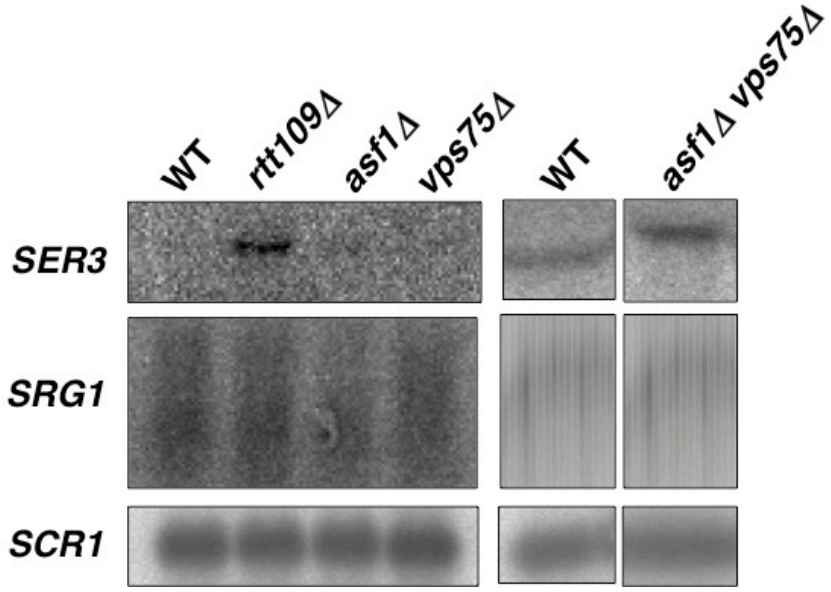

Figure 36: Effect of Rtt109, Asf1, and Vps75 on *SER3* repression.

Northern analysis of *SER3*, *SRG1*, and *SCR1* levels in wild type (FY5) *rtt109*Δ (YP043), *asf1*Δ (YP049), *vps75*Δ (YP057), and *asf1*Δ *vps75*Δ (YP069) strains.

Since Asf1 and Vps75 did not seem to be required for the function of Rtt109 in *SER3* repression, I tested the role of certain targets of Rtt109 HAT activity. I performed Northern analysis on strains in which one copy of histone H3 is deleted and the other has the lysine 9 or lysine 56 residues mutated to arginine, a conservative mutation that retains the relative size and charge of the side chain while eliminating acetylation (Figure 37) (Dai et al. 2008). Compared to a control strain lacking one copy of histone H3, these mutations had no effect on *SER3* levels. Other Rtt109 targets, K4 and K27, were also tested and had no effect on *SER3* levels (Hainer and Martens 2011a). This result was not surprising, since Asf1 and Vps75 are required for these modifications and they have little effect on *SER3* levels, but these results together suggested a role for Rtt109 outside of its known roles in these histone acetylation marks.

5.3.2 Rtt109, Asf1, and Vps75 are weakly associated with *SRG1* transcription during *SER3* repression

I performed ChIP experiments to determine if Rtt109 was physically associated with the *SER3* locus in order to determine whether it could be acting directly. I utilized strains from the yeast TAP collection, which were confirmed by PCR and for expression by Western analysis (Data not shown). TAP tagged versions of Rtt109, Asf1, and Vps75 were pulled down with anti-protein A antibody and the associated DNA was analyzed by real time PCR (Figure 38). All three factors were equally associated with the *SER3* promoter but not the ORF. I also examined occupancy over two highly transcribed genes, *PMA1* and *ADH1*, and two poorly transcribed genes, *GAL1* and *CYC1* (Figure 38B). All three factors also associate to the same degree with the highly transcribed genes, suggesting they may be associated with active transcription, as has been previously suggested (Schneider et al. 2006). It is not clear if this level of association is

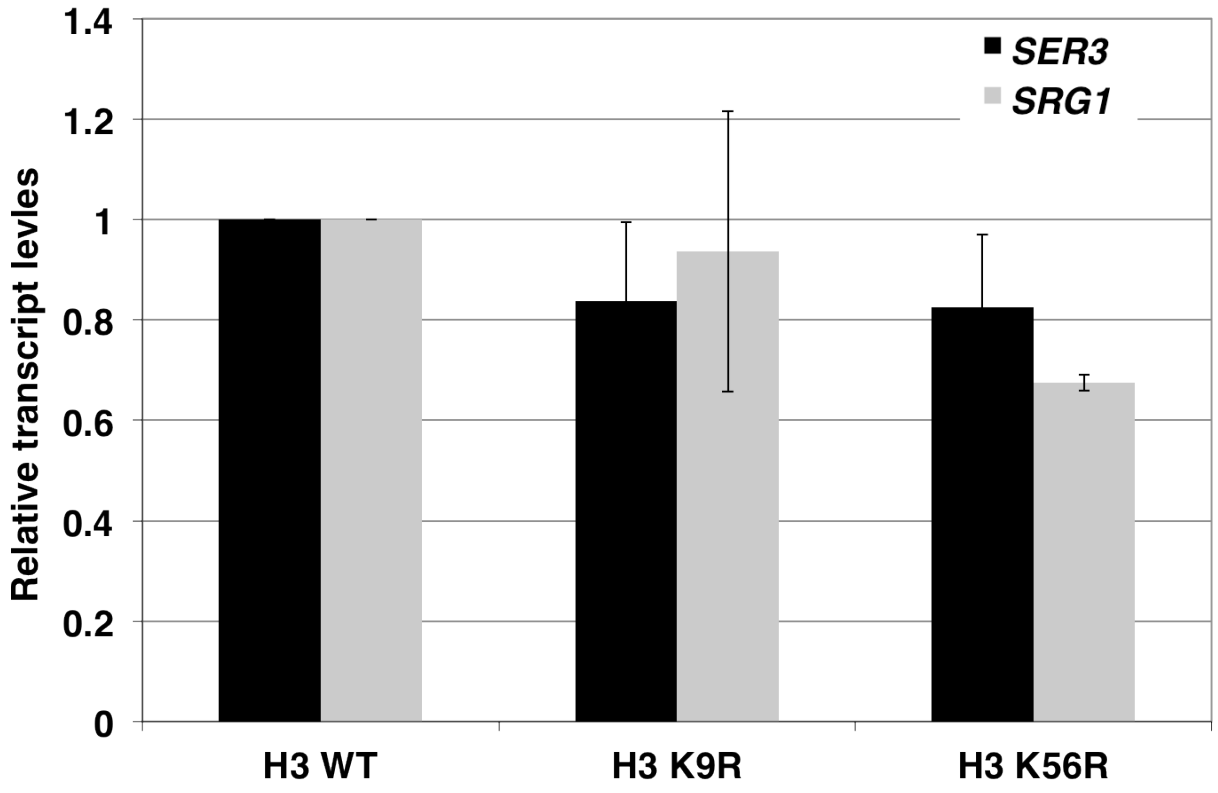

Figure 37: Northern analysis of histone H3 K9R and K56R strains.

Quantitation of results of Northern analyses from H3 K9R and H3 K56R strains compared to control strains lacking one copy of the gene encoding histone H3. The values shown are the mean *SER3* (black) and *SRG1* (grey) transcript levels from three independent experiments that have been normalized to the *SCR1* loading control and made relative to the wild-type strains. Error bars indicate SEM.

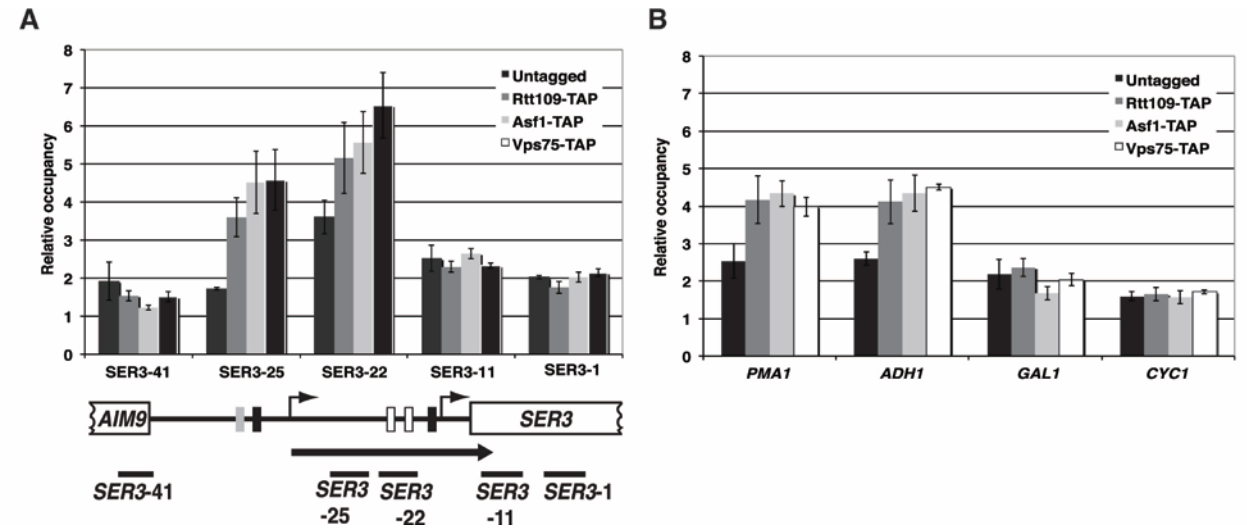

Figure 38: Rtt019, Asf1, and Vps75 weakly associate with active transcription.

A) Results of real-time qPCR from a ChIP experiment in which Rtt109, Asf1, and Vps65 were immunoprecipitated from TAP-tagged strains and an untagged control strain (OY8). Isolated DNA was amplified using primers that contain different regions of the *SRG1*/*SER3* locus as well as control region (No ORF). Bars represent mean of at least three independent experiments relative to No ORF. Error bars indicate SEM. B) Same ChIP as (A) looking at occupancy over two highly transcribed genes, *PMA1* and *ADH1*, and two poorly transcribed genes, *GAL1* and *CYC1*. Bars represent mean of at least three independent experiments relative to No ORF. Error bars indicate SEM.

significant since Rtt109 mostly functions on free histones, not those incorporated into nucleosomes.

5.3.3 Rtt109 does not affect nucleosome occupancy over the *SER3* promoter

To determine any effect of Rtt109 on *SER3* nucleosome architecture, I performed nucleosome scanning assays on *rtt109*Δ strains and compared it to wild type strains (Figure 39). The pattern of nucleosome occupancy almost completely overlaps in the wild type and mutant strains except over the *SER3* promoter. At this region, there is a small but reproducible decrease in nucleosome occupancy. This small difference may account for the effect seen in *rtt109* deletion stains but it is unlikely.

5.3.4 The effect of *rtt109*Δ on *SER3* repression is variable and may be through an indirect mechanism

In my investigations of *rtt109*Δ strains and their effect on *SER3*, I noticed the derepression of *SER3* was not always consistent. There was variability in the extent of derepression between different *rtt109*Δ strains obtained from the same cross, as well as different effects of the same strain grown at different times. For example, the Northern in Figure 40 shows fairly consistent results for *asf1*Δ and *vps75*Δ strains, but some *rtt109*Δ strains have strong effects and others are more similar to *asf1*Δ strains.

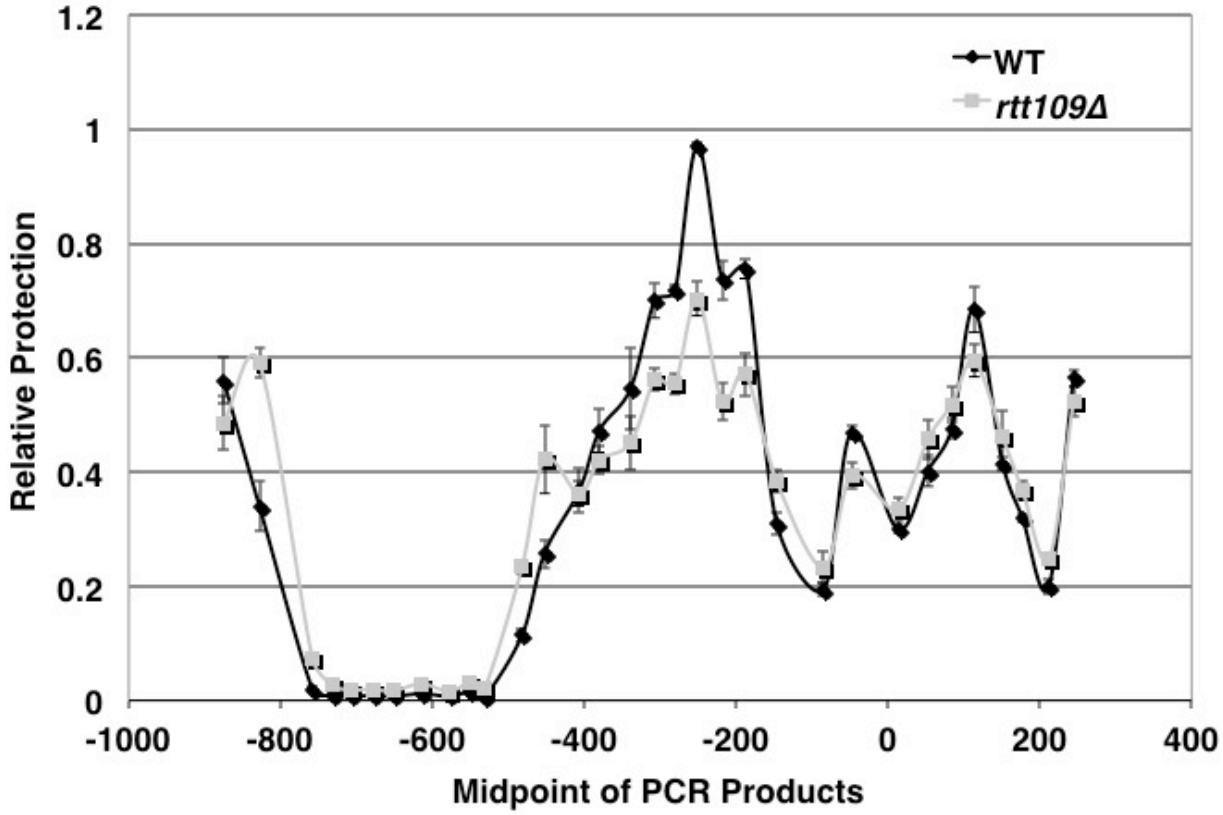

Figure 39: Effect of *rtt109*Δ on nucleosome occupancy of the *SER3* promoter.

Nucleosome scanning assay was performed on wild type (FY4, FY5) and *rtt109*Δ (YP042, YP044, YP045, YP047) strains. MNase protection across the *SER3* locus was calculated relative to a positioned nucleosome within the *GAL1* promoter using qPCR as described in Materials and Methods. The mean +/- SEM from at least three biological replicates is plotted at the midpoint for each PCR product.

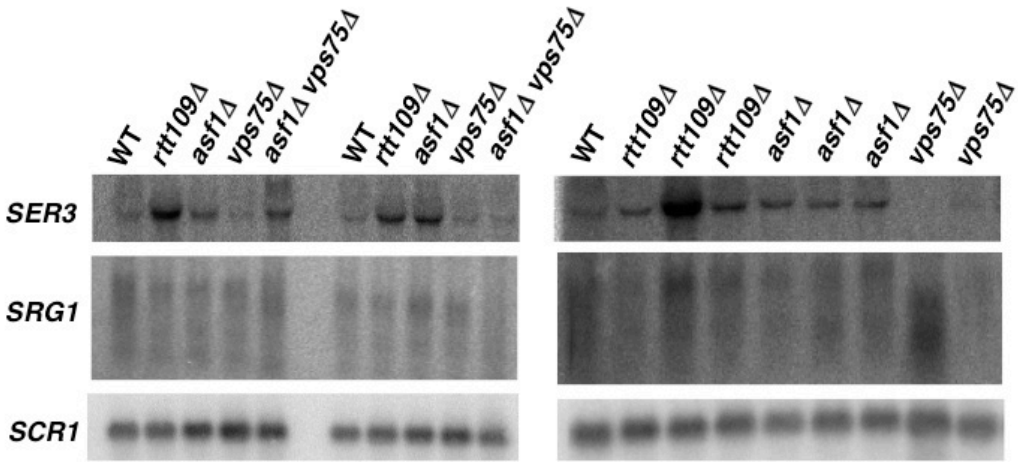

Figure 40: Variability in the effect of *rtt109*Δ on *SER3* repression.

Northern analysis of *SER3*, *SRG1*, and *SCR1* levels in wild type (FY3, FY4) *rtt109*Δ (YP043, YP044, YP045, YP046, YP047) *asf1*Δ (YP049, YP050, YP051, YP052, YP053) *vps75*Δ (YP055, YP056, YP057, YP058) *asf1*Δ *vps75*Δ (YP070, YP071) strains.

To reduce the degree of variability in *rtt109*Δ experiments and determine the true effect on *SER3* repression, I remade the *rtt109*Δ deletion allele in a diploid strain and covered the deletion with a *URA3*-marked plasmid containing the wild-type *RTT109* gene (pRTT109). The same diploid strain was also transformed with an empty vector control (pRS416) and plasmids containing *rtt109* mutants that lack HAT activity (D89A and DD287-288AA) (Han et al. 2007). The four diploid strains were sporulated to obtain wild type and *rtt109*Δ haploid strains containing each plasmid.

To test for complementation of the deletion, I performed a serial dilution assay to determine if a known *rtt109*Δ phenotype (HU sensitivity) is rescued by addition of the wild type *RTT109* on a plasmid (Figure 41A). All strains grew on control plates and none of the wild type strains were sensitive to HU suggesting there was no effect of overexpressing *RTT109* or any dominant negative effect of the catalytic mutants. *rtt109*Δ strains containing the empty vector were sensitive to HU while those containing pRTT109 grew like wild type suggesting complete rescue. As expected, based on previously published results, the catalytic mutants are unable to rescue phenotypes associated with the complete deletion (Han et al. 2007). These results indicated the deletion and plasmids were behaving as expected in the experiment. Surprisingly, by Northern analysis there was no derepression of *SER3* in any of the strains tested (Figure 41B,C). While this result was expected for the wild type strains and the *rtt109*Δ strain complemented by pRTT109, I expected the *rtt109*Δ strain containing the empty vector pRS416 to show the same 8-fold derepression I had seen previously. I did not know whether the catalytic mutants would complement this phenotype, so I was interested to see if HAT activity was required for *SER3* repression. These results suggest that initially there is little to no effect of *rtt109*Δ on *SER3* levels and our previous results were due to secondary or indirect effects.

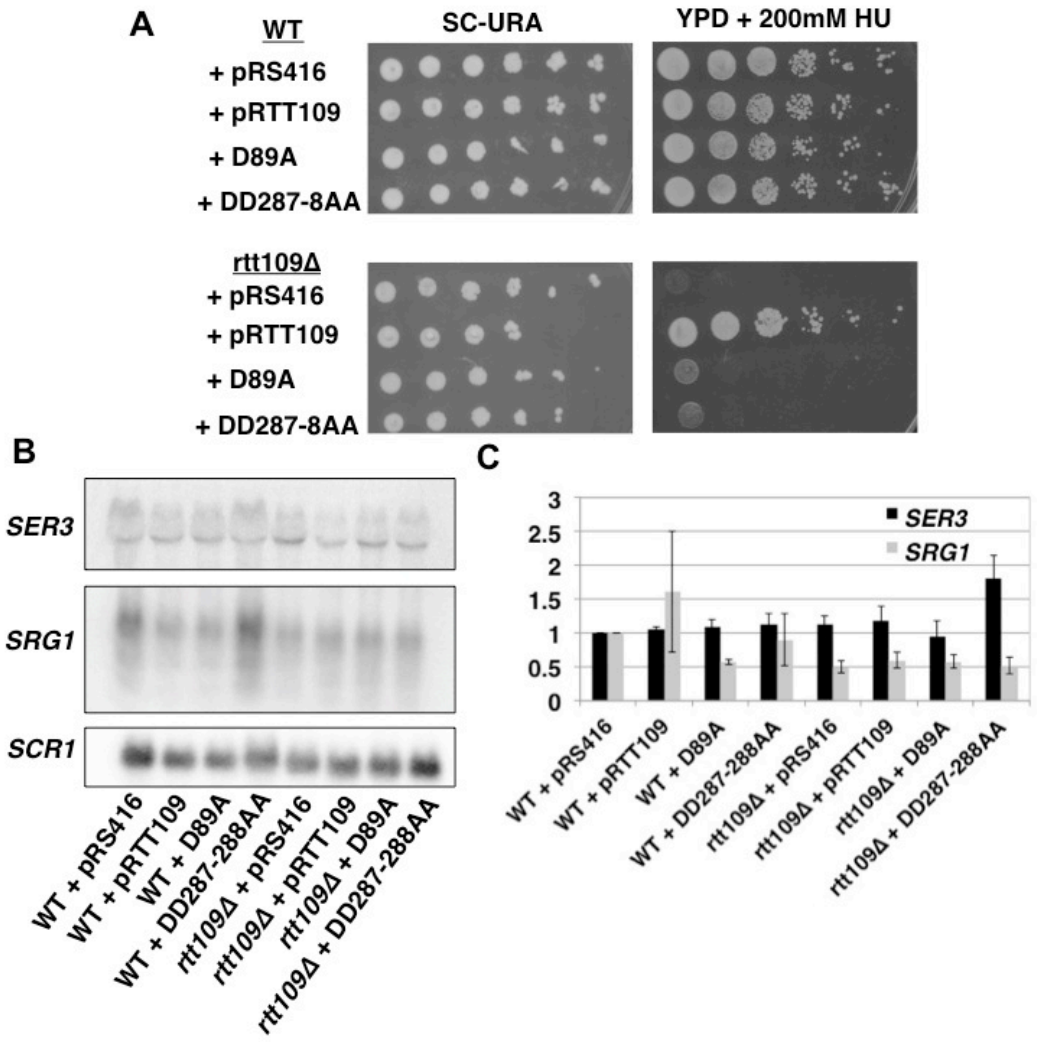

Figure 41: Plasmid complementation of *rtt109*Δ strains.

A) Serial dilution analysis of wild type or *rtt109*Δ strains transformed with empty vector (pRS416), wild type *RTT109* (pRTT109), or catalytic mutants of *RTT109* (D89A or DD287-288AA) expressed from a plasmid. 10-fold serial dilutions of each strain were spotted onto SC-ura (control) and YPD plates containing 200mM HU and incubated for 3 days at 30°C. B) Representative Northern analysis of *SER3*, *SRG1*, and *SCR1* levels in strains used in A. C) Quantitation of Northern results from 4 independent strains. The values shown are the mean *SER3* (black) and *SRG1* (grey) transcript levels that have been normalized to the *SCR1* loading control and made relative to the wild-type strains. Error bars indicate SEM.

Although the newly created *rtt109*Δ strain with pRS416 showed no effect on *SER3* levels, I carried out the initial plan to test new deletions of *RTT109, ASF1*, and *VPS75*. I started with the *rtt109*Δ strains complemented with pRTT109 and passaged them on non-selective media to allow for loss of the *URA3*-marked plasmid. I selected for loss on 5FOA plates and immediately grew these strains, and harvested RNA for Northern analysis. At the same time, I also created new *asf1*Δ and *vps75*Δ strains to retest their phenotypes. By Northern analysis, these *rtt109*Δ strains did show some effect on *SER3* levels, but it was the same effect as *asf1*Δ strains (2-3fold) suggesting Rtt109 may play a small role in *SER3* repression, but it is likely through a previously known function involving Asf1 (Figure 42).

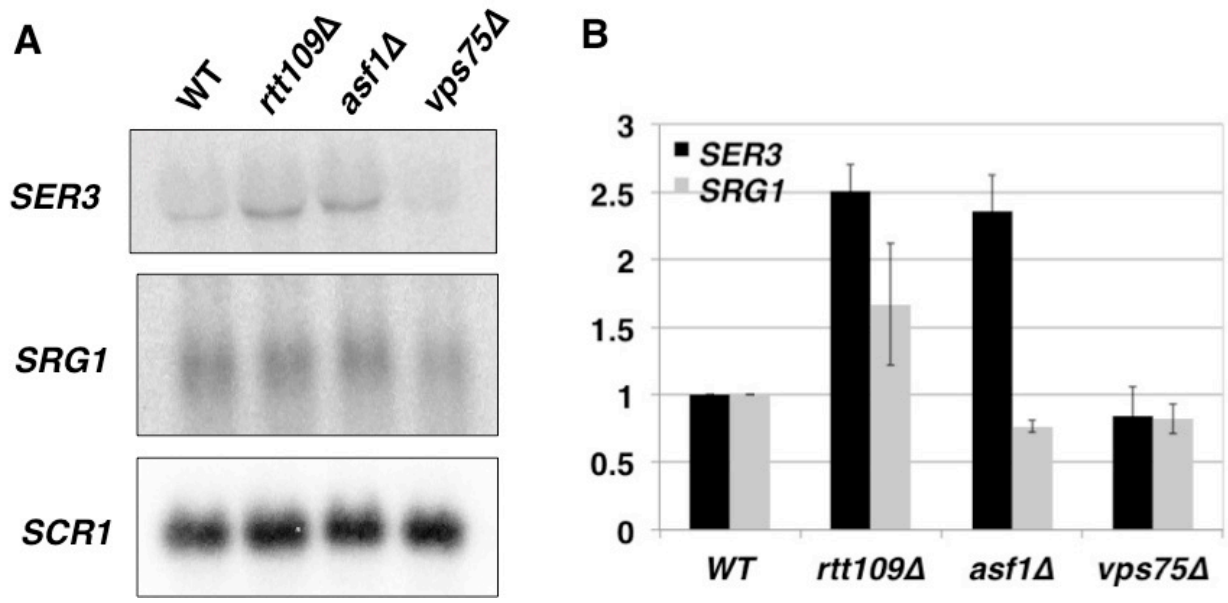

Figure 42: Northern analysis of newly created *rtt109*Δ *asf1*Δ and *vps75*Δ strains.

A) Representative Northern analysis of *SER3*, *SRG1*, and *SCR1* levels in wild type (FY4, FY5, YJ770, YJ771) and newly created *rtt109*Δ (YP286, YP287, YP288, YP289), *asf1*Δ (YP306, YP307, YP308, YP309), and *vps75*Δ (YP310, YP311, YP312, YP313) strains. B) Quantitation of Northern results from 4 independent strains. The values shown are the mean *SER3* (black) and *SRG1* (grey) transcript levels that have been normalized to the *SCR1* loading control and made relative to the wild-type strains. Error bars indicate SEM.

5.4 CONCLUSIONS

I identified Rtt109 in my genetic screen as a candidate factor involved in *SER3* repression. Initial results were promising as *rtt109*Δ strains showed strong derepression of *SER3*. However, deletion of known interacting partners, Asf1 and Vps75, or strains lacking Rtt109 acetylation targets, H3 K9 or K56, had only minor effects on *SER3* levels. This suggested a role for Rtt109 outside of its known roles in histone acetylation. Further studies showed Rtt109, Asf1, and Vps75 all weakly associate with *SRG1* transcription during *SER3* repression and they do not have much effect on the chromatin structure over the *SER3* promoter.

Unfortunately, the effect of *rtt109*Δ on *SER3* was not always reproducible; it varied from strain to strain and from experiment to experiment. This could be due to the inherent genomic instability of *rtt109*Δ strains due to defects in replication and DNA repair (Driscoll et al. 2007; Han et al. 2007). *rtt109*Δ strains also have shorter lifespans than wild type yeast suggesting aging of colonies on plates in between or during experiments could alter phenotypic results (Feser et al. 2010). Another possibility is through the role of Rtt109 in regulating expression of certain histone genes (Fillingham et al. 2009). We know that an imbalance in histone levels can lead to *SER3* derepression (Chapter 3.1) and may have contributed to the effects I have seen.

In order to best evaluate the effect of *rtt109*Δ strains on *SER3* and reduce experimental variability, I recreated the *rtt109*Δ::KanMX allele in strains that were complemented by expression of wild type *RTT109* from a *URA3*-marked plasmid. This allowed me to maintain *RTT109* expression until just before an experiment when I could select for loss of the plasmid and start with a fresh *rtt109*Δ strain. By doing this, my hypothesis was that the large defect in *SER3* repression would be seen in the new strains, indicating the lack of a defect seen in previous experiments was due to indirect effects or suppression in those strains. Conversely, my

experiments showed that these newly created *rtt109*Δ strains had only a minor effect on *SER3* derepression (2-3 fold). This suggests the stronger results were anomalies, and may be the result of second site mutations or altered histone levels in those strains. This may also be the case for the *rtt109*Δ strain in the deletion collection. Because Asf1 was not identified in the screen, a 2-3 fold derepression of *SER3* might have been below the limit of detection, suggesting Rtt109 was identified due to these stronger indirect effects.

The fact remains that Rtt109 does have a minor role in *SER3* repression but it is similar to Asf1. Although these two factors both affect histone expression levels, this is not likely to be related to *SER3* repression as Rtt109 plays a role in activating histone genes, while Asf1 is involved in their repression (Fillingham et al. 2009). Alternatively, these factors could contribute to *SER3* repression through their role in histone exchange during transcription. Genome-wide studies demonstrate a role for K56 acetylation in histone exchange during transcription as well as over promoter regions (Rufiange et al. 2007), and there is evidence of some exchange over the *SER3* promoter region (Thebault et al. 2011). My results would suggest that this exchange only plays a minor role in *SER3* repression suggesting that exchange is not a major source of the nucleosomes required for *SER3* repression (Chapter 3.4). Overall, these investigations of the role of Rtt109, Asf1, and Vps75 in *SER3* repression have yielded some interesting results, but will likely not be a significant avenue of future research.

6.0 DISCUSSION AND FUTURE DIRECTIONS

My studies into the mechanism of *SER3* repression by *SRG1* intergenic transcription have yielded new insights into this intriguing regulatory system. Using a genetic screen, I identified 21 factors required for full repression of *SER3* and further characterized a number of them. Many of these factors (H2A, Spt10, Spt21, Rtt106, Rtt109) are involved in the regulation of histone gene expression, highlighting the importance of maintaining proper histone levels, and implicating chromatin in the repression mechanism. This led us to uncover the basis of *SER3* repression, in which the intergenic transcription alone is not sufficient for repression, but acts to establish and maintain nucleosomes over the *SER3* promoter to prevent transcription factor binding (Hainer et al. 2011). Other results from the screen demonstrated a role for the transcription elongation complexes, Bur1/Bur2 and Spt4/Spt5, and led us to the Paf1 complex, which acts in the same pathway. I uncovered a role for the Paf1 complex in mediating repression of *SER3* by maintaining nucleosome occupancy over its promoter, possibly through the recruitment of Spt6 and Spt16 (Pruneski et al. 2011). These studies have contributed to our understanding of both the factors involved and the molecular details of *SER3* repression and laid the groundwork for future studies (Figure 43).

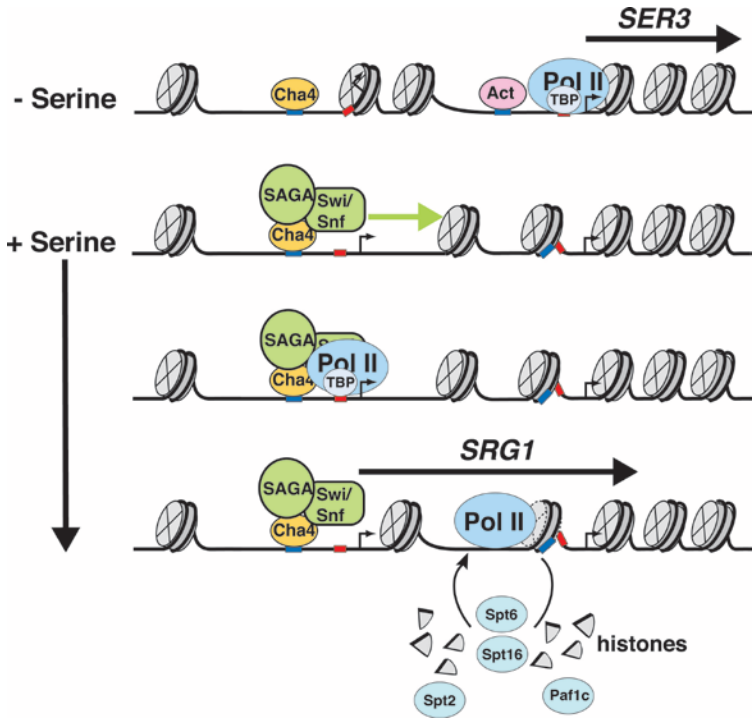

Figure 43: Model of *SER3* repression by *SRG1* intergenic transcription.

In the absence of serine, the Cha4 activator is bound to the *SRG1* promoter but is unable to initiate transcription. The *SER3* promoter is depleted of nucleosomes allowing proteins, either an as yet unknown sequence-specific activator or general transcription factors, to bind and activate *SER3* transcription. In response to serine, Cha4 recruits SAGA and Swi/Snf to reposition the nucleosomes at the 5` end of *SRG1* towards the *SER3* promoter, permitting initiation of *SRG1* transcription. These repositioned nucleosomes are then disassembled ahead of the transcribing RNA Pol II and reassembled after passage of RNA Pol II by the Spt6 and Spt16 histone chaperones. The Paf1 complex and Spt2 are required for nucleosome occupancy, possibly through the recruitment or activity of Spt6 and Spt16. The nucleosomes being maintained by *SRG1* transcription occlude the *SER3* promoter, preventing the binding of transcription factors and *SER3* transcription. Modified from (Pruneski and Martens 2011).

The widespread transcriptional activity in eukaryotic genomes can have profound effects on gene regulation; both through the ncRNAs produced as well the process of transcription itself. The regulation of *SER3* by *SRG1* intergenic transcription represents the first and best characterized case of intergenic transcription regulating neighboring gene expression. This mechanism allows for the rapid activation and repression of *SER3* in response to changes in cellular serine levels. Moreover, the coordinated regulation of *SRG1* and *CHA1* by Cha4 allows a single activator to both activate and repress opposing pathways in response to a single environmental stimulus.

Since the discovery of *SER3* regulation by intergenic transcription, similar mechanisms have been seen at other yeast genes and in higher eukaryotes, suggesting the mechanism may be common and conserved (Ashe et al. 1997; Gribnau et al. 2000; Schmitt et al. 2005; Bird et al. 2006; Petruk et al. 2006). Indeed, since budding yeast lack the traditional RNAi pathways of higher eukaryotes, other mechanisms of regulation by non-coding transcription may perform these regulatory functions (Harrison et al. 2009). Interestingly, some ncRNAs are stable in the cell and have been detected associating with polyribosomes, suggesting they could be translated and might form the basis for evolution of new protein-coding genes (Thompson and Parker 2007). To fully understand the extent and impact of non-coding transcription, it will be important, not only to discover new cases of regulation, but also to understand the detailed mechanisms by which transcription of ncDNA can regulate expression of protein-coding genes.

Our studies of the mechanism of *SER3* repression by a repressive nucleosome structure that is established and maintained by *SRG1* transcription have given insight into the regulation of transcription beyond this locus. *SRG1-SER3* has proven to be a good model system to study the general process of transcription-dependent chromatin dynamics (Hainer and Martens 2011a;

Pruneski et al. 2011). *SRG1* transcription has interesting properties compared to transcription over protein coding genes as it is involved in maintaining nucleosomes rather than removing them. It also allows nucleosomes to occupy a promoter region that inherently disfavors nucleosome formation. The instability of the nucleosomes placed over the *SER3* promoter, therefore, makes them very sensitive to changes in nucleosome dynamics and allows us to study effects that may be too subtle to be seen at other locations. This feature can be exploited to uncover the details of transcription-dependent nucleosome disassembly and assembly, such as the order of factors required and the source of the nucleosomes during this process.

If *SRG1* transcription from intergenic DNA is required to maintain nucleosomes over the *SER3* UAS, then from where might these nucleosomes originate? An intriguing source of these nucleosomes would be those positioned over the *SRG1* transcription start site and TATA (Figure 43), which likely inhibit *SRG1* transcription in the absence of serine. Based on this study and our previous work (Martens and Winston 2002; Martens et al. 2004; Martens et al. 2005), Swi/Snf, when recruited to the *SRG1* promoter in response to serine, may slide these nucleosomes toward *SER3*, to facilitate pre-initiation complex assembly and *SRG1* transcription. Once RNA Pol II begins to transcribe *SRG1*, the nucleosomes originally moved by Swi/Snf are disassembled to allow passage of RNA Pol II and then reassembled behind RNA Pol II by Spt6 and Spt16 (Figure 43). Therefore, the activities of Swi/Snf, Spt6/Spn1, and FACT may combine to establish and maintain nucleosomes over the *SER3* promoter, which interfere with transcription factor binding to this region. This scenario would also explain the difference in nucleosome occupancy at the 5' end of *SRG1* observed for wild-type cells grown in the serine starvation media as compared to *srg1-1* cells grown in serine-rich media, two conditions in which *SER3* is strongly derepressed. In contrast to wild-type cells grown in serine starvation

medium where it is no longer recruited, Swi/Snf is presumably still recruited by Cha4 in the *srg1-1* (*SRG1* TATA mutant) cells that are grown in serine-rich media. Thus, Swi/Snf can remodel the nucleosomes at the 5' end of *SRG1*; however, these nucleosomes cannot be maintained in the absence of *SRG1* transcription.

My investigations into the role of the Paf1 complex in *SER3* repression have revealed a potentially novel function for this well-studied complex. The Paf1 complex is required to maintain nucleosome occupancy over the *SER3* promoter during repression. This activity relies mostly on the Paf1 and Ctr9 subunits with little contribution from Paf1 complex-regulated histone modifications or any of its other known functions, such as CTD phosphorylation or transcription termination.

How might Paf1 and Ctr9 promote *SRG1* transcription-dependent nucleosome occupancy at the *SER3* promoter? One possibility is that Paf1 regulates the histone chaperones Spt6 and/or Spt16, which are required for the maintenance of nucleosomes over the *SER3* promoter (Hainer et al. 2011). In yeast, the Paf1 complex has genetic interactions with Spt6 (Kaplan et al. 2005) and both physical and genetic interactions with Spt16 (Squazzo et al. 2002; Pavri et al. 2006). The Paf1 complex has also been shown in *Drosophila* to be required for full recruitment of Spt6 and the FACT subunit SSRP1 during transcription (Adelman et al. 2006). These connections led me to examine whether the recruitment of these factors is affected in Paf1 complex mutants in yeast. ChIP experiments revealed that Spt16 occupancy over *SRG1* is strongly dependent on Paf1 but not Rtf1, which correlates with the effect that each of these factors have on *SER3* repression. Western analyses and RNA Pol II ChIP data indicate that this reduction in Spt16 occupancy in *paf1*Δ cells is not caused by a reduction in Spt16 protein levels or by a reduction in RNA Pol II levels across *SRG1*. Taken together, my results support a role for Paf1 and Ctr9 in

promoting FACT occupancy across actively transcribed *SRG1*. In contrast to Spt16, I found Spt6 occupancy at *SRG1* to be only modestly dependent on Paf1. Therefore, while Paf1 and Ctr9 may play a prominent role in Spt16 occupancy at *SRG1*, other factors are likely to contribute significantly to Spt6 occupancy of this region. Spt6 is known to associate with elongating Pol II through a direct interaction with Pol II CTD containing Ser2-P (Diebold et al. 2010; Sun et al. 2010). Although it is unlikely to be part of the Paf1-dependent pathway that represses *SER3* as I discussed earlier, this mark may contribute to Spt6 occupancy at *SRG1* in a Paf1-independent pathway. Interestingly, Thebault et al. recently reported that Spt6 occupancy at *SRG1* is also partially dependent on Spt2, an HMG-like transcription elongation factor (Thebault et al. 2011). Although I cannot rule out the possibility that Spt2 contributes to Spt6 occupancy in a pathway with Paf1, Spt2 may also facilitate Spt6 recruitment independently of Paf1. Overall, my results are consistent with those observed in *Drosophila*, in which depletion of Paf1, and to a lesser extent, Rtf1, led to reduced association of Spt6 and FACT over an actively transcribed gene, without affecting Pol II association or global protein levels (Adelman et al. 2006). Interestingly, localization of the Paf1 complex to actively transcribed genes has also been shown to be partially dependent on Spt6 and Spt16 (Kaplan et al. 2005; Pavri et al. 2006). Therefore, it is possible that recruitment of Spt6, Spt16, and the Paf1 complex is interdependent, where the disruption of one of these factors results in reduced association of the others. Detailed studies will be necessary to determine their precise order or recruitment, how interdependent they are on each other, and at which step in the process each factor functions.

It is also not clear if these factors function together for transcription-dependent nucleosome assembly at other genes. In addition to *SRG1*, I assayed the effect of deleting *PAF1* on histone H3, Spt6, and Spt16 occupancy over the transcribed regions of two other highly

transcribed genes, *PMA1* and *ADH1*. Similar to my results for *SRG1*, I detected reduced Spt6 and Spt16 occupancy in cells lacking Paf1. However, in contrast to what I observed at *SRG1*, RNA Pol II levels at these genes were also slightly reduced in *paf1*Δ cells. Gene-specific differences in RNA Pol II occupancy have been previously reported for *paf1*Δ mutants (Mueller et al. 2004). When normalized to RNA Pol II levels, there is no longer a reduction in Spt6 occupancy, while Spt16 occupancy is reduced to similar levels in both *paf1*Δ and *rtf1*Δ mutants. Furthermore, histone H3 occupancy was unaffected at *ADH1* and only moderately reduced at *PMA1* in *paf1*Δ cells. These studies suggest that there are likely to be additional factors that influence the role of the Paf1 complex in regulating transcription-coupled nucleosome assembly at specific genes. One factor that may contribute to these gene-specific differences is the properties of the transcribed DNA. *SRG1* is transcribed across the promoter region of *SER3*. In general, promoter regions tend to be comprised of sequences that are refractory to nucleosome formation, whereas the sequence of ORFs generally do not contain these properties (Segal and Widom 2009). This would explain the inherent instability of nucleosomes over the *SER3* promoter in the absence of *SRG1* transcription. This characteristic of the *SRG1* transcription unit makes it unique compared to the transcription of most protein-coding genes and may have allowed me to uncover this new role for the Paf1 complex that may not be readily detectable at other transcribed regions of the genome. My results indicate there may be gene-specific requirements for these factors, but more genes will have to be tested to understand the reasons why some regions are affected and others are not.

The *SER3* promoter is contained within the *SRG1* transcription unit, a setup that resembles cryptic promoters within protein-coding genes that can be spuriously activated in the absence of nucleosome reassembly by Spt6/Spn1 and FACT or a cascade of transcription-

dependent post-translational modifications of histones (Lee and Shilatifard 2007; Li et al. 2007a). However, our studies show that *SER3* repression appears to be independent of at least some of these marks, including Set1-mediated methylation of histone H3 K4, Set2-mediated methylation of K36, and the removal of histone H3 and H4 acetylation by the Rpd3S and Set3C histone deacetylase complexes. Although we cannot rule out the possibility that other post-translational histone modifications may be involved, our results indicate a difference in the requirement of transcription-dependent post-translational histone modifications between *SER3* repression by *SRG1* transcription and repression of cryptic intragenic transcription. This difference may be related to the fact that *SRG1* is a relatively short transcription unit (~400 base pairs) that is highly transcribed. It has been recently reported that cryptic intragenic transcription preferentially occurs at lowly transcribed genes (Li et al. 2007b; Cheung et al. 2008; Lickwar et al. 2009). Therefore, it is possible that highly transcribed *SRG1* may not be dependent on H3K36 methylation and subsequent histone deacetylation for protection from intragenic transcription because of the frequent passage of RNA Pol II. Alternatively, short, highly transcribed genes may never establish this histone mark since histone H3 K36 methylation predominates towards the 3' ends of transcribed genes (Pokholok et al. 2005). In support of this possibility, genome-wide analyses of K36 methylation indicate little K36 trimethylation at *SRG1* (Pokholok et al. 2005).

The importance of both nucleosome architecture and the Paf1 complex during *SER3* repression raises the question of whether these mechanisms are necessary for other cases of yeast genes regulated by intergenic transcription. Since some of the other examples have already been discovered and others are likely to be found in the future, it will be interesting to determine what factors are shared among all mechanisms and which are locus-dependent. Our results

demonstrate that transcription of non-coding DNA can affect chromatin structure and involves factors, such as Spt6, Spt16, Spt2, and the Paf1 complex, that are evolutionarily conserved and important for the transcription of many genes. This highlights the importance of considering the effect pervasive transcription can have on a genome, not just on gene expression, but on every DNA-mediated process.

BIBLIOGRAPHY

Adelman K, Wei W, Ardehali MB, Werner J, Zhu B, Reinberg D, Lis JT. 2006. Drosophila Paf1 modulates chromatin structure at actively transcribed genes. *Mol Cell Biol* **26**: 250-260.

Adkins MW, Tyler JK. 2006. Transcriptional activators are dispensable for transcription in the absence of Spt6-mediated chromatin reassembly of promoter regions. *Mol Cell* **21**: 405-416.

Adkins MW, Williams SK, Linger J, Tyler JK. 2007. Chromatin disassembly from the PHO5 promoter is essential for the recruitment of the general transcription machinery and coactivators. *Mol Cell Biol* **27**: 6372-6382.

Ahmad M, Bussey H. 1986. Yeast arginine permease: nucleotide sequence of the CAN1 gene. *Curr Genet* **10**: 587-592.

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.

Akhtar MS, Heidemann M, Tietjen JR, Zhang DW, Chapman RD, Eick D, Ansari AZ. 2009. TFIIH kinase places bivalent marks on the carboxy-terminal domain of RNA polymerase II. *Mol Cell* **34**: 387-393.

Albaugh BN, Arnold KM, Lee S, Denu JM. 2011. Autoacetylation of the histone acetyltransferase Rtt109. *J Biol Chem* **286**: 24694-24701.

Albers E, Laize V, Blomberg A, Hohmann S, Gustafsson L. 2003. Ser3p (Yer081wp) and Ser33p (Yil074cp) are phosphoglycerate dehydrogenases in Saccharomyces cerevisiae. *J Biol Chem* **278**: 10264-10272.

Albert I, Mavrich TN, Tomsho LP, Qi J, Zanton SJ, Schuster SC, Pugh BF. 2007. Translational and rotational settings of H2A.Z nucleosomes across the Saccharomyces cerevisiae genome. *Nature* **446**: 572-576.

Ansari A, Hampsey M. 2005. A role for the CPF 3'-end processing machinery in RNAP II-dependent gene looping. *Genes Dev* **19**: 2969-2978.

Ardehali MB, Yao J, Adelman K, Fuda NJ, Petesch SJ, Webb WW, Lis JT. 2009. Spt6 enhances the elongation rate of RNA polymerase II in vivo. *EMBO J* **28**: 1067-1077.

Arigo JT, Carroll KL, Ames JM, Corden JL. 2006. Regulation of yeast NRD1 expression by premature transcription termination. *Mol Cell* **21**: 641-651.

Ashe HL, Monks J, Wijgerde M, Fraser P, Proudfoot NJ. 1997. Intergenic transcription and transinduction of the human beta-globin locus. *Genes Dev* **11**: 2494-2509.

Ausubel F, Brent F, Kingston R, Moore D, Seidman J, Smith J, Struhl K. 1991. Current Protocols in Molecular Biology. John Wiley and Sons, New York.

Avvakumov N, Nourani A, Cote J. 2011. Histone chaperones: modulators of chromatin marks. *Mol Cell* **41**: 502-514.

Ban N, Nissen P, Hansen J, Moore PB, Steitz TA. 2000. The complete atomic structure of the large ribosomal subunit at 2.4 A resolution. *Science* **289**: 905-920.

Basehoar AD, Zanton SJ, Pugh BF. 2004. Identification and distinct regulation of yeast TATA box-containing genes. *Cell* **116**: 699-709.

Basrai MA, Kingsbury J, Koshland D, Spencer F, Hieter P. 1996. Faithful chromosome transmission requires Spt4p, a putative regulator of chromatin structure in Saccharomyces cerevisiae. *Mol Cell Biol* **16**: 2838-2847.

Belotserkovskaya R, Oh S, Bondarenko VA, Orphanides G, Studitsky VM, Reinberg D. 2003. FACT facilitates transcription-dependent nucleosome alteration. *Science* **301**: 1090-1093.

Bengtson MH, Joazeiro CA. 2010. Role of a ribosome-associated E3 ubiquitin ligase in protein quality control. *Nature* **467**: 470-473.

Berndsen CE, Tsubota T, Lindner SE, Lee S, Holton JM, Kaufman PD, Keck JL, Denu JM. 2008. Molecular functions of the histone acetyltransferase chaperone complex Rtt109-Vps75. *Nat Struct Mol Biol* **15**: 948-956.

Berretta J, Morillon A. 2009. Pervasive transcription constitutes a new level of eukaryotic genome regulation. *EMBO Rep* **10**: 973-982.

Bird AJ, Gordon M, Eide DJ, Winge DR. 2006. Repression of ADH1 and ADH3 during zinc deficiency by Zap1-induced intergenic RNA transcripts. *EMBO J* **25**: 5726-5734.

Bortvin A, Winston F. 1996. Evidence that Spt6p controls chromatin structure by a direct interaction with histones. *Science* **272**: 1473-1476.

Bowman A, Ward R, Wiechens N, Singh V, El-Mkami H, Norman DG, Owen-Hughes T. 2011. The histone chaperones Nap1 and Vps75 bind histones H3 and H4 in a tetrameric conformation. *Mol Cell* **41**: 398-408.

Brachmann CB, Davies A, Cost GJ, Caputo E, Li J, Hieter P, Boeke JD. 1998. Designer deletion strains derived from Saccharomyces cerevisiae S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast* **14**: 115-132.

Braun MA, Costa PJ, Crisucci EM, Arndt KM. 2007. Identification of Rkr1, a nuclear RING domain protein with functional connections to chromatin modification in Saccharomyces cerevisiae. *Mol Cell Biol* **27**: 2800-2811.

Breslow DK, Cameron DM, Collins SR, Schuldiner M, Stewart-Ornstein J, Newman HW, Braun S, Madhani HD, Krogan NJ, Weissman JS. 2008. A comprehensive strategy enabling high-resolution functional analysis of the yeast genome. *Nat Meth* **5**: 711-718.

Brickner DG, Cajigas I, Fondufe-Mittendorf Y, Ahmed S, Lee PC, Widom J, Brickner JH. 2007. H2A.Z-mediated localization of genes at the nuclear periphery confers epigenetic memory of previous transcriptional state. *PLoS Biol* **5**: e81.

Bumgarner SL, Dowell RD, Grisafi P, Gifford DK, Fink GR. 2009. Toggle involving cis-interfering noncoding RNAs controls variegated gene expression in yeast. *Proc Natl Acad Sci U S A* **106**: 18321-18326.

Buratowski S. 2009. Progression through the RNA polymerase II CTD cycle. *Mol Cell* **36**: 541-546.

Burgess RJ, Zhou H, Han J, Zhang Z. 2010. A role for Gcn5 in replication-coupled nucleosome assembly. *Mol Cell* **37**: 469-480.

Cairns BR. 2009. The logic of chromatin architecture and remodelling at promoters. *Nature* **461**: 193-198.

Callahan KP, Butler JS. 2010. TRAMP complex enhances RNA degradation by the nuclear exosome component Rrp6. *J Biol Chem* **285**: 3540-3547.

Camblong J, Beyrouthy N, Guffanti E, Schlaepfer G, Steinmetz LM, Stutz F. 2009. Trans-acting antisense RNAs mediate transcriptional gene cosuppression in S. cerevisiae. *Genes Dev* **23**: 1534-1545.

Camblong J, Iglesias N, Fickentscher C, Dieppois G, Stutz F. 2007. Antisense RNA stabilization induces transcriptional gene silencing via histone deacetylation in S. cerevisiae. *Cell* **131**: 706-717.

Campos EI, Reinberg D. 2009. Histones: annotating chromatin. *Annu Rev Genet* **43**: 559-599.

Carrozza MJ, Li B, Florens L, Suganuma T, Swanson SK, Lee KK, Shia WJ, Anderson S, Yates J, Washburn MP et al. 2005. Histone H3 methylation by Set2 directs deacetylation of coding regions by Rpd3S to suppress spurious intragenic transcription. *Cell* **123**: 581-592.

Chai B, Huang J, Cairns BR, Laurent BC. 2005. Distinct roles for the RSC and Swi/Snf ATP-dependent chromatin remodelers in DNA double-strand break repair. *Genes Dev* **19**: 1656-1661.

Chapman RD, Heidemann M, Albert TK, Mailhammer R, Flatley A, Meisterernst M, Kremmer E, Eick D. 2007. Transcribing RNA polymerase II is phosphorylated at CTD residue serine-7. *Science* **318**: 1780-1782.

Chaudhary K, Deb S, Moniaux N, Ponnusamy MP, Batra SK. 2007. Human RNA polymerase II-associated factor complex: dysregulation in cancer. *Oncogene* **26**: 7499-7507.

Chen Y, Yamaguchi Y, Tsugeno Y, Yamamoto J, Yamada T, Nakamura M, Hisatake K, Handa H. 2009. DSIF, the Paf1 complex, and Tat-SF1 have nonredundant, cooperative roles in RNA polymerase II elongation. *Genes Dev* **23**: 2765-2777.

Cheng YL, Chen RH. 2010. The AAA-ATPase Cdc48 and cofactor Shp1 promote chromosome bi-orientation by balancing Aurora B activity. *J Cell Sci* **123**: 2025-2034.

Cheung V, Chua G, Batada NN, Landry CR, Michnick SW, Hughes TR, Winston F. 2008. Chromatin- and transcription-related factors repress transcription from within coding regions throughout the Saccharomyces cerevisiae genome. *PLoS Biol* **6**: e277.

Cho EJ, Kobor MS, Kim M, Greenblatt J, Buratowski S. 2001. Opposing effects of Ctk1 kinase and Fcp1 phosphatase at Ser 2 of the RNA polymerase II C-terminal domain. *Genes Dev* **15**: 3319-3329.

Chu Y, Simic R, Warner MH, Arndt KM, Prelich G. 2007. Regulation of histone modification and cryptic transcription by the Bur1 and Paf1 complexes. *EMBO J* **26**: 4646-4656.

Chu Y, Sutton A, Sternglanz R, Prelich G. 2006. The BUR1 cyclin-dependent protein kinase is required for the normal pattern of histone methylation by SET2. *Mol Cell Biol* **26**: 3029-3038.

Churchman LS, Weissman JS. 2011. Nascent transcript sequencing visualizes transcription at nucleotide resolution. *Nature* **469**: 368-373.

Clapier CR, Cairns BR. 2009. The biology of chromatin remodeling complexes. *Annu Rev Biochem* **78**: 273-304.

Collins LJ. 2011. The RNA infrastructure: an introduction to ncRNA networks. *Advan Exper Med Biol* **722**: 1-19.

Costa PJ, Arndt KM. 2000. Synthetic lethal interactions suggest a role for the Saccharomyces cerevisiae Rtf1 protein in transcription elongation. *Genetics* **156**: 535-547.

Cote J, Peterson CL, Workman JL. 1998. Perturbation of nucleosome core structure by the SWI/SNF complex persists after its detachment, enhancing subsequent transcription factor binding. *Proc Natl Acad Sci U S A* **95**: 4947-4952.

Cote J, Quinn J, Workman JL, Peterson CL. 1994. Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. *Science* **265**: 53-60.

Couoh-Cardel SJ, Uribe-Carvajal S, Wilkens S, Garcia-Trejo JJ. 2010. Structure of dimeric F1F0-ATP synthase. *J Biol Chem* **285**: 36447-36455.

Cox JS, Chapman RE, Walter P. 1997. The unfolded protein response coordinates the production of endoplasmic reticulum protein and endoplasmic reticulum membrane. *Mol Biol Cell* **8**: 1805-1814.

Cramer P, Armache KJ, Baumli S, Benkert S, Brueckner F, Buchen C, Damsma GE, Dengl S, Geiger SR, Jasiak AJ et al. 2008. Structure of eukaryotic RNA polymerases. *Annu Rev Biophys* **37**: 337-352.

Cramer P, Bushnell DA, Fu J, Gnatt AL, Maier-Davis B, Thompson NE, Burgess RR, Edwards AM, David PR, Kornberg RD. 2000. Architecture of RNA polymerase II and implications for the transcription mechanism. *Science* **288**: 640-649.

Cramer P, Bushnell DA, Kornberg RD. 2001. Structural basis of transcription: RNA polymerase II at 2.8 angstrom resolution. *Science* **292**: 1863-1876.

Crisucci EM, Arndt KM. 2011. The Paf1 complex represses ARG1 transcription in Saccharomyces cerevisiae by promoting histone modifications. *Eukaryot Cell* **10**: 712-723.

Dai J, Hyland EM, Yuan DS, Huang H, Bader JS, Boeke JD. 2008. Probing nucleosome function: a highly versatile library of synthetic histone H3 and H4 mutants. *Cell* **134**: 1066-1078.

David L, Huber W, Granovskaia M, Toedling J, Palm CJ, Bofkin L, Jones T, Davis RW, Steinmetz LM. 2006. A high-resolution map of transcription in the yeast genome. *Proc Natl Acad Sci U S A* **103**: 5320-5325.

Davis CA, Ares M, Jr. 2006. Accumulation of unstable promoter-associated transcripts upon loss of the nuclear exosome subunit Rrp6p in Saccharomyces cerevisiae. *Proc Natl Acad Sci U S A* **103**: 3262-3267.

Diebold ML, Loeliger E, Koch M, Winston F, Cavarelli J, Romier C. 2010. Noncanonical tandem SH2 enables interaction of elongation factor Spt6 with RNA polymerase II. *J Biol Chem* **285**: 38389-38398.

Ding L, Paszkowski-Rogacz M, Nitzsche A, Slabicki MM, Heninger AK, de Vries I, Kittler R, Junqueira M, Shevchenko A, Schulz H et al. 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.

Dollard C, Ricupero-Hovasse SL, Natsoulis G, Boeke JD, Winston F. 1994. SPT10 and SPT21 are required for transcription of particular histone genes in Saccharomyces cerevisiae. *Mol Cell Biol* **14**: 5223-5228.

Driscoll R, Hudson A, Jackson SP. 2007. Yeast Rtt109 promotes genome stability by acetylating histone H3 on lysine 56. *Science* **315**: 649-652.

Durairaj G, Chaurasia P, Lahudkar S, Malik S, Shukla A, Bhaumik SR. 2010. Regulation of chromatin assembly/disassembly by Rtt109p, a histone H3 Lys56-specific acetyltransferase, in vivo. *J Biol Chem* **285**: 30472-30479.

Dvir A. 2002. Promoter escape by RNA polymerase II. *Biochim Biophys Acta* **1577**: 208-223.

Egloff S, O'Reilly D, Chapman RD, Taylor A, Tanzhaus K, Pitts L, Eick D, Murphy S. 2007. Serine-7 of the RNA polymerase II CTD is specifically required for snRNA gene expression. *Science* **318**: 1777-1779.

Eitoku M, Sato L, Senda T, Horikoshi M. 2008. Histone chaperones: 30 years from isolation to elucidation of the mechanisms of nucleosome assembly and disassembly. *Cell Mol Life Sci* **65**: 414-444.

English CM, Adkins MW, Carson JJ, Churchill ME, Tyler JK. 2006. Structural basis for the histone chaperone activity of Asf1. *Cell* **127**: 495-508.

Eriksson PR, Mendiratta G, McLaughlin NB, Wolfsberg TG, Marino-Ramirez L, Pompa TA, Jainerin M, Landsman D, Shen CH, Clark DJ. 2005. Global regulation by the yeast Spt10 protein is mediated through chromatin structure and the histone upstream activating sequence elements. *Mol Cell Biol* **25**: 9127-9137.

Fassler JS, Winston F. 1988. Isolation and analysis of a novel class of suppressor of Ty insertion mutations in Saccharomyces cerevisiae. *Genetics* **118**: 203-212.

Ferreira ME, Flaherty K, Prochasson P. 2011. The Saccharomyces cerevisiae histone chaperone Rtt106 mediates the cell cycle recruitment of SWI/SNF and RSC to the HIR-dependent histone genes. *PLoS One* **6**: e21113.

Feser J, Truong D, Das C, Carson JJ, Kieft J, Harkness T, Tyler JK. 2010. Elevated histone expression promotes life span extension. *Mol Cell* **39**: 724-735.

Field Y, Kaplan N, Fondufe-Mittendorf Y, Moore IK, Sharon E, Lubling Y, Widom J, Segal E. 2008. Distinct modes of regulation by chromatin encoded through nucleosome positioning signals. *PLoS Comput Biol* **4**: e1000216.

Fillingham J, Kainth P, Lambert JP, van Bakel H, Tsui K, Pena-Castillo L, Nislow C, Figeys D, Hughes TR, Greenblatt J et al. 2009. Two-color cell array screen reveals interdependent roles for histone chaperones and a chromatin boundary regulator in histone gene repression. *Mol Cell* **35**: 340-351.

Fillingham J, Recht J, Silva AC, Suter B, Emili A, Stagljar I, Krogan NJ, Allis CD, Keogh MC, Greenblatt JF. 2008. Chaperone control of the activity and specificity of the histone H3 acetyltransferase Rtt109. *Mol Cell Biol* **28**: 4342-4353.

Floer M, Wang X, Prabhu V, Berrozpe G, Narayan S, Spagna D, Alvarez D, Kendall J, Krasnitz A, Stepansky A et al. 2010. A RSC/nucleosome complex determines chromatin architecture and facilitates activator binding. *Cell* **141**: 407-418.

Formosa T, Eriksson P, Wittmeyer J, Ginn J, Yu Y, Stillman DJ. 2001. Spt16-Pob3 and the HMG protein Nhp6 combine to form the nucleosome-binding factor SPN. *EMBO J* **20**: 3506-3517.

Gambus A, Jones RC, Sanchez-Diaz A, Kanemaki M, van Deursen F, Edmondson RD, Labib K. 2006. GINS maintains association of Cdc45 with MCM in replisome progression complexes at eukaryotic DNA replication forks. *Nat Cell Biol* **8**: 358-366.

Gelbart ME, Kuroda MI. 2009. Drosophila dosage compensation: a complex voyage to the X chromosome. *Development* **136**: 1399-1410.

Ghaemmaghami S, Huh WK, Bower K, Howson RW, Belle A, Dephoure N, O'Shea EK, Weissman JS. 2003. Global analysis of protein expression in yeast. *Nature* **425**: 737-741.

Ghildiyal M, Zamore PD. 2009. Small silencing RNAs: an expanding universe. *Nat Rev Genet* **10**: 94-108.

Gnatt AL, Cramer P, Fu J, Bushnell DA, Kornberg RD. 2001. Structural basis of transcription: an RNA polymerase II elongation complex at 3.3 A resolution. *Science* **292**: 1876-1882.

Goodrich JA, Kugel JF. 2009. From bacteria to humans, chromatin to elongation, and activation to repression: The expanding roles of noncoding RNAs in regulating transcription. *Crit Rev Biochem Mol Biol* **44**: 3-15.

Greger IH, Aranda A, Proudfoot N. 2000. Balancing transcriptional interference and initiation on the GAL7 promoter of Saccharomyces cerevisiae. *Proc Natl Acad Sci U S A* **97**: 8415-8420.

Gribnau J, Diderich K, Pruzina S, Calzolari R, Fraser P. 2000. Intergenic transcription and developmental remodeling of chromatin subdomains in the human beta-globin locus. *Mol Cell* **5**: 377-386.

Groth A, Corpet A, Cook AJ, Roche D, Bartek J, Lukas J, Almouzni G. 2007. Regulation of replication fork progression through histone supply and demand. *Science* **318**: 1928-1931.

Guelin E, Chevallier J, Rigoulet M, Guerin B, Velours J. 1993. ATP synthase of yeast mitochondria. Isolation and disruption of the ATP epsilon gene. *J Biol Chem* **268**: 161-167.

Guillemette B, Bataille AR, Gevry N, Adam M, Blanchette M, Robert F, Gaudreau L. 2005. Variant histone H2A.Z is globally localized to the promoters of inactive yeast genes and regulates nucleosome positioning. *PLoS Biol* **3**: e384.

Guillemette B, Drogaris P, Lin HH, Armstrong H, Hiragami-Hamada K, Imhof A, Bonneil E, Thibault P, Verreault A, Festenstein RJ. 2011. H3 lysine 4 is acetylated at active gene promoters and is regulated by H3 lysine 4 methylation. *PLoS Genet* **7**: e1001354.

Guttman M, Amit I, Garber M, French C, Lin MF, Feldser D, Huarte M, Zuk O, Carey BW, Cassady JP et al. 2009. Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. *Nature* **458**: 223-227.

Guttman M, Garber M, Levin JZ, Donaghey J, Robinson J, Adiconis X, Fan L, Koziol MJ, Gnirke A, Nusbaum C et al. 2010. Ab initio reconstruction of cell type-specific transcriptomes in mouse reveals the conserved multi-exonic structure of lincRNAs. *Nat Biotech* **28**: 503-510.

Haase E, Brendel M. 1990. A recessive mutant allele of the HNM1 gene of Saccharomyces cerevisiae is responsible for hyper-resistance to nitrogen mustard. *Curr Genet* **18**: 187-192.

Hainer SJ, Martens JA. 2011a. Identification of histone mutants that are defective for transcription-coupled nucleosome occupancy. *Mol Cell Biol* **31**: 3557-3568.

Hainer SJ, Martens JA. 2011b. Transcription of ncDNA: Many roads lead to local gene regulation. *Transcription* **2**: 120-123.

Hainer SJ, Pruneski JA, Mitchell RD, Monteverde RM, Martens JA. 2011. Intergenic transcription causes repression by directing nucleosome assembly. *Genes Dev* **25**: 29-40.

Han J, Zhou H, Horazdovsky B, Zhang K, Xu RM, Zhang Z. 2007. Rtt109 acetylates histone H3 lysine 56 and functions in DNA replication. *Science* **315**: 653-655.

Harrison BR, Yazgan O, Krebs JE. 2009. Life without RNAi: noncoding RNAs and their functions in Saccharomyces cerevisiae. *Biochem Cell Biol* **87**: 767-779.

Hartzog GA, Wada T, Handa H, Winston F. 1998. Evidence that Spt4, Spt5, and Spt6 control transcription elongation by RNA polymerase II in Saccharomyces cerevisiae. *Genes Dev* **12**: 357-369.

He Y, Vogelstein B, Velculescu VE, Papadopoulos N, Kinzler KW. 2008. The antisense transcriptomes of human cells. *Science* **322**: 1855-1857.

Henry KW, Wyce A, Lo WS, Duggan LJ, Emre NC, Kao CF, Pillus L, Shilatifard A, Osley MA, Berger SL. 2003. Transcriptional activation via sequential histone H2B ubiquitylation and deubiquitylation, mediated by SAGA-associated Ubp8. *Genes Dev* **17**: 2648-2663.

Hess D, Liu B, Roan NR, Sternglanz R, Winston F. 2004. Spt10-dependent transcriptional activation in Saccharomyces cerevisiae requires both the Spt10 acetyltransferase domain and Spt21. *Mol Cell Biol* **24**: 135-143.

Holmberg S, Schjerling P. 1996. Cha4p of Saccharomyces cerevisiae activates transcription via serine/threonine response elements. *Genetics* **144**: 467-478.

Holstege FC, Jennings EG, Wyrick JJ, Lee TI, Hengartner CJ, Green MR, Golub TR, Lander ES, Young RA. 1998. Dissecting the regulatory circuitry of a eukaryotic genome. *Cell* **95**: 717-728.

Hongay CF, Grisafi PL, Galitski T, Fink GR. 2006. Antisense transcription controls cell fate in Saccharomyces cerevisiae. *Cell* **127**: 735-745.

Hou H, Yu H. 2010. Structural insights into histone lysine demethylation. *Current opinion in structural biology* **20**: 739-748.

Houseley J, Rubbi L, Grunstein M, Tollervey D, Vogelauer M. 2008. A ncRNA modulates histone modification and mRNA induction in the yeast GAL gene cluster. *Mol Cell* **32**: 685-695.

Huang S, Zhou H, Katzmann D, Hochstrasser M, Atanasova E, Zhang Z. 2005. Rtt106p is a histone chaperone involved in heterochromatin-mediated silencing. *Proc Natl Acad Sci U S A* **102**: 13410-13415.

Huang S, Zhou H, Tarara J, Zhang Z. 2007. A novel role for histone chaperones CAF-1 and Rtt106p in heterochromatin silencing. *EMBO J* **26**: 2274-2283.

Huang YC, Chen HT, Teng SC. 2010. Intragenic transcription of a noncoding RNA modulates expression of ASP3 in budding yeast. *RNA* **16**: 2085-2093.

Imbeault D, Gamar L, Rufiange A, Paquet E, Nourani A. 2008. The Rtt106 histone chaperone is functionally linked to transcription elongation and is involved in the regulation of spurious transcription from cryptic promoters in yeast. *J Biol Chem* **283**: 27350-27354.

Ioshikhes IP, Albert I, Zanton SJ, Pugh BF. 2006. Nucleosome positions predicted through comparative genomics. *Nat Genet* **38**: 1210-1215.

Izban MG, Luse DS. 1991. Transcription on nucleosomal templates by RNA polymerase II in vitro: inhibition of elongation with enhancement of sequence-specific pausing. *Genes Dev* **5**: 683-696.

Jacquier A. 2009. The complex eukaryotic transcriptome: unexpected pervasive transcription and novel small RNAs. *Nat Rev Genet* **10**: 833-844.

Jaehning JA. 2010. The Paf1 complex: platform or player in RNA polymerase II transcription? *Biochim Biophys Acta* **1799**: 379-388.

Jamai A, Puglisi A, Strubin M. 2009. Histone chaperone spt16 promotes redeposition of the original h3-h4 histones evicted by elongating RNA polymerase. *Mol Cell* **35**: 377-383.

Jenks MH, O'Rourke TW, Reines D. 2008. Properties of an intergenic terminator and start site switch that regulate IMD2 transcription in yeast. *Mol Cell Biol* **28**: 3883-3893.

Joshi AA, Struhl K. 2005. Eaf3 chromodomain interaction with methylated H3-K36 links histone deacetylation to Pol II elongation. *Mol Cell* **20**: 971-978.

Kao CF, Hillyer C, Tsukuda T, Henry K, Berger S, Osley MA. 2004. Rad6 plays a role in transcriptional activation through ubiquitylation of histone H2B. *Genes Dev* **18**: 184-195.

Kaplan CD, Holland MJ, Winston F. 2005. Interaction between transcription elongation factors and mRNA 3'-end formation at the Saccharomyces cerevisiae GAL10-GAL7 locus. *J Biol Chem* **280**: 913-922.

Kaplan CD, Laprade L, Winston F. 2003. Transcription elongation factors repress transcription initiation from cryptic sites. *Science* **301**: 1096-1099.

Kaplan N, Moore IK, Fondufe-Mittendorf Y, Gossett AJ, Tillo D, Field Y, LeProust EM, Hughes TR, Lieb JD, Widom J et al. 2009. The DNA-encoded nucleosome organization of a eukaryotic genome. *Nature* **458**: 362-366.

Kapranov P, Willingham AT, Gingeras TR. 2007. Genome-wide transcription and the implications for genomic organization. *Nat Rev Genet* **8**: 413-423.

Keck KM, Pemberton LF. 2011. Interaction with the histone chaperone Vps75 promotes nuclear localization and HAT activity of Rtt109 in vivo. *Traffic* **12**: 826-839.

Keogh MC, Kurdistani SK, Morris SA, Ahn SH, Podolny V, Collins SR, Schuldiner M, Chin K, Punna T, Thompson NJ et al. 2005. Cotranscriptional set2 methylation of histone H3 lysine 36 recruits a repressive Rpd3 complex. *Cell* **123**: 593-605.

Kettenberger H, Armache KJ, Cramer P. 2004. Complete RNA polymerase II elongation complex structure and its interactions with NTP and TFIIS. *Mol Cell* **16**: 955-965.

Kim J, Guermah M, Roeder RG. 2010. The human PAF1 complex acts in chromatin transcription elongation both independently and cooperatively with SII/TFIIS. *Cell* **140**: 491-503.

Kim M, Ahn SH, Krogan NJ, Greenblatt JF, Buratowski S. 2004. Transitions in RNA polymerase II elongation complexes at the 3' ends of genes. *EMBO J* **23**: 354-364.

Kim T, Buratowski S. 2009. Dimethylation of H3K4 by Set1 recruits the Set3 histone deacetylase complex to 5' transcribed regions. *Cell* **137**: 259-272.

Kim TH, Barrera LO, Zheng M, Qu C, Singer MA, Richmond TA, Wu Y, Green RD, Ren B. 2005. A high-resolution map of active promoters in the human genome. *Nature* **436**: 876-880.

Kiss T. 2002. Small nucleolar RNAs: an abundant group of noncoding RNAs with diverse cellular functions. *Cell* **109**: 145-148.

Kizer KO, Phatnani HP, Shibata Y, Hall H, Greenleaf AL, Strahl BD. 2005. A novel domain in Set2 mediates RNA polymerase II interaction and couples histone H3 K36 methylation with transcript elongation. *Mol Cell Biol* **25**: 3305-3316.

Klopotowski T, Wiater A. 1965. Synergism of aminotriazole and phosphate on the inhibition of yeast imidazole glycerol phosphate dehydratase. *Arch Biochem Biophys* **112**: 562-566.

Komarnitsky P, Cho EJ, Buratowski S. 2000. Different phosphorylated forms of RNA polymerase II and associated mRNA processing factors during transcription. *Genes Dev* **14**: 2452-2460.

Kornberg RD. 1974. Chromatin structure: a repeating unit of histones and DNA. *Science* **184**: 868-871.

Kornberg RD. 2005. Mediator and the mechanism of transcriptional activation. *Trends Biochem Sci* **30**: 235-239.

Kornberg RD. 2007. The molecular basis of eukaryotic transcription. *Proc Natl Acad Sci U S A* **104**: 12955-12961.

Kornberg RD, Lorch Y. 1999. Twenty-five years of the nucleosome, fundamental particle of the eukaryote chromosome. *Cell* **98**: 285-294.

Krebs JE. 2007. Moving marks: dynamic histone modifications in yeast. *Molecular bioSystems* **3**: 590-597.

Krick R, Bremer S, Welter E, Schlotterhose P, Muehe Y, Eskelinen EL, Thumm M. 2010. Cdc48/p97 and Shp1/p47 regulate autophagosome biogenesis in concert with ubiquitin-like Atg8. *J Cell Biol* **190**: 965-973.

Krishnamurthy S, Hampsey M. 2009. Eukaryotic transcription initiation. *Curr Biol* **19**: R153-156.

Krishnamurthy S, He X, Reyes-Reyes M, Moore C, Hampsey M. 2004. Ssu72 Is an RNA polymerase II CTD phosphatase. *Mol Cell* **14**: 387-394.

Krogan NJ, Dover J, Wood A, Schneider J, Heidt J, Boateng MA, Dean K, Ryan OW, Golshani A, Johnston M et al. 2003. The Paf1 complex is required for histone H3 methylation by COMPASS and Dot1p: linking transcriptional elongation to histone methylation. *Mol Cell* **11**: 721-729.

Krogan NJ, Kim M, Ahn SH, Zhong G, Kobor MS, Cagney G, Emili A, Shilatifard A, Buratowski S, Greenblatt JF. 2002. RNA polymerase II elongation factors of Saccharomyces cerevisiae: a targeted proteomics approach. *Mol Cell Biol* **22**: 6979-6992.

Kruger W, Herskowitz I. 1991. A negative regulator of HO transcription, SIN1 (SPT2), is a nonspecific DNA-binding protein related to HMG1. *Mol Cell Biol* **11**: 4135-4146.

Kuehner JN, Brow DA. 2008. Regulation of a eukaryotic gene by GTP-dependent start site selection and transcription attenuation. *Mol Cell* **31**: 201-211.

Kuehner JN, Pearson EL, Moore C. 2011. Unravelling the means to an end: RNA polymerase II transcription termination. *Nat Rev Mol Cell Biol* **12**: 283-294.

Kuo MH, Brownell JE, Sobel RE, Ranalli TA, Cook RG, Edmondson DG, Roth SY, Allis CD. 1996. Transcription-linked acetylation by Gcn5p of histones H3 and H4 at specific lysines. *Nature* **383**: 269-272.

LaCava J, Houseley J, Saveanu C, Petfalski E, Thompson E, Jacquier A, Tollervey D. 2005. RNA degradation by the exosome is promoted by a nuclear polyadenylation complex. *Cell* **121**: 713-724.

Laribee RN, Krogan NJ, Xiao T, Shibata Y, Hughes TR, Greenblatt JF, Strahl BD. 2005. BUR kinase selectively regulates H3 K4 trimethylation and H2B ubiquitylation through recruitment of the PAF elongation complex. *Curr Biol* **15**: 1487-1493.

Lee CK, Shibata Y, Rao B, Strahl BD, Lieb JD. 2004. Evidence for nucleosome depletion at active regulatory regions genome-wide. *Nat Genet* **36**: 900-905.

Lee JS, Shilatifard A. 2007. A site to remember: H3K36 methylation a mark for histone deacetylation. *Mutat Res* **618**: 130-134.

Lee JT. 2009. Lessons from X-chromosome inactivation: long ncRNA as guides and tethers to the epigenome. *Genes Dev* **23**: 1831-1842.

Lee W, Tillo D, Bray N, Morse RH, Davis RW, Hughes TR, Nislow C. 2007. A high-resolution atlas of nucleosome occupancy in yeast. *Nat Genet* **39**: 1235-1244.

Li B, Carey M, Workman JL. 2007a. The role of chromatin during transcription. *Cell* **128**: 707-719.

Li B, Gogol M, Carey M, Pattenden SG, Seidel C, Workman JL. 2007b. Infrequently transcribed long genes depend on the Set2/Rpd3S pathway for accurate transcription. *Genes Dev* **21**: 1422-1430.

Li B, Pattenden SG, Lee D, Gutierrez J, Chen J, Seidel C, Gerton J, Workman JL. 2005. Preferential occupancy of histone variant H2AZ at inactive promoters influences local histone modifications and chromatin remodeling. *Proc Natl Acad Sci U S A* **102**: 18385-18390.

Li Q, Zhou H, Wurtele H, Davies B, Horazdovsky B, Verreault A, Zhang Z. 2008. Acetylation of histone H3 lysine 56 regulates replication-coupled nucleosome assembly. *Cell* **134**: 244-255.

Li Z, Brendel M. 1993. Co-regulation with genes of phospholipid biosynthesis of the CTR/HNM1-encoded choline/nitrogen mustard permease in Saccharomyces cerevisiae. *Mol Gen Genet* **241**: 680-684.

Li Z, Vizeacoumar FJ, Bahr S, Li J, Warringer J, Vizeacoumar FS, Min R, Vandersluis B, Bellay J, Devit M et al. 2011. Systematic exploration of essential yeast gene function with temperature-sensitive mutants. *Nat Biotech* **29**: 361-367.

Li ZY, Haase E, Brendel M. 1991. Hyper-resistance to nitrogen mustard in Saccharomyces cerevisiae is caused by defective choline transport. *Curr Genet* **19**: 423-427.

Lia G, Praly E, Ferreira H, Stockdale C, Tse-Dinh YC, Dunlap D, Croquette V, Bensimon D, Owen-Hughes T. 2006. Direct observation of DNA distortion by the RSC complex. *Mol Cell* **21**: 417-425.

Libuda DE, Winston F. 2006. Amplification of histone genes by circular chromosome formation in Saccharomyces cerevisiae. *Nature* **443**: 1003-1007.

Libuda DE, Winston F. 2010. Alterations in DNA replication and histone levels promote histone gene amplification in Saccharomyces cerevisiae. *Genetics* **184**: 985-997.

Lickwar CR, Rao B, Shabalin AA, Nobel AB, Strahl BD, Lieb JD. 2009. The Set2/Rpd3S pathway suppresses cryptic transcription without regard to gene length or transcription frequency. *PLoS One* **4**: e4886.

Lin C, Yuan YA. 2008. Structural insights into histone H3 lysine 56 acetylation by Rtt109. *Structure* **16**: 1503-1510.

Lin L, Zhang JH, Panicker LM, Simonds WF. 2008. The parafibromin tumor suppressor protein inhibits cell proliferation by repression of the c-myc proto-oncogene. *Proc Natl Acad Sci U S A* **105**: 17420-17425.

Lindstrom DL, Squazzo SL, Muster N, Burckin TA, Wachter KC, Emigh CA, McCleery JA, Yates JR, 3rd, Hartzog GA. 2003. Dual roles for Spt5 in pre-mRNA processing and transcription elongation revealed by identification of Spt5-associated proteins. *Mol Cell Biol* **23**: 1368-1378.

Liu X, Bushnell DA, Silva DA, Huang X, Kornberg RD. 2011. Initiation complex structure and promoter proofreading. *Science* **333**: 633-637.

Liu Y, Warfield L, Zhang C, Luo J, Allen J, Lang WH, Ranish J, Shokat KM, Hahn S. 2009. Phosphorylation of the transcription elongation factor Spt5 by yeast Bur1 kinase stimulates recruitment of the PAF complex. *Mol Cell Biol* **29**: 4852-4863.

Lohr D. 1984. Organization of the GAL1-GAL10 intergenic control region chromatin. *Nucleic Acids Res* **12**: 8457-8474.

Longtine MS, McKenzie A, 3rd, Demarini DJ, Shah NG, Wach A, Brachat A, Philippsen P, Pringle JR. 1998. Additional modules for versatile and economical PCR-based gene deletion and modification in Saccharomyces cerevisiae. *Yeast* **14**: 953-961.

Luger K, Mader AW, Richmond RK, Sargent DF, Richmond TJ. 1997. Crystal structure of the nucleosome core particle at 2.8 A resolution. *Nature* **389**: 251-260.

Malone EA, Clark CD, Chiang A, Winston F. 1991. Mutations in SPT16/CDC68 suppress cis- and trans-acting mutations that affect promoter function in Saccharomyces cerevisiae. *Mol Cell Biol* **11**: 5710-5717.

Martens JA, Laprade L, Winston F. 2004. Intergenic transcription is required to repress the Saccharomyces cerevisiae SER3 gene. *Nature* **429**: 571-574.

Martens JA, Winston F. 2002. Evidence that Swi/Snf directly represses transcription in S. cerevisiae. *Genes Dev* **16**: 2231-2236.

Martens JA, Wu PY, Winston F. 2005. Regulation of an intergenic transcript controls adjacent gene transcription in Saccharomyces cerevisiae. *Genes Dev* **19**: 2695-2704.

Marton HA, Desiderio S. 2008. The Paf1 complex promotes displacement of histones upon rapid induction of transcription by RNA polymerase II. *BMC Mol Biol* **9**: 4.

Mason PB, Struhl K. 2003. The FACT complex travels with elongating RNA polymerase II and is important for the fidelity of transcriptional initiation in vivo. *Mol Cell Biol* **23**: 8323-8333.

Masumoto H, Hawke D, Kobayashi R, Verreault A. 2005. A role for cell-cycle-regulated histone H3 lysine 56 acetylation in the DNA damage response. *Nature* **436**: 294-298.

Max T, Sogaard M, Svejstrup JQ. 2007. Hyperphosphorylation of the C-terminal repeat domain of RNA polymerase II facilitates dissociation of its complex with mediator. *J Biol Chem* **282**: 14113-14120.

Mayer A, Lidschreiber M, Siebert M, Leike K, Soding J, Cramer P. 2010. Uniform transitions of the general RNA polymerase II transcription complex. *Nat Struct Mol Biol* **17**: 1272-1278.

Mazo A, Hodgson JW, Petruk S, Sedkov Y, Brock HW. 2007. Transcriptional interference: an unexpected layer of complexity in gene regulation. *J Cell Sci* **120**: 2755-2761.

Mercer TR, Dinger ME, Mattick JS. 2009. Long non-coding RNAs: insights into functions. *Nat Rev Genet* **10**: 155-159.

Miura F, Kawaguchi N, Sese J, Toyoda A, Hattori M, Morishita S, Ito T. 2006. A large-scale full-length cDNA analysis to explore the budding yeast transcriptome. *Proc Natl Acad Sci U S A* **103**: 17846-17851.

Mnaimneh S, Davierwala AP, Haynes J, Moffat J, Peng WT, Zhang W, Yang X, Pootoolal J, Chua G, Lopez A et al. 2004. Exploration of essential gene functions via titratable promoter alleles. *Cell* **118**: 31-44.

Moniaux N, Nemos C, Schmied BM, Chauhan SC, Deb S, Morikane K, Choudhury A, Vanlith M, Sutherlin M, Sikela JM et al. 2006. The human homologue of the RNA polymerase II-associated factor 1 (hPaf1), localized on the 19q13 amplicon, is associated with tumorigenesis. *Oncogene* **25**: 3247-3257.

Moran L, Norris D, Osley MA. 1990. A yeast H2A-H2B promoter can be regulated by changes in histone gene copy number. *Genes Dev* **4**: 752-763.

Morillon A, Karabetsou N, O'Sullivan J, Kent N, Proudfoot N, Mellor J. 2003. Isw1 chromatin remodeling ATPase coordinates transcription elongation and termination by RNA polymerase II. *Cell* **115**: 425-435.

Morris KV, Santoso S, Turner AM, Pastori C, Hawkins PG. 2008. Bidirectional transcription directs both transcriptional gene activation and suppression in human cells. *PLoS Genet* **4**: e1000258.

Mosley AL, Pattenden SG, Carey M, Venkatesh S, Gilmore JM, Florens L, Workman JL, Washburn MP. 2009. Rtr1 is a CTD phosphatase that regulates RNA polymerase II during the transition from serine 5 to serine 2 phosphorylation. *Mol Cell* **34**: 168-178.

Mueller CL, Jaehning JA. 2002. Ctr9, Rtf1, and Leo1 are components of the Paf1/RNA polymerase II complex. *Mol Cell Biol* **22**: 1971-1980.

Mueller CL, Porter SE, Hoffman MG, Jaehning JA. 2004. The Paf1 complex has functions independent of actively transcribing RNA polymerase II. *Mol Cell* **14**: 447-456.

Mulder KW, Brenkman AB, Inagaki A, van den Broek NJ, Timmers HT. 2007. Regulation of histone H3K4 tri-methylation and PAF complex recruitment by the Ccr4-Not complex. *Nucleic Acids Res* **35**: 2428-2439.

Murray S, Udupa R, Yao S, Hartzog G, Prelich G. 2001. Phosphorylation of the RNA polymerase II carboxy-terminal domain by the Bur1 cyclin-dependent kinase. *Mol Cell Biol* **21**: 4089-4096.

Nagalakshmi U, Wang Z, Waern K, Shou C, Raha D, Gerstein M, Snyder M. 2008. The transcriptional landscape of the yeast genome defined by RNA sequencing. *Science* **320**: 1344-1349.

Natsoulis G, Dollard C, Winston F, Boeke JD. 1991. The products of the SPT10 and SPT21 genes of Saccharomyces cerevisiae increase the amplitude of transcriptional regulation at a large number of unlinked loci. *New Biol* **3**: 1249-1259.

Natsoulis G, Winston F, Boeke JD. 1994. The SPT10 and SPT21 genes of Saccharomyces cerevisiae. *Genetics* **136**: 93-105.

Neil H, Malabat C, d'Aubenton-Carafa Y, Xu Z, Steinmetz LM, Jacquier A. 2009. Widespread bidirectional promoters are the major source of cryptic transcripts in yeast. *Nature* **457**: 1038-1042.

Neuwald AF, Landsman D. 1997. GCN5-related histone N-acetyltransferases belong to a diverse superfamily that includes the yeast SPT10 protein. *Trends Biochem Sci* **22**: 154-155.

Ng HH, Dole S, Struhl K. 2003a. The Rtf1 component of the Paf1 transcriptional elongation complex is required for ubiquitination of histone H2B. *J Biol Chem* **278**: 33625-33628.

Ng HH, Robert F, Young RA, Struhl K. 2003b. Targeted recruitment of Set1 histone methylase by elongating Pol II provides a localized mark and memory of recent transcriptional activity. *Mol Cell* **11**: 709-719.

Ng HH, Xu RM, Zhang Y, Struhl K. 2002. Ubiquitination of histone H2B by Rad6 is required for efficient Dot1-mediated methylation of histone H3 lysine 79. *J Biol Chem* **277**: 34655-34657.

Nikawa J, Hosaka K, Tsukagoshi Y, Yamashita S. 1990. Primary structure of the yeast choline transport gene and regulation of its expression. *J Biol Chem* **265**: 15996-16003.

Nikawa J, Tsukagoshi Y, Yamashita S. 1986. Cloning of a gene encoding choline transport in Saccharomyces cerevisiae. *J Bacter* **166**: 328-330.

Nissen P, Hansen J, Ban N, Moore PB, Steitz TA. 2000. The structural basis of ribosome activity in peptide bond synthesis. *Science* **289**: 920-930.

Nordick K, Hoffman MG, Betz JL, Jaehning JA. 2008. Direct interactions between the Paf1 complex and a cleavage and polyadenylation factor are revealed by dissociation of Paf1 from RNA polymerase II. *Eukaryot Cell* **7**: 1158-1167.

Norris D, Osley MA. 1987. The two gene pairs encoding H2A and H2B play different roles in the Saccharomyces cerevisiae life cycle. *Mol Cell Biol* **7**: 3473-3481.

Nourani A, Robert F, Winston F. 2006. Evidence that Spt2/Sin1, an HMG-like factor, plays roles in transcription elongation, chromatin structure, and genome stability in Saccharomyces cerevisiae. *Mol Cell Biol* **26**: 1496-1509.

O'Sullivan JM, Tan-Wong SM, Morillon A, Lee B, Coles J, Mellor J, Proudfoot NJ. 2004. Gene loops juxtapose promoters and terminators in yeast. *Nat Genet* **36**: 1014-1018.

Ozdemir A, Masumoto H, Fitzjohn P, Verreault A, Logie C. 2006. Histone H3 lysine 56 acetylation: a new twist in the chromosome cycle. *Cell Cycle* **5**: 2602-2608.

Palmer AC, Egan JB, Shearwin KE. 2011. Transcriptional interference by RNA polymerase pausing and dislodgement of transcription factors. *Transcription* **2**: 9-14.

Papamichos-Chronakis M, Krebs JE, Peterson CL. 2006. Interplay between Ino80 and Swr1 chromatin remodeling enzymes regulates cell cycle checkpoint adaptation in response to DNA damage. *Genes Dev* **20**: 2437-2449.

Park NJ, Tsao DC, Martinson HG. 2004. The two steps of poly(A)-dependent termination, pausing and release, can be uncoupled by truncation of the RNA polymerase II carboxyl-terminal repeat domain. *Mol Cell Biol* **24**: 4092-4103.

Pavri R, Zhu B, Li G, Trojer P, Mandal S, Shilatifard A, Reinberg D. 2006. Histone H2B monoubiquitination functions cooperatively with FACT to regulate elongation by RNA polymerase II. *Cell* **125**: 703-717.

Peckham HE, Thurman RE, Fu Y, Stamatoyannopoulos JA, Noble WS, Struhl K, Weng Z. 2007. Nucleosome positioning signals in genomic DNA. *Genome Res* **17**: 1170-1177.

Penheiter KL, Washburn TM, Porter SE, Hoffman MG, Jaehning JA. 2005. A posttranscriptional role for the yeast Paf1-RNA polymerase II complex is revealed by identification of primary targets. *Mol Cell* **20**: 213-223.

Perocchi F, Xu Z, Clauder-Munster S, Steinmetz LM. 2007. Antisense artifacts in transcriptome microarray experiments are resolved by actinomycin D. *Nucleic Acids Res* **35**: e128.

Petruk S, Sedkov Y, Riley KM, Hodgson J, Schweisguth F, Hirose S, Jaynes JB, Brock HW, Mazo A. 2006. Transcription of bxd noncoding RNAs promoted by trithorax represses Ubx in cis by transcriptional interference. *Cell* **127**: 1209-1221.

Pfaffl MW. 2001. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* **29**: e45.

Pheasant M, Mattick JS. 2007. Raising the estimate of functional human sequences. *Genome Res* **17**: 1245-1253.

Pinskaya M, Gourvennec S, Morillon A. 2009. H3 lysine 4 di- and tri-methylation deposited by cryptic transcription attenuates promoter activation. *EMBO J* **28**: 1697-1707.

Pokholok DK, Hannett NM, Young RA. 2002. Exchange of RNA polymerase II initiation and elongation factors during gene expression in vivo. *Mol Cell* **9**: 799-809.

Pokholok DK, Harbison CT, Levine S, Cole M, Hannett NM, Lee TI, Bell GW, Walker K, Rolfe PA, Herbolsheimer E et al. 2005. Genome-wide map of nucleosome acetylation and methylation in yeast. *Cell* **122**: 517-527.

Polo SE, Almouzni G. 2006. Chromatin assembly: a basic recipe with various flavours. *Curr Opin Genet Dev* **16**: 104-111.

Pruneski JA, Hainer SJ, Petrov KO, Martens JA. 2011. The Paf1 complex represses SER3 transcription in Saccharomyces cerevisiae by facilitating intergenic transcription-dependent nucleosome occupancy of the SER3 promoter. *Eukaryot Cell* **10**: 1283-1294.

Pruneski JA, Martens JA. 2011. Transcription of intergenic DNA deposits nucleosomes on promoter to silence gene expression. *Cell Cycle* **10**: 1021-1022.

Qiu H, Hu C, Hinnebusch AG. 2009. Phosphorylation of the Pol II CTD by KIN28 enhances BUR1/BUR2 recruitment and Ser2 CTD phosphorylation near promoters. *Mol Cell* **33**: 752-762.

Qiu H, Hu C, Wong CM, Hinnebusch AG. 2006. The Spt4p subunit of yeast DSIF stimulates association of the Paf1 complex with elongating RNA polymerase II. *Mol Cell Biol* **26**: 3135-3148.

Raisner RM, Hartley PD, Meneghini MD, Bao MZ, Liu CL, Schreiber SL, Rando OJ, Madhani HD. 2005. Histone variant H2A.Z marks the 5' ends of both active and inactive genes in euchromatin. *Cell* **123**: 233-248.

Rao B, Shibata Y, Strahl BD, Lieb JD. 2005. Dimethylation of histone H3 at lysine 36 demarcates regulatory and nonregulatory chromatin genome-wide. *Mol Cell Biol* **25**: 9447-9459.

Rinn JL, Kertesz M, Wang JK, Squazzo SL, Xu X, Brugmann SA, Goodnough LH, Helms JA, Farnham PJ, Segal E et al. 2007. Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. *Cell* **129**: 1311-1323.

Rodriguez-Navarro S. 2009. Insights into SAGA function during gene expression. *EMBO Rep* **10**: 843-850.

Rondon AG, Gallardo M, Garcia-Rubio M, Aguilera A. 2004. Molecular evidence indicating that the yeast PAF complex is required for transcription elongation. *EMBO Rep* **5**: 47-53.

Rondon AG, Garcia-Rubio M, Gonzalez-Barrera S, Aguilera A. 2003. Molecular evidence for a positive role of Spt4 in transcription elongation. *EMBO J* **22**: 612-620.

Rose MD, F. Winston and P. Heiter. 1991. Methods in Yeast Genetics: a Laboratory Course Manual. in *Cold Spring Harbor Laboratory Press*.

Rossi ML, Bambara RA. 2006. Reconstituted Okazaki fragment processing indicates two pathways of primer removal. *J Biol Chem* **281**: 26051-26061.

Royo H, Cavaille J. 2008. Non-coding RNAs in imprinted gene clusters. *Biol Cell* **100**: 149-166.

Rufiange A, Jacques PE, Bhat W, Robert F, Nourani A. 2007. Genome-wide replication-independent histone H3 exchange occurs predominantly at promoters and implicates H3 K56 acetylation and Asf1. *Mol Cell* **27**: 393-405.

Sabet N, Tong F, Madigan JP, Volo S, Smith MM, Morse RH. 2003. Global and specific transcriptional repression by the histone H3 amino terminus in yeast. *Proc Natl Acad Sci U S A* **100**: 4084-4089.

Saha A, Wittmeyer J, Cairns BR. 2002. Chromatin remodeling by RSC involves ATP-dependent DNA translocation. *Genes Dev* **16**: 2120-2134.

Schmitt S, Prestel M, Paro R. 2005. Intergenic transcription through a polycomb group response element counteracts silencing. *Genes Dev* **19**: 697-708.

Schneider J, Bajwa P, Johnson FC, Bhaumik SR, Shilatifard A. 2006. Rtt109 is required for proper H3K56 acetylation: a chromatin mark associated with the elongating RNA polymerase II. *J Biol Chem* **281**: 37270-37274.

Scholes DT, Banerjee M, Bowen B, Curcio MJ. 2001. Multiple regulators of Ty1 transposition in Saccharomyces cerevisiae have conserved roles in genome maintenance. *Genetics* **159**: 1449-1465.

Schuberth C, Richly H, Rumpf S, Buchberger A. 2004. Shp1 and Ubx2 are adaptors of Cdc48 involved in ubiquitin-dependent protein degradation. *EMBO Rep* **5**: 818-824.

Schwabish MA, Struhl K. 2004. Evidence for eviction and rapid deposition of histones upon transcriptional elongation by RNA polymerase II. *Mol Cell Biol* **24**: 10111-10117.

Schwabish MA, Struhl K. 2007. The Swi/Snf complex is important for histone eviction during transcriptional activation and RNA polymerase II elongation in vivo. *Mol Cell Biol* **27**: 6987-6995.

Segal E, Fondufe-Mittendorf Y, Chen L, Thastrom A, Field Y, Moore IK, Wang JP, Widom J. 2006. A genomic code for nucleosome positioning. *Nature* **442**: 772-778.

Segal E, Widom J. 2009. What controls nucleosome positions? *Trends Genet* **25**: 335-343.

Sekinger EA, Moqtaderi Z, Struhl K. 2005. Intrinsic histone-DNA interactions and low nucleosome density are important for preferential accessibility of promoter regions in yeast. *Mol Cell* **18**: 735-748.

Sellick CA, Reece RJ. 2005. Eukaryotic transcription factors as direct nutrient sensors. *Trends Biochem Sci* **30**: 405-412.

Shahbazian MD, Zhang K, Grunstein M. 2005. Histone H2B ubiquitylation controls processive methylation but not monomethylation by Dot1 and Set1. *Mol Cell* **19**: 271-277.

Sheldon KE, Mauger DM, Arndt KM. 2005. A Requirement for the Saccharomyces cerevisiae Paf1 complex in snoRNA 3' end formation. *Mol Cell* **20**: 225-236.

Sherwood PW, Tsang SV, Osley MA. 1993. Characterization of HIR1 and HIR2, two genes required for regulation of histone gene transcription in Saccharomyces cerevisiae. *Mol Cell Biol* **13**: 28-38.

Shi X, Chang M, Wolf AJ, Chang CH, Frazer-Abel AA, Wade PA, Burton ZF, Jaehning JA. 1997. Cdc73p and Paf1p are found in a novel RNA polymerase II-containing complex distinct from the Srbp-containing holoenzyme. *Mol Cell Biol* **17**: 1160-1169.

Shim EY, Hong SJ, Oum JH, Yanez Y, Zhang Y, Lee SE. 2007. RSC mobilizes nucleosomes to improve accessibility of repair machinery to the damaged chromatin. *Mol Cell Biol* **27**: 1602-1613.

Shirra MK, Rogers SE, Alexander DE, Arndt KM. 2005. The Snf1 protein kinase and Sit4 protein phosphatase have opposing functions in regulating TATA-binding protein association with the Saccharomyces cerevisiae INO1 promoter. *Genetics* **169**: 1957-1972.

Sikorski TW, Buratowski S. 2009. The basal initiation machinery: beyond the general transcription factors. *Curr Opin Cell Biol* **21**: 344-351.

Simic R, Lindstrom DL, Tran HG, Roinick KL, Costa PJ, Johnson AD, Hartzog GA, Arndt KM. 2003. Chromatin remodeling protein Chd1 interacts with transcription elongation factors and localizes to transcribed genes. *EMBO J* **22**: 1846-1856.

Smerdon MJ. 1991. DNA repair and the role of chromatin structure. *Curr Opin Cell Biol* **3**: 422-428.

Spector MS, Raff A, DeSilva H, Lee K, Osley MA. 1997. Hir1p and Hir2p function as transcriptional corepressors to regulate histone gene transcription in the Saccharomyces cerevisiae cell cycle. *Mol Cell Biol* **17**: 545-552.

Squazzo SL, Costa PJ, Lindstrom DL, Kumer KE, Simic R, Jennings JL, Link AJ, Arndt KM, Hartzog GA. 2002. The Paf1 complex physically and functionally associates with transcription elongation factors in vivo. *EMBO J* **21**: 1764-1774.

Stavropoulos P, Nagy V, Blobel G, Hoelz A. 2008. Molecular basis for the autoregulation of the protein acetyl transferase Rtt109. *Proc Natl Acad Sci U S A* **105**: 12236-12241.

Steinmetz EJ, Warren CL, Kuehner JN, Panbehi B, Ansari AZ, Brow DA. 2006. Genome-wide distribution of yeast RNA polymerase II and its control by Sen1 helicase. *Mol Cell* **24**: 735-746.

Stillman B. 1986. Chromatin assembly during SV40 DNA replication in vitro. *Cell* **45**: 555-565.

Stolinski LA, Eisenmann DM, Arndt KM. 1997. Identification of RTF1, a novel gene important for TATA site selection by TATA box-binding protein in Saccharomyces cerevisiae. *Mol Cell Biol* **17**: 4490-4500.

Strahl BD, Allis CD. 2000. The language of covalent histone modifications. *Nature* **403**: 41-45.

Strohner R, Wachsmuth M, Dachauer K, Mazurkiewicz J, Hochstatter J, Rippe K, Langst G. 2005. A 'loop recapture' mechanism for ACF-dependent nucleosome remodeling. *Nat Struct Mol Biol* **12**: 683-690.

Struhl K. 1989. Molecular mechanisms of transcriptional regulation in yeast. *Annu Rev Biochem* **58**: 1051-1077.

Struhl K. 2007. Transcriptional noise and the fidelity of initiation by RNA polymerase II. *Nat Struct Mol Biol* **14**: 103-105.

Struhl K, Davis RW. 1977. Production of a functional eukaryotic enzyme in Escherichia coli: cloning and expression of the yeast structural gene for imidazole-glycerolphosphate dehydratase (his3). *Proc Natl Acad Sci U S A* **74**: 5255-5259.

Sudarsanam P, Iyer VR, Brown PO, Winston F. 2000. Whole-genome expression analysis of snf/swi mutants of Saccharomyces cerevisiae. *Proc Natl Acad Sci U S A* **97**: 3364-3369.

Suganuma T, Workman JL. 2008. Crosstalk among Histone Modifications. *Cell* **135**: 604-607.

Sun M, Lariviere L, Dengl S, Mayer A, Cramer P. 2010. A tandem SH2 domain in transcription elongation factor Spt6 binds the phosphorylated RNA polymerase II C-terminal repeat domain (CTD). *J Biol Chem* **285**: 41597-41603.

Sun ZW, Allis CD. 2002. Ubiquitination of histone H2B regulates H3 methylation and gene silencing in yeast. *Nature* **418**: 104-108.

Swanson MS, Winston F. 1992. SPT4, SPT5 and SPT6 interactions: effects on transcription and viability in Saccharomyces cerevisiae. *Genetics* **132**: 325-336.

Sychrova H, Chevallier MR. 1993. Cloning and sequencing of the Saccharomyces cerevisiae gene LYP1 coding for a lysine-specific permease. *Yeast* **9**: 771-782.

Talbert PB, Henikoff S. 2010. Histone variants--ancient wrap artists of the epigenome. *Nat Rev Mol Cell Biol* **11**: 264-275.

Tang Y, Holbert MA, Delgoshaie N, Wurtele H, Guillemette B, Meeth K, Yuan H, Drogaris P, Lee EH, Durette C et al. 2011. Structure of the Rtt109-AcCoA/Vps75 complex and implications for chaperone-mediated histone acetylation. *Structure* **19**: 221-231.

Tang Y, Holbert MA, Wurtele H, Meeth K, Rocha W, Gharib M, Jiang E, Thibault P, Verreault A, Cole PA et al. 2008. Fungal Rtt109 histone acetyltransferase is an unexpected structural homolog of metazoan p300/CBP. *Nat Struct Mol Biol* **15**: 998.

Tenney K, Gerber M, Ilvarsonn A, Schneider J, Gause M, Dorsett D, Eissenberg JC, Shilatifard A. 2006. Drosophila Rtf1 functions in histone methylation, gene expression, and Notch signaling. *Proc Natl Acad Sci U S A* **103**: 11970-11974.

Thebault P, Boutin G, Bhat W, Rufiange A, Martens J, Nourani A. 2011. Transcription regulation by the noncoding RNA SRG1 requires Spt2-dependent chromatin deposition in the wake of RNA polymerase II. *Mol Cell Biol* **31**: 1288-1300.

Thompson DM, Parker R. 2007. Cytoplasmic decay of intergenic transcripts in Saccharomyces cerevisiae. *Mol Cell Biol* **27**: 92-101.

Tomson BN, Davis CP, Warner MH, Arndt KM. 2011. Identification of a role for histone H2B ubiquitylation in noncoding RNA 3'-end formation through mutational analysis of Rtf1 in Saccharomyces cerevisiae. *Genetics* **188**: 273-289.

Tong AH, Boone C. 2006. Synthetic genetic array analysis in Saccharomyces cerevisiae. *Methods Mol Biol* **313**: 171-192.

Tong AH, Evangelista M, Parsons AB, Xu H, Bader GD, Page N, Robinson M, Raghibizadeh S, Hogue CW, Bussey H et al. 2001. Systematic genetic analysis with ordered arrays of yeast deletion mutants. *Science* **294**: 2364-2368.

Tous C, Rondon AG, Garcia-Rubio M, Gonzalez-Aguilera C, Luna R, Aguilera A. 2011. A novel assay identifies transcript elongation roles for the Nup84 complex and RNA processing factors. *EMBO J* **30**: 1953-1964.

Tran HG, Steger DJ, Iyer VR, Johnson AD. 2000. The chromo domain protein chd1p from budding yeast is an ATP-dependent chromatin-modifying factor. *EMBO J* **19**: 2323-2331.

Tsubota T, Berndsen CE, Erkmann JA, Smith CL, Yang L, Freitas MA, Denu JM, Kaufman PD. 2007. Histone H3-K56 acetylation is catalyzed by histone chaperone-dependent complexes. *Mol Cell* **25**: 703-712.

Uhler JP, Hertel C, Svejstrup JQ. 2007. A role for noncoding transcription in activation of the yeast PHO5 gene. *Proc Natl Acad Sci U S A* **104**: 8011-8016.

van Bakel H, Nislow C, Blencowe BJ, Hughes TR. 2010. Most "dark matter" transcripts are associated with known genes. *PLoS Biol* **8**: e1000371.

van Dijk EL, Chen CL, d'Aubenton-Carafa Y, Gourvennec S, Kwapisz M, Roche V, Bertrand C, Silvain M, Legoix-Ne P, Loeillet S et al. 2011. XUTs are a class of Xrn1-sensitive antisense regulatory non-coding RNA in yeast. *Nature* **475**: 114-117.

Vanacova S, Wolf J, Martin G, Blank D, Dettwiler S, Friedlein A, Langen H, Keith G, Keller W. 2005. A new yeast poly(A) polymerase complex involved in RNA quality control. *PLoS Biol* **3**: e189.

Vincent JA, Kwong TJ, Tsukiyama T. 2008. ATP-dependent chromatin remodeling shapes the DNA replication landscape. *Nat Struct Mol Biol* **15**: 477-484.

Wada T, Takagi T, Yamaguchi Y, Ferdous A, Imai T, Hirose S, Sugimoto S, Yano K, Hartzog GA, Winston F et al. 1998. DSIF, a novel transcription elongation factor that regulates RNA polymerase II processivity, is composed of human Spt4 and Spt5 homologs. *Genes Dev* **12**: 343-356.

Wang G, Cui Y, Zhang G, Garen A, Song X. 2009. Regulation of proto-oncogene transcription, cell proliferation, and tumorigenesis in mice by PSF protein and a VL30 noncoding RNA. *Proc Natl Acad Sci U S A* **106**: 16794-16798.

Wang KC, Chang HY. 2011. Molecular mechanisms of long noncoding RNAs. *Mol Cell* **43**: 904-914.

Wang P, Bowl MR, Bender S, Peng J, Farber L, Chen J, Ali A, Zhang Z, Alberts AS, Thakker RV et al. 2008. Parafibromin, a component of the human PAF complex, regulates growth factors and is required for embryonic development and survival in adult mice. *Mol Cell Biol* **28**: 2930-2940.

Warner MH, Roinick KL, Arndt KM. 2007. Rtf1 is a multifunctional component of the Paf1 complex that regulates gene expression by directing cotranscriptional histone modification. *Mol Cell Biol* **27**: 6103-6115.

West ML, Corden JL. 1995. Construction and analysis of yeast RNA polymerase II CTD deletion and substitution mutations. *Genetics* **140**: 1223-1233.

Whitehouse I, Tsukiyama T. 2006. Antagonistic forces that position nucleosomes in vivo. *Nat Struct Mol Biol* **13**: 633-640.

Will CL, Luhrmann R. 2011. Spliceosome structure and function. *Cold Spring Harb Perspect Biol* **3**.

Williams SK, Truong D, Tyler JK. 2008. Acetylation in the globular core of histone H3 on lysine-56 promotes chromatin disassembly during transcriptional activation. *Proc Natl Acad Sci U S A* **105**: 9000-9005.

Willingham AT, Gingeras TR. 2006. TUF love for "junk" DNA. *Cell* **125**: 1215-1220.

Winston F, Chaleff DT, Valent B, Fink GR. 1984. Mutations affecting Ty-mediated expression of the HIS4 gene of Saccharomyces cerevisiae. *Genetics* **107**: 179-197.

Winston F, Dollard C, Ricupero-Hovasse SL. 1995. Construction of a set of convenient Saccharomyces cerevisiae strains that are isogenic to S288C. *Yeast* **11**: 53-55.

Winzeler EA, Shoemaker DD, Astromoff A, Liang H, Anderson K, Andre B, Bangham R, Benito R, Boeke JD, Bussey H et al. 1999. Functional characterization of the S. cerevisiae genome by gene deletion and parallel analysis. *Science* **285**: 901-906.

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. *J Biol Chem* **278**: 34739-34742.

Wood A, Schneider J, Dover J, Johnston M, Shilatifard A. 2005. The Bur1/Bur2 complex is required for histone H2B monoubiquitination by Rad6/Bre1 and histone methylation by COMPASS. *Mol Cell* **20**: 589-599.

Wood A, Shilatifard A. 2006. Bur1/Bur2 and the Ctk complex in yeast: the split personality of mammalian P-TEFb. *Cell Cycle* **5**: 1066-1068.

Wu X, Wang Z. 1999. Relationships between yeast Rad27 and Apn1 in response to apurinic/apyrimidinic (AP) sites in DNA. *Nucleic Acids Res* **27**: 956-962.

Wyers F, Rougemaille M, Badis G, Rousselle JC, Dufour ME, Boulay J, Regnault B, Devaux F, Namane A, Seraphin B et al. 2005. Cryptic pol II transcripts are degraded by a nuclear quality control pathway involving a new poly(A) polymerase. *Cell* **121**: 725-737.

Wyrick JJ, Holstege FC, Jennings EG, Causton HC, Shore D, Grunstein M, Lander ES, Young RA. 1999. Chromosomal landscape of nucleosome-dependent gene expression and silencing in yeast. *Nature* **402**: 418-421.

Xiao T, Kao CF, Krogan NJ, Sun ZW, Greenblatt JF, Osley MA, Strahl BD. 2005. Histone H2B ubiquitylation is associated with elongating RNA polymerase II. *Mol Cell Biol* **25**: 637-651.

Xie Y, Liu Y, Argueso JL, Henricksen LA, Kao HI, Bambara RA, Alani E. 2001. Identification of rad27 mutations that confer differential defects in mutation avoidance, repeat tract instability, and flap cleavage. *Mol Cell Biol* **21**: 4889-4899.

Xin H, Takahata S, Blanksma M, McCullough L, Stillman DJ, Formosa T. 2009. yFACT induces global accessibility of nucleosomal DNA without H2A-H2B displacement. *Mol Cell* **35**: 365-376.

Xu Z, Wei W, Gagneur J, Clauder-Munster S, Smolik M, Huber W, Steinmetz LM. 2011. Antisense expression increases gene expression variability and locus interdependency. *Mol Syst Biol* **7**: 468.

Xu Z, Wei W, Gagneur J, Perocchi F, Clauder-Munster S, Camblong J, Guffanti E, Stutz F, Huber W, Steinmetz LM. 2009. Bidirectional promoters generate pervasive transcription in yeast. *Nature* **457**: 1033-1037.

Yamashita I. 1993. Isolation and characterization of the SUD1 gene, which encodes a global repressor of core promoter activity in Saccharomyces cerevisiae. *Mol Gen Genet* **241**: 616-626.

Yao S, Neiman A, Prelich G. 2000. BUR1 and BUR2 encode a divergent cyclin-dependent kinase-cyclin complex important for transcription in vivo. *Mol Cell Biol* **20**: 7080-7087.

Youdell ML, Kizer KO, Kisseleva-Romanova E, Fuchs SM, Duro E, Strahl BD, Mellor J. 2008. Roles for Ctk1 and Spt6 in regulating the different methylation states of histone H3 lysine 36. *Mol Cell Biol* **28**: 4915-4926.

Yuan GC, Liu YJ, Dion MF, Slack MD, Wu LF, Altschuler SJ, Rando OJ. 2005. Genome-scale identification of nucleosome positions in S. cerevisiae. *Science* **309**: 626-630.

Yun M, Wu J, Workman JL, Li B. 2011. Readers of histone modifications. *Cell Res* **21**: 564-578.

Zhang H, Roberts DN, Cairns BR. 2005. Genome-wide dynamics of Htz1, a histone H2A variant that poises repressed/basal promoters for activation through histone loss. *Cell* **123**: 219-231.

Zhang S, Guha S, Volkert FC. 1995. The Saccharomyces SHP1 gene, which encodes a regulator of phosphoprotein phosphatase 1 with differential effects on glycogen metabolism, meiotic differentiation, and mitotic cell cycle progression. *Mol Cell Biol* **15**: 2037-2050.

Zhang Y, Smith CL, Saha A, Grill SW, Mihardja S, Smith SB, Cairns BR, Peterson CL, Bustamante C. 2006. DNA translocation and loop formation mechanism of chromatin remodeling by SWI/SNF and RSC. *Mol Cell* **24**: 559-568.

Zheng L, Shen B. 2011. Okazaki fragment maturation: nucleases take centre stage. *J Mol Cell Biol* **3**: 23-30.

Zheng S, Wyrick JJ, Reese JC. 2010. Novel trans-tail regulation of H2B ubiquitylation and H3K4 methylation by the N terminus of histone H2A. *Mol Cell Biol* **30**: 3635-3645.

Zhou K, Kuo WH, Fillingham J, Greenblatt JF. 2009. Control of transcriptional elongation and cotranscriptional histone modification by the yeast BUR kinase substrate Spt5. *Proc Natl Acad Sci U S A* **106**: 6956-6961.